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Brief report

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

Mathilde Couteaudier [1,2], Diego Calzada-Fraile [1,2], Thomas Montange [1,2], Antoine Gessain [1,2], and Florence Buseyne [1,2]*

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

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

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

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

Abstract

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

Keywords

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

Introduction

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

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

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

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

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

Results

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

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

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

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

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

Human hematopoietic cell lines are poorly susceptible to infection with SFVs

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

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

SFV Env-mediated fusion is inducible by short exposure to acidic pH when expressed at the cell surface [33]. The fusion process is much slower for macaque SFV Env than CI-PFV Env [34]. We thus tested whether changing the pH could enhance infection with viral particles. GFAB 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 to acidic pH had no effect on the number of infected GI-D468 cells and decreased the number of those infected with CI-PFV (Figure 2A). Cationic polymers can also increase the adsorption of virions on target cell membranes, leading to the enhancement of infection of certain retroviruses [35]. We infected GFAB cells with GI-D468 or CI-PFV in the presence of polyethylenimine, polybrene, or DEAE dextran at concentrations ranging from 0.5 to 8 µg/mL. Cationic polymers did not increase the frequency of infection with GI-D468 or CI-PFV, and even decreased infectivity at the highest concentrations (Figure 2C).

IFN inhibitors enhance infection with gorilla SFVs

We addressed whether SFV replication is sensitive to type I IFN-mediated restriction by adding small molecules that inhibit kinases involved in IFN induction (TBK-1 and IKK2) or signaling (JAK1/2) [36-38]. Inhibitors of JAK1/2 (Ruxolitinib), TBK-1 (BX795), or IKK2 (TPCA-1 and BMS345541) were added to cultures 4 h before GFAB cell infection and maintained during the 72 h-long culture. Ruxolitinib and BX795 increased the number of GFAB cells infected with GI-D468 and GII-BAK74 (t test, $P < 0.05$, Figure 3A-B). There was no additive effect if both molecules were mixed (data not shown). At the highest concentrations tested, there was a cytotoxic effect that was more evident 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 shwon). 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 understimation 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

Discussion

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

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

Our primary aim was to enhance the *in vitro* growth of primary gorilla SFV. We thus included the laboratory-adapted chimpanzee CI-PFV strain as a positive control of infection in every experiment. Its susceptibility to IFN blockade differed from that of gorilla SFV strains. Indeed, CI-PFV infection was not enhanced by ruxolitinib or BX795. Several nonexclusive mechanisms could account for such a strain-specific effect. As discussed above, pharmacological inhibitors may block several pathways and/or have several targets with opposite effects. For example, JAK/STAT inhibitors 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

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

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

Materials and Methods

Cells

K562 (ECACC 89121407, human erythroleukemia cells) and BHK-21 (ATCC-CLL-10, hamster kidney fibroblasts) cells were obtained from colleagues at the Institut Pasteur. BHK-21-derived gorilla foamy virus-activated β-galactosidase (GFAB) cells were generated in our laboratory [23]. Other cell lines were purchased from LGC standards or Sigma-Aldrich and stocks were produced after less than 10 passages. The cells were screened for mycoplasma infection every 10 passages with a bioluminescent assay (#LT07-418, Lonza). The HT1080 cells (ECACC 85111505, human fibrosarcoma) were cultivated in Eagle's Minimum Essential Medium with Earle's Balanced Salts and L-Glutamine (EMEM-EBSS, Lonza) supplemented with 10% fetal bovine serum (FBS, PAA Laboratories) and 1% nonessential amino acids (NEAA, Invitrogen). The K562, HEL 92.1.7 (ATCC-TIB-180, human erythroleukemia cells), THP-1 (ECACC 88081201, human monocytic leukemia cells), Raji (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 Dulbeco'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 $MgCl_2$;
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 µl 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 µl 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

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

Flow cytometry

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

Statistics

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

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Conflicts of Interest

The authors have no conflicting interests relevant to the study.

References

1. Gessain, A., R. Rua, E. Betsem, J. Turpin, and R. Mahieux, HTLV-3/4 and simian foamy retroviruses in humans: Discovery, epidemiology, cross-species transmission and molecular virology. Virology, 2013. **435**: 187-99.
2. Betsem, E., R. Rua, P. Tortevoye, A. Froment, and A. Gessain, Frequent and recent human acquisition of simian foamy viruses through apes' bites in Central Africa. PLoS Pathog, 2011. **7**: e1002306.
3. Rua, R. and A. Gessain, Origin, evolution and innate immune control of simian foamy viruses in humans. Curr Opin Virol, 2015. **10**: 47-55.
4. Filippone, C., E. Betsem, P. Tortevoye, O. Cassar, S. Bassot, A. Froment, A. Fontanet, and A. Gessain, A severe bite from a nonhuman primate is a major risk factor for HTLV-1 infection in hunters from Central Africa. Clin Infect Dis, 2015. **60**: 1667-76.
5. Pinto-Santini, D.M., C.R. Stenbak, and M.L. Linial, Foamy virus zoonotic infections. Retrovirology, 2017. **14**: 55.
6. Buseyne, F., E. Betsem, T. Montange, R. Njouom, C. Bilounga Ndongo, O. Hermine, and A. Gessain, Clinical signs and blood test results among humans infected with zoonotic simian foamy virus: a case-control study. J Inf Dis, 2018. **218**: 144-151.
7. Schweizer, M., V. Falcone, J. Gange, R. Turek, and D. Neumann-Haefelin, Simian foamy virus isolated from an accidentally infected human individual. J Virol, 1997. **71**: 4821-4824.
8. Boneva, R.S., W.M. Switzer, T.J. Spira, V.B. Bhullar, V. Shanmugam, M.E. Cong, L. Lam, W. Heneine, T.M. Folks, and L.E. Chapman, Clinical and virological characterization of persistent human infection with simian foamy viruses. AIDS Res Hum Retrov, 2007. **23**: 1330-1337.
9. Rua, R., E. Betsem, S. Calattini, A. Saib, and A. Gessain, Genetic characterization of simian foamy viruses infecting humans. J Virol, 2012. **86**: 13350-13359.
10. Calattini, S., E.B.A. Betsem, A. Froment, P. Mauclere, P. Tortevoye, C. Schmitt, R. Njouom, A. Saib, and A. Gessain, Simian foamy virus transmission from apes to humans, rural Cameroon. Emerg Inf Dis, 2007. **13**: 1314-1320.
11. Jones-Engel, L., C.C. May, G.A. Engel, K.A. Steinkraus, M.A. Schillaci, A. Fuentes, A. Rompis, M.K. Chalise, N. Aggimarangsee, M.M. Feeroz, R. Grant, J.S. Allan, A. Putra, I.N. Wandia, R. Watanabe, L. Kuller, S. Thongsawat, R. Chaiwarith, R.C. Kyes, and M.L. Linial, Diverse contexts of zoonotic transmission of simian foamy viruses in Asia. Emerg Inf Dis, 2008. **14**: 1200-1208.
12. Switzer, W.M., S.H. Tang, S. Ahuka-Mundeke, A. Shankar, D.L. Hanson, H.Q. Zheng, A. Ayouba, N.D. Wolfe, M. LeBreton, C.F. Djoko, U. Tamoufe, A. Esteban, W. Heneine, M. Peeters, L.L. Wright, J.J. Muyembe-Tamfum, E.O. Wemakoy, P. Mulembakani, N.A. Hoff, and A.W. Rimoin, Novel simian foamy virus infections from multiple monkey species in women from the Democratic Republic of Congo. Retrovirology, 2012. **9**: 100.
13. Engel, G.A., C.T. Small, K. Soliven, M.M. Feeroz, X. Wang, M. Kamrul Hasan, G. Oh, S.M. Rabiul Alam, K.L. Craig, D.L. Jackson, F.A. Matsen Iv, M.L. Linial, and L. Jones-Engel, Zoonotic simian foamy virus in Bangladesh reflects diverse patterns of transmission and co-infection. Emerg Microb Infect, 2013. **2**: e58.
14. Rua, R., E. Betsem, T. Montange, F. Buseyne, and A. Gessain, *In vivo* cellular tropism of gorilla simian foamy virus in blood of infected humans. J Virol, 2014. **88**: 13429-35.

15. Rua, R., A. Lepelley, A. Gessain, and O. Schwartz, Innate sensing of foamy viruses by human hematopoietic cells. J Virol, 2012. **86**: 909-918.
16. Lambert, C., M. Couteaudier, J. Gouzil, L. Richard, T. Montange, E. Betsem, R. Rua, J. Tobaly-Tapiero, D. Lindemann, R. Njouom, A. Mouinga-Ondeme, A. Gessain, and F. Buseyne, Potent neutralizing antibodies in humans infected with zoonotic simian foamy viruses target conserved epitopes located in the dimorphic domain of the surface envelope protein. PLoS Pathog, 2018. **14**: e1007293.
17. Kabat, D., S.L. Kozak, K. Wehrly, and B. Chesebro, Differences in CD4 dependence for infectivity of laboratory-adapted and primary patient isolates of human immunodeficiency virus type 1. J Virol, 1994. **68**: 2570-2577.
18. Wrin, T., T.P. Loh, J.C. Vennari, H. Schuitemaker, and J.H. Nunberg, Adaptation to persistent growth in the H9 cell line renders a primary isolate of human immunodeficiency virus type 1 sensitive to neutralization by vaccine sera. J Virol, 1995. **69**: 39-48.
19. Cook, R.F., S.L. Berger, K.E. Rushlow, J.M. McManus, S.J. Cook, S. Harrold, M.L. Raabe, R.C. Montelaro, and C.J. Issel, Enhanced sensitivity to neutralizing antibodies in a variant of equine infectious-anemia virus is linked to amino-acid substitutions in the surface unit envelope glycoprotein. J Virol, 1995. **69**: 1493-1499.
20. Mascola, J.R. and B.F. Haynes, HIV-1 neutralizing antibodies: understanding nature's pathways. Immunol Rev, 2013. **254**: 225-244.
21. Mouinga-Ondeme, A., M. Caron, D. Nkoghe, P. Telfer, P. Marx, A. Saib, E. Leroy, J.P. Gonzalez, A. Gessain, and M. Kazanji, Cross-species transmission of simian foamy virus to humans in rural Gabon, Central Africa. J Virol, 2012. **86**: 1255-60.
22. Bieniasz, P.D., A. Rethwilm, R. Pitman, M.D. Daniel, I. Chrystie, and M.O. McClure, A comparative study of higher primate foamy viruses, including a new virus from a gorilla. Virology, 1995. **207**: 217-228.
23. Lambert, C., R. Rua, A. Gessain, and F. Buseyne, A new sensitive indicator cell line reveals cross-transactivation of the viral LTR by gorilla and chimpanzee simian foamy viruses. Virology, 2016. **496**: 219-226.
24. Schneider, W.M., M. Chevilotte, and C.M. Rice, Interferon-stimulated genes: a complex beb of host defenses. Ann Rev Immunol, 2014. **32**: 513-545.
25. McNab, F., K. Mayer-Barber, A. Sher, A. Wack, and A. O'Garra, Type I interferons in infectious disease. Nat Rev Immunol, 2015. **15**: 87-103.
26. Matthes, D., T. Wiktorowicz, J. Zahn, J. Bodem, N. Stanke, D. Lindemann, and A. Rethwilm, Basic residues in the foamy virus Gag protein. J Virol, 2011. **85**: 3986-3995.
27. Bähr, A., A. Singer, A. Hain, A.A.J. Vasudevan, M. Schilling, J. Reh, M. Riess, S. Panitz, V. Serrano, M. Schweizer, R. König, S. Chanda, D. Häussinger, G. Kochs, D. Lindemann, and C. Münk, Interferon but not MxB inhibits foamy retroviruses. Virology, 2016. **488**: 51-60.
28. Lochelt, M., F. Romen, P. Bastone, H. Muckenfuss, N. Kirchner, Y.B. Kim, U. Truyen, U. Rosler, M. Battenberg, A. Saib, E. Flory, K. Cichutek, and C. Munk, The antiretroviral activity of APOBEC3 is inhibited by the foamy virus accessory Bet protein. PNAS, 2005. **102**: 7982-7987.
29. Russell, R.A., H.L. Wiegand, M.D. Moore, A. Schafer, M.O. McClure, and B.R. Cullen, Foamy virus Bet proteins function as novel inhibitors of the APOBEC3 family of innate antiretroviral defense factors. J Virol, 2005. **79**: 8724-8731.

30. Berka, U., M.V. Hamann, and D. Lindemann, Early events in foamy virus-host interaction and intracellular trafficking. Viruses, 2013. **5**: 1055-74.
31. Kane, M., Trinity M. Zang, Suzannah J. Rihn, F. Zhang, T. Kueck, M. Alim, J. Schoggins, Charles M. Rice, Sam J. Wilson, and Paul D. Bieniasz, Identification of interferon-stimulated genes with antiretroviral activity. Cell Host Mic, 2016. **20**: 392-405.
32. Plochmann, K., A. Horn, E. Gschmack, N. Armbruster, J. Krieg, T. Wiktorowicz, C. Weber, K. Stirnnagel, D. Lindemann, A. Rethwilm, and C. Scheller, Heparan sulfate is an attachment factor for foamy virus entry. J Virol, 2012. **86**: 10028-10035.
33. Picard-Maureau, M., G. Jarmy, A. Berg, A. Rethwilm, and D. Lindemann, Foamy virus envelope glycoprotein-mediated entry involves a pH-dependent fusion process. J Virol, 2003. **77**: 4722-4730.
34. Stirnnagel, K., D. Schupp, A. Dupont, V. Kudryavtsev, J. Reh, E. Mullers, D.C. Lamb, and D. Lindemann, Differential pH-dependent cellular uptake pathways among foamy viruses elucidated using dual-colored fluorescent particles. Retrovirology, 2012. **9**: 71.
35. Toyoshima, K. and P.K. Vogt, Enhancement and inhibition of avian sarcoma viruses by polycations and polyanions. Virology, 1969. **38**: 414-26.
36. Clark, K., L. Plater, M. Peggie, and P. Cohen, Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB kinase epsilon: a distinct upstream kinase mediates Ser-172 phosphorylation and activation. J Biol Chem, 2009. **284**: 14136-46.
37. Stewart, C.E., R.E. Randall, and C.S. Adamson, Inhibitors of the interferon response enhance virus replication in vitro. PLoS One, 2014. **9**: e112014.
38. Cataldi, M., N.R. Shah, S.A. Felt, and V.Z. Grdzelishvili, Breaking resistance of pancreatic cancer cells to an attenuated vesicular stomatitis virus through a novel activity of IKK inhibitor TPCA-1. Virology, 2015. **485**: 340-54.
39. Ma, Z., S.E. Hopcraft, F. Yang, A. Petrucelli, H. Guo, J.P. Ting, D.P. Dittmer, and B. Damania, NLRX1 negatively modulates type I IFN to facilitate KSHV reactivation from latency. PLoS Pathog, 2017. **13**: e1006350.
40. Decalf, J., M. Desdouits, V. Rodrigues, F.X. Gobert, M. Gentili, S. Marques-Ladeira, C. Chamontin, M. Mougel, B. Cunha de Alencar, and P. Benaroch, Sensing of HIV-1 entry triggers a type I interferon response in human primary macrophages. J Virol, 2017.
41. Rawlings, J.S., K.M. Rosler, and D.A. Harrison, The JAK/STAT signaling pathway. J Cell Sci, 2004. **117**: 1281-3.
42. Trobridge, G. and D.W. Russell, Cell cycle requirements for transduction by foamy virus vectors compared to those of oncovirus and lentivirus vectors. J Virol, 2004. **78**: 2327-2335.
43. Jaishankar, D., A.M. Yakoub, T. Yadavalli, A. Agelidis, N. Thakkar, S. Hadigal, J. Ames, and D. Shukla, An off-target effect of BX795 blocks herpes simplex virus type 1 infection of the eye. Sci Transl Med, 2018. **10**.
44. Nan, J., Y. Du, X. Chen, Q. Bai, Y. Wang, X. Zhang, N. Zhu, J. Zhang, J. Hou, Q. Wang, and J. Yang, TPCA-1 is a direct dual inhibitor of STAT3 and NF-kappaB and regresses mutant EGFR-associated human non-small cell lung cancers. Mol Cancer Ther, 2014. **13**: 617-29.
45. Quan, Y.D., H.T. Xu, Y.S. Han, T. Mesplede, and M.A. Wainberg, JAK-STAT Signaling Pathways and Inhibitors Affect Reversion of Envelope-Mutated HIV-1. J Virol, 2017. **91**.

46. Gavegnano, C., M. Detorio, C. Montero, A. Bosque, V. Planelles, and R.F. Schinazi, Ruxolitinib and tofacitinib are potent and selective inhibitors of HIV-1 replication and virus reactivation *in vitro*. Antimicrob Agents Chemother, 2014. **58**: 1977-1986.
47. Jakobsdottir, G.M., M. Iliopoulou, R. Nolan, L. Alvarez, A.A. Compton, and S. Padilla-Parra, On the Whereabouts of HIV-1 Cellular Entry and Its Fusion Ports. Trends Mol Med, 2017. **23**: 932-944.
48. Rhodes-Feuillette, A., F. Saal, J. Lasneret, M. Santillana-Hayat, and J. Peries, Studies on in vitro interferon induction capacity and interferon sensitivity of simian foamy viruses. Arch Virol, 1987. **97**: 77-84.
49. Sabile, A., A. Rhodes-Feuillette, F.Z. Jaoui, J. Tobaly-Tapiero, M.L. Giron, J. Lasneret, J. Peries, and M. Canivet, In vitro studies on interferon-inducing capacity and sensitivity to IFN of human foamy virus. Res Virol, 1996. **147**: 29-37.
50. Loh, P.C. and K.S. Ang, Replication of human syncytium-forming virus in human cells: effect of certain biological factors and selective chemicals. J Med Virol, 1981. **7**: 67-73.
51. Hill, C.L., P.D. Bieniasz, and M.O. McClure, Properties of human foamy virus relevant to its development as a vector for gene therapy. J Gen Virol, 1999. **80**: 2003-2009.
52. Falcone, V., M. Schweizer, A. Toniolo, D. Neumann-Haefelin, and A. Meyerhans, Gamma interferon is a major suppressive factor produced by activated human peripheral blood lymphocytes that is able to inhibit foamy virus-induced cytopathic effects. J Virol, 1999. **73**: 1724-1728.
53. Herchenroder, O., M. Lochelt, F. Buseyne, A. Gessain, M.A. Soares, A.S. Khan, and D. Lindemann, Twelfth international foamy virus conference-meeting report. Viruses, 2019. **11**.

Figure legends

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

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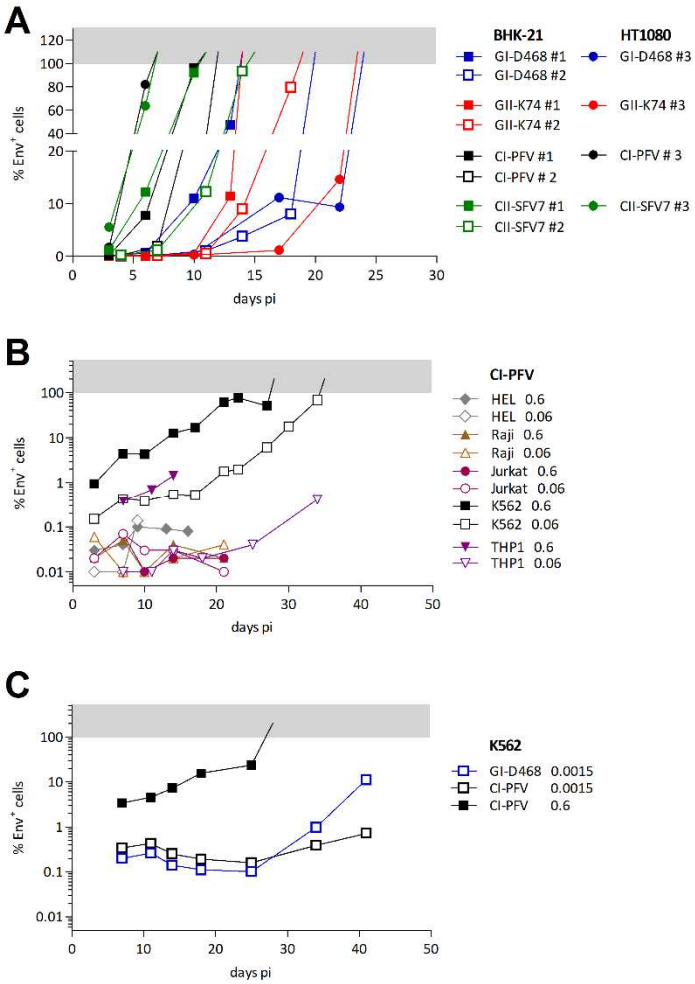
504 Figure 1



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507 Figure 2

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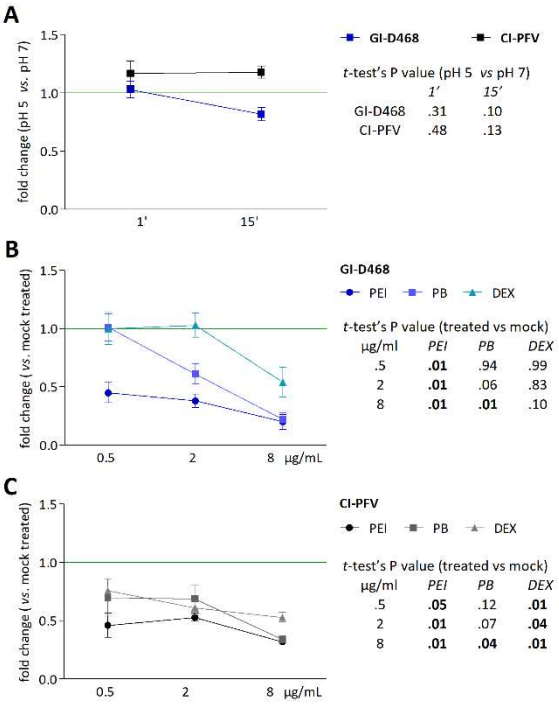

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512 Figure 3

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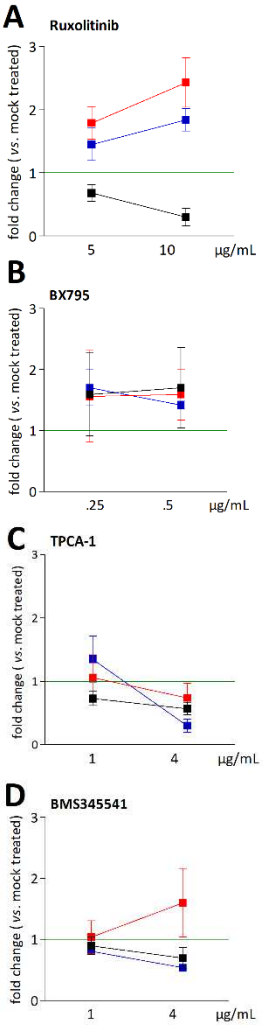

t-test's P value (treated vs mock)

µg/ml	GI-D468	GII-K74	CI-PFV
5	.22	**.05**	.13
10	**.03**	**.002**	.12

t-test's P value (treated vs mock)

µg/ml	GI-D468	GII-K74	CI-PFV
.25	.13	.20	.54
.5	**.01**	**.05**	.48

t-test's P value (treated vs mock)

µg/ml	GI-D468	GII-K74	CI-PFV
1	.52	.66	.25
4	.06	.11	**.04**

t-test's P value (treated vs mock)

µg/ml	GI-D468	GII-K74	CI-PFV
1	.25	.84	.63
4	**.12**	.25	.33

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518 Figure 4

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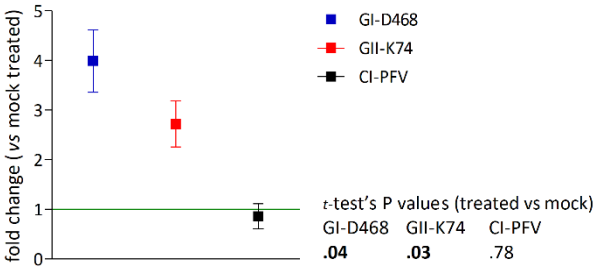

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