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## A new sensitive indicator cell line reveals cross-transactivation of the viral LTR by gorilla and chimpanzee simian foamy viruses

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1 **VIRO-16-284-Revised**

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3 **Presented as short report**

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6 **A new sensitive indicator cell line reveals cross-transactivation of the viral LTR by gorilla**  
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8 **and chimpanzee simian foamy viruses**  
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## Abstract

The majority of currently identified simian foamy virus (SFV)-infected **Cameroonian and Gabonese** individuals harbor SFV from the gorilla lineage. We constructed an indicator cell line for the quantification of gorilla SFVs, in which the U3 sequence of a gorilla SFV directs the expression of the  $\beta$ -galactosidase protein. The gorilla foamy virus activated  $\beta$ -galactosidase (GFAB) cells efficiently quantified two zoonotic primary gorilla isolates and SFVs from three chimpanzee subspecies. Primary gorilla SFVs replicated more slowly and at lower levels than primary chimpanzee SFVs. Analysis of previously described motifs of Tas proteins and U3 LTRs involved in viral gene synthesis revealed conservation of such motifs in Tas proteins from gorilla and chimpanzee SFVs, but little sequence homology in the LTR regions previously shown to interact with viral and cellular factors.

Keywords: foamy viruses, Gorilla, zoonotic infection, viral transcription, long terminal repeat

## Introduction

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4 Foamy viruses (FVs) are complex retroviruses that infect nonhuman primates (NHPs), cats,  
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6 cows, and horses (Kehl et al., 2013; Liu et al., 2013; Rethwilm and Bodem, 2013). Human  
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8 infection occurs following cross-species transmission of simian foamy viruses (SFVs) with  
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10 bites from infected animals representing the main route of infection (Betsem et al., 2011;  
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12 Gessain et al., 2013; Khan, 2009; Rua and Gessain, 2015). SFVs establish lifelong infection in  
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14 humans, with persistent detection of SFV DNA and SFV-specific antibodies in peripheral  
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16 blood (Betsem et al., 2011; Cummins et al., 2005; Switzer et al., 2012). Neither pathogenicity  
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18 nor human-to-human transmission of SFVs have yet been demonstrated. Human  
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20 immunodeficiency viruses and human T-lymphotropic viruses have emerged from simian  
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22 reservoirs, and cross-species transmission of simian retroviruses to humans is an ongoing  
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24 process in many parts of the world (Locatelli and Peeters, 2012). The study of zoonotic SFV  
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26 infection is relevant as a marker of exposure of humans to NHP pathogens and as a model to  
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28 understand host-pathogen interactions in the new human host following cross-species  
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30 transmission of retroviruses. SFVs also merit study for their own potential impact on human  
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32 health, as vectors for gene therapy and vaccination (Olszko and Trobridge, 2013), and  
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34 because co-infection with other retroviruses has been reported in humans (Filippone et al.,  
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36 2015; Switzer et al., 2008). Such a co-infection with SFV has deleterious consequences in  
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38 macaques experimentally infected with SIV (Choudhary et al., 2013).  
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51 We have been conducting epidemiological studies on SFVs in exposed human populations in  
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53 Cameroon and Gabon, focusing both on risk factors of infection and viral characteristics. In  
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55 these studies, about 75% of SFV-infected Central African hunters harbored an SFV strain  
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57 from the gorilla lineage (Betsem et al., 2011; Calattini et al., 2007; Mouinga-Ondeme et al.,  
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2012). We previously set-up a gorilla SFV-specific quantitative PCR assay (Betsem et al.,  
2011) that allowed the detection of SFV DNA in blood and saliva cells and showed  
preferential tropism to both B and T lymphocytes (Rua et al., 2013; Rua et al., 2014). For  
historical reasons, the most studied SFV strains were derived from host-species housed in  
zoo and primate research centers, *i.e.* chimpanzee, macaques, and African green monkeys.  
In contrast, gorilla SFVs have been poorly studied despite infecting the majority of African  
individuals identified as carrying such viruses (Betsem et al., 2011; Calattini et al., 2007;  
Mouinga-Ondeme et al., 2012). A single study has reported the *in vitro* replication of a gorilla  
SFV (Bieniasz et al., 1995), of which the full length sequence was recently published (Schulze  
et al., 2011). We isolated two replication-competent gorilla SFVs from infected Cameroonian  
hunters and described their genetic characteristics (Rua et al., 2012) and also studied the  
genetic diversity of the *env* gene from 36 zoonotic gorilla SFVs (Richard et al., 2015).

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Here, we aimed to conduct further studies that require quantification of replicating SFV. For  
this purpose, we have constructed an indicator cell line expressing the  $\beta$ -galactosidase  
protein under the control of a zoonotic gorilla SFV promoter, adapting a previously described  
method (Yu and Linial, 1993). This system was able to efficiently quantify cell infection with  
autologous SFV. This new cell line also efficiently quantified chimpanzee SFVs, revealing  
cross-transactivation of LTR-driven viral gene expression by heterologous SFV transactivator  
proteins. These properties offered the opportunity to compare primary SFV strains from  
both host-specific lineages as well as laboratory-adapted and primary chimpanzee SFVs.

## Materials and Methods

### Cells

Baby hamster kidney cells (BHK-21) were cultured in Dulbecco's modified Eagle medium (DMEM-Glutamax, Gibco, Courtaboeuf, France) supplemented with 5% fetal bovine serum (FBS, PAA Laboratories, Velizy Villacoublay, France). The foamy virus activated  $\beta$ -galactosidase (FAB) cells are BHK-21-derived cells containing the  $\beta$ -Galactosidase gene under the control of the LTR from SFVpsc-hu.PFV (Yu and Linial, 1993). They were cultured in DMEM supplemented with 5% fetal bovine serum and 50  $\mu$ g/ml hygromycin B (Sigma-Aldrich, Lyon, France). The gorilla foamy virus activated  $\beta$ -galactosidase (GFAB) cells (described below) were cultured in DMEM supplemented with 5% fetal bovine serum and 300  $\mu$ g/ml G418 (Sigma-Aldrich). We sequenced the LTR inserted in the FAB cell line to detect any possible sequence changes relative to Genbank sequence U21247.1. We found no nucleotide changes in the regions analyzed in the present study (data not shown).

### Viruses

SFV isolates used for the study are presented in Table 1. The PFV (SFVpsc-hu.PFV) and SFV7 (SFVpve-7.pan2) strains were kindly provided by A. Saïb and A. Rethwilm. These strains have been extensively passaged *in vitro* and will be referred to as laboratory-adapted strains. Two chimpanzee, two gorilla, and one Cercopithecus SFV strain were isolated from peripheral blood cells of infected humans studied in our laboratory (Calattini et al., 2007; Rua et al., 2012). Original and first passage supernatants were used to produce the viral stocks used in this study, and these strains are referred to as primary strains.

## Construction of the GFAB cell line

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3 The 653 nt long fragment of SFVggo-hu.BAK74 LTR (referred to as short U3 (S2) in (Rua et al.,  
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5 2012)), homologous to the region from -533 to +20 nt of the HSRV13 molecular clone of  
6  
7 SFVpsc-hu.PFV LTR (Yu and Linial, 1993), was inserted into the pCIneo plasmid (Promega) to  
8  
9 replace the CMV promoter and the intron initially present. The coding sequence of the  
10  
11 nuclear localization signal MPKKKRK from simian virus 40 T antigen was inserted between  
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13 the LTR and  $\beta$ -Galactosidase coding sequence (optimized for expression in Hamster cells: 5'-  
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15 ATGCCCAAGAAGAAAAGGAAA-3'). A neomycin resistance gene driven by an SV40 promoter  
16  
17 was inserted into the plasmid for the purpose of selection in eukaryotic cells. This reporter  
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19 plasmid, 140412-BAK, was transfected into BHK-21 cells using the LipoD293Reagent  
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21 (SignaGen Laboratories, Rockville, MD, United States) according to the manufacturer's  
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23 instructions. Twenty-four hours later, G418 was added (1 mg/ml) in culture medium and the  
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25 cells were selected for 20 days. G418-resistant cells were seeded at 1, 0.5, and 0.25  
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27 cells/well in DMEM-5% FBS with 300  $\mu$ g/ml G418. Forty clones were selected from 96-well  
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29 plates with growing cells in less than 30% of the wells. These clones were screened for high  
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31 level of X-Gal staining following infection with SFVggo-hu.BAK74 and SFVggo-hu.BAD468  
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33 strains and for the absence of staining of mock-infected cells. Of the 12 cellular clones  
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35 specifically expressing  $\beta$ -galactosidase following gorilla SFV infection, one clone (# 35) was  
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37 chosen on the basis of its high susceptibility to infection and stability over time, and was  
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39 further amplified.  
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## Detection of SFV-infected cells

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53 Virus titers were determined by infecting GFAB cells at 30-40% confluence in flat bottom 96-  
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55 well plates with 30  $\mu$ l/well of serially diluted viral solutions prepared in DMEM. After a 2 h  
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1 incubation, 170 µl of DMEM supplemented with 5% FBS were added to each well. Tests were  
2 performed in triplicate. The cells were fixed after 72 h with 0.5% glutaraldehyde in  
3 phosphate-buffered saline solution (PBS) for 10 min at room temperature (RT). Cells were  
4 washed with PBS and incubated 1 h at 37 °C with a X-Gal staining solution [2 mM MgCl<sub>2</sub>; 10  
5 mM Potassium ferricyanide, 10 mM Potassium ferrocyanide; 0.5 mg/ml 5-Bromo-4-chloro-3-  
6 indolyl-B-D-galactopyranoside, in PBS]. An Ultimate UV Image analyzer (CTL Europe, Bonn,  
7 Germany) was used for counting stained cells. One infectious unit was defined as a blue cell  
8 or syncytia. Quantification was considered to be valid if the mean was > 30 cells/well and the  
9 coefficient of variation (CV = 100\*SD/mean) was < 30%.

## 23 Results

### 27 Construction of the GFAB cell line for the quantification of gorilla SFVs

30 The GFAB cell line was designed following the model of the FAB cell line that expresses the  
31 β-galactosidase gene under the transcriptional control of U3 sequences from the LTR of the  
32 HSRV13 molecular clone of SFVpsc-hu.PFV (Yu and Linial, 1993) (Figure 1A). **This molecular**  
33 **clone harbors a deletion variant of LTR U3, referred to as short U3 (Schmidt et al., 1997).** We  
34 selected the naturally occurring short LTR U3 from the primary SFVggo-hu.BAK74 strain (Rua  
35 et al., 2012), and inserted the sequence upstream of a nuclear localization signal and the β-  
36 galactosidase gene in a mammalian expression vector. We obtained stably-transfected BHK-  
37 21 cells. The reporter gene was expressed 72 h after infection with the autologous SFVggo-  
38 hu.BAK74 and blue cell foci were observed upon **staining** (Figure 1B). Similar results were  
39 obtained with another zoonotic primary gorilla isolate, SFVggo-hu.BAD468 (Figure 1B). Serial  
40 two-fold dilutions showed an overall linear relationship between 30 to 1000 infectious  
41 units/well (data not shown), although the best precision for automatic counting was



1 obtained for approximately 100-200 cells/well. The GFAB cells were infected at a moi of 0.02  
2 (100 infectious units per P96 well ≈5000 cells) with several viral strains in triplicate on 12  
3 different plates. The intraplate CV was from 3% to 27% (median 15%) and the interplate CV  
4 from 28% to 36% (median 30%) for this moi.  
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10 The GFAB cell line quantifies infection by both gorilla and chimpanzee SFVs.  
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13 We then tested the specificity of the GFAB and FAB cell lines for the detection of viral strains  
14 from various host-specific lineages. Four SFV isolates from three different chimpanzee  
15 subspecies (*Pan troglodytes schweinfurthii*, *P.t. verus* and *P.t. troglodytes*) efficiently  
16 transactivated the gorilla SFV LTR inserted in the GFAB cell line: the laboratory-adapted  
17 prototype PFV (SFVpsc-hu.PFV) and SFV7 (SFVpve-7.pan2) strains, and two primary zoonotic  
18 isolates (SFVptr-hu.AG15 and SFVptr-hu.BAD327) (Figure 2). The two primary zoonotic gorilla  
19 SFV strains reciprocally transactivated the chimpanzee SFVpsc-hu.PFV LTR inserted in the  
20 FAB cells, but the number of stained FAB cells was lower than that of stained GFAB cells  
21 (Figure 2). To confirm this finding, we quantified the same viral strains using a second  
22 indicator cell line constructed with another fragment of the short SFVpsc-hu.PFV LTR  
23 (Bieniasz et al., 1995). The viral titers were lower but proportional to those obtained with  
24 the FAB cells (data not shown). The primary *Cercopithecus* SFVcni-hu.AG16 isolate was not  
25 detected by the FAB or GFAB cells (Figure 2).  
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48 Although chimpanzee and gorilla SFV were able to transactivate the LTR from both the FAB  
49 and GFAB cell lines, the efficiency of cross-transactivation was not equivalent for both  
50 strains: the ratio of GFAB to FAB infected cells was close to one for the two laboratory-  
51 adapted chimpanzee isolates, approximately 1.5 for the two zoonotic chimpanzee isolates,  
52 and approximately three to five for the two zoonotic gorilla SFVs. In conclusion, efficient  
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1 activation of a gorilla SFV LTR by heterologous chimpanzee SFVs was observed in GFAB cells,  
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3 whereas the reciprocal interaction was detectable but weaker in FAB cells.  
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### 5 Sequences of Tas and LTR motifs from gorilla and chimpanzee SFVs 6

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8 We searched for the molecular basis of the cross-transactivation of SFV LTRs by Tas proteins  
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10 from different strains. A consensus DNA binding sequence common to simian, feline and  
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12 bovine foamy viruses responsible for the interaction between the Tas protein and the LTR  
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14 has been reported (Löchelt, 2003). Eight out of the eleven positions from this consensus  
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16 sequence are fully conserved, and three harbor conservative changes between chimpanzee  
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18 and gorilla SFVs (Figure 3A): L<sub>99</sub> versus F<sub>98</sub>, D<sub>144</sub> versus E<sub>143</sub> and I<sub>156</sub> versus V<sub>155</sub>. The R<sub>199</sub>H<sub>200</sub>  
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20 motif involved in DNA binding (Ma et al., 2014) was conserved between both lineages.  
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22 Further alignments of strains described in this work confirmed these observations  
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24 (Supplementary Figure 1). In contrast, the *Cercopithecus* SFVcni-hu.AG16 strain that was not  
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26 detected by either GFAB or FAB cells has a nonconservative H<sub>200</sub> to A<sub>200</sub> change as well as  
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28 conservative changes in the consensus DNA binding motif. Altogether, previously defined  
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30 functional motifs in the Tas protein were well conserved between chimpanzee and gorilla  
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32 SFVs.  
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36 In the SFVpsc-hu.PFV U3 region proximal to the transcription initiation site, a Tas binding  
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38 element has been mapped to position -65 to -35 by DNA footprinting and gel retardation  
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40 analysis (He et al., 1996). This region contains a heptamer sequence shown to be essential  
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42 for Tas binding by mutagenesis studies (He et al., 1996) as well as a stretch of 11 nucleotides  
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44 conserved between chimpanzee and gorilla sequences (Schulze et al., 2011). However, these  
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46 sequences were not conserved between SFVpsc-hu.PFV and SFVggo-hu.BAK74 (Figure 3B).  
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1 Additional differences in nucleotide sequences were present across the chimpanzee SFVs  
2 (Supplementary Figure 2).  
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5 Several, more distant and longer Tas responsive elements (TRE) and one negative regulatory  
6 element (NRE), have been described by analysis of reporter gene expression under the  
7 control of a mutated LTR (Erlwein and Rethwilm, 1993; Lee et al., 1993). The primary SFV U3  
8 nucleotide sequences contained stretches of both conserved and variable sequences.  
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10 Overall, the TRE sequences were not more conserved than non-TRE sequences (Figure 3B).  
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12 The U3 of chimpanzee SFV contained three AP-1 binding sites (Maurer et al., 1991), of which  
13 two are mutated in gorilla SFVs. The putative Ets-1 binding site present in the short LTR U3  
14 (Schmidt et al., 1997) was conserved in chimpanzee SFVs only. In conclusion, U3 sequences,  
15 proposed or shown to be response elements to viral and cellular transcription factors, were  
16 globally poorly conserved **between SFV strains demonstrating cross-transactivation of**  
17 **promoters.**  
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34 **Gorilla SFVs replicate more slowly and at a lower level than chimpanzee SFVs**  
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37 **We titrated several SFV stocks using the GFAB cell line and normalized the production to the**  
38 **extent of cell layer destruction. Viral titers differed markedly between isolates: those of**  
39 **laboratory-adapted chimpanzee SFVs ranged from 10<sup>5</sup>-10<sup>6</sup> IU/ml, those of primary**  
40 **chimpanzee SFVs from 10<sup>4</sup> to 10<sup>5</sup> IU/ml, and those of primary gorilla SFVs from 10<sup>3</sup> to 10<sup>4</sup>**  
41 **IU/ml. We titrated each viral stock at several time points using GFAB cells. There was no**  
42 **signal after 24 h. Gorilla SFV-infected cells were barely detectable after 48 h, but could be**  
43 **quantified 72 h post-infection. We detected significant viral titers (> 1000 infectious unit/ml)**  
44 **for chimpanzee SFVs after 48 h, which increased further after 72 h. Cell destruction after 96**  
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h precluded further quantification. Thus, gorilla SFVs replicate more slowly than both primary and laboratory-adapted chimpanzee SFVs on BHK-21-derived GFAB cells.

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## Discussion

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4 We generated a novel indicator cell line for the detection of SFVs, the GFAB cell line that  
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6 encodes  $\beta$ -galactosidase under the control of a the U3 region from a gorilla SFV isolated in  
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8 our laboratory. The GFAB cell line is able to detect gorilla SFV infection with high sensitivity  
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10 as well as SFV isolates from another host-species lineage – chimpanzee. The sensitivity of the  
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12 cell line was critical for the quantification of zoonotic primary gorilla SFVs with slow and/or  
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14 low level replication. This tool will provide valuable information on strains that persistently  
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16 infect humans and for molecular studies of these viruses.  
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22 The quantitative bioassay of viruses based on functional detection of an early synthesized  
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24 viral protein is efficient for the detection of gorilla SFVs, consistent with results obtained  
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26 with FV from several mammalian species (Jones-Engel et al., 2007; Stenbak et al., 2014;  
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28 Zemba et al., 2000). Parallel titration showed similar sensitivity of GFAB and FAB cells for the  
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30 quantification of chimpanzee SFVs, and a higher sensitivity of GFAB cells for the  
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32 quantification of gorilla SFVs. The use of the same cell line to quantify several isolates is  
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34 important to decrease assay variation due to variability in the ability of cell lines to support  
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36 SFV entry and replication, and time-dependent evolution of cell lines over long term culture.  
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### Gorilla SFVs replicate more slowly and at lower levels than chimpanzee SFVs in GFAB cells

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49 The only previous *in vitro* study of gorilla SFV was performed with the SFVggo-Gg strain  
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51 isolated from a captive animal that was detected with a chimpanzee SFV indicator cell line  
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53 (Bieniasz et al., 1995). Comparison of X-gal staining and cytopathic effect following culture  
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55 with SFVggo-Gg-infected cells led to an estimated 10-fold lower transactivation of SFVpsc-  
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57 hu.PFV LTR by SFVggo-Gg than by chimpanzee strains (Bieniasz et al., 1995). Furthermore,  
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1 SFVggo-Gg could only be detected after cell-mediated infection, but not after incubation  
2 with cell-free virus. We also report the lower and/or slower replication of gorilla SFVs  
3 relative to chimpanzee SFVs. The difference was not related to the zoonotic origin of gorilla  
4 strains, as primary zoonotic chimpanzee strains reached titers that were tenfold higher than  
5 those of the primary zoonotic gorilla SFVs. We also noted significant differences in viral titers  
6 between primary and laboratory-adapted chimpanzee SFVs. Cautious interpretation is  
7 needed when comparing viral isolates for which the properties could change during *in vitro*  
8 culture. Of note, viral stocks were produced from the primary viruses used in this study using  
9 vials from either the original stock or the first passage. Evolution during the 4-6 week period  
10 before obtainment of the first stock is limited, as shown by a comparison of the sequences  
11 from these viruses with those in the donor's buffy coat (Rua et al., 2012).

### 28 [Cross-transactivation of viral LTRs by heterologous SFVs](#)

31 One important result of this report is the transactivation of the LTR from a gorilla SFV by a  
32 chimpanzee SFV, and the transactivation of the LTR of a chimpanzee SFV by a gorilla SFV,  
33 albeit less efficiently. Furthermore, the four chimpanzee strains were isolated from three  
34 subspecies of chimpanzee SFVs and showed similar patterns of transactivation of the two U3  
35 sequences tested. This functional conservation was observed despite overall low  
36 conservation of primary sequences of Tas protein and LTR. The percent identities were over  
37 90% between gorilla SFVs, from 80 to 90% between chimpanzee SFVs from different animal  
38 subspecies, and from 55 to 70% between gorilla and chimpanzee SFVs (supplementary  
39 Figures 1 and 2). Chimpanzee SFVs cross-transactivated the heterologous LTR more  
40 efficiently than gorilla SFVs. This may reflect that gorilla SFVs have delayed and/or reduced  
41 synthesis of viral proteins relative to those of chimpanzee SFVs. It may also reflect the

1 binding of Tas proteins on distinct sequences in LTR from different host-specific SFV lineages.

2 The cross-transactivation of LTR by heterologous viral strains is neither unique to gorilla and  
3 chimpanzee SFVs, nor restricted to strains from related host-species lineages: it was  
4 previously reported for macaque and *Cercopithecus* SFVs (Renne et al., 1993), for HIV and  
5 chimpanzee SFVs (Keller et al., 1992; Lee et al., 1992), and for HIV, SIV, and macaque SFVs  
6 (Mergia et al., 1992). However, no cross-transactivation has been observed between HTLV-1  
7 and chimpanzee SFVs (Keller et al., 1991; Venkatesh et al., 1991), nor between primate and  
8 nonprimate FVs (Bodem et al., 2004; Omoto et al., 2004; Winkler et al., 1997).

9  
10 The search for the molecular basis of cross-transactivation between gorilla and chimpanzee  
11 SFVs demonstrate the conservation of previously identified functional motifs in the Tas  
12 protein, whereas sequence homologies in the U3 TRE were difficult to find. In particular, the  
13 sequence previously identified as a functional Tas binding domain on the SFVpsc-hu.PFV (He  
14 et al., 1996) was not conserved between gorilla and chimpanzee SFVs. The absence of  
15 primary LTR sequence homology despite functional similarity is in agreement with previously  
16 published data. First, the sequences of TRE on LTRs and/or internal promoters present no or  
17 few homologies. Second, studies defining the DNA binding specificity of Tas to the internal  
18 promoter have illustrated the plasticity of the interaction between Tas and its DNA target as  
19 many nucleotide mutations did not affect this interaction, or even increased Tas-mediated  
20 transactivation (Kang and Cullen, 1998). Identification of sequence homologies related to  
21 cross-transactivation has been further hindered by the complexity of LTR regions from  
22 retroviruses that have numerous viral and cellular protein binding sites (Erlwein and  
23 Rethwilm, 1993; Lee et al., 1993), and by the lack of knowledge on the Tas-TRE binding  
24 mode.

1 Our observation raises the question of whether the interaction between Tas proteins and  
2 heterologous SFV LTR is an intrinsic property or depends upon extrinsic factors known to  
3 regulate transactivation: the lytic/persistence cellular infection state, cell-type specific  
4 factors, and U3 length polymorphisms. Indeed, the activity of the two SFV promoters differ  
5 in lytic and persistent infection *in vitro*, with higher internal promoter activity in persistently  
6 infected cells and higher LTR activity during lytic infection (Meiering et al., 2001). Activation  
7 of the SFV promoter varies in different cell lines, with up to 30-fold differences between the  
8 fibroblast cell line BHK and the lymphocytic Jurkat cells (Meiering et al., 2001). In addition,  
9 the presence of cell-type specific negative regulatory elements in LTR have been suggested  
10 through comparison of short and long LTR from SFVpsc-hu.PFV (Schmidt et al., 1997). Finally,  
11 length polymorphisms in U3 may affect their interaction with Tas protein. The U3 sequence  
12 used to construct the GFAB cell line is a deletion variant (Rua et al., 2012; Schmidt et al.,  
13 1997). *In vitro*, the various forms of the LTR are functional, with the full length LTR being  
14 possibly more sensitive to Tas protein (De Celis-Kosmas et al., 1997).

## 36 Conclusion

37 The novel GFAB cell line is a valuable research tool for gorilla and chimpanzee SFV  
38 quantification and the identification of key viral properties across primary strains. Here, we  
39 report differences between laboratory-adapted and primary viral strains. **Our results**  
40 **underline the importance of extending current knowledge beyond the most studied**  
41 **prototype strain. Indeed**, Tas interacts with promoters from host genes (Colas et al., 1995;  
42 Kido et al., 2002; Wagner et al., 2000; Wang et al., 2010), may be involved in clinically  
43 relevant interactions with lentiviruses (Choudhary et al., 2013), and is a potential (but still  
44 controversial) target of the immune system (Meiering and Linial, 2003; Regad et al., 2001).



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## Figure Legends

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4 **Figure 1. The GFAB cell line.** A. Principle of the GFAB indicator cell line 1. Schematic  
5  
6 representation of the transgene stably transfected into BHK-21 cells: the 653 nt long  
7  
8 fragment of SFVggo-hu.BAK74 LTR (referred to as short U3 (S2) in (Rua et al., 2012)),  
9  
10 homologous to the -533 to +20 nt region of the HSRV13 molecular clone of SFVpsc-hu.PFV  
11  
12 LTR (Yu and Linial, 1993), was inserted in the pCIneo plasmid (Promega), followed by the  
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14 coding sequence of the nuclear localization signal (NLS)MPKKKRK, and the  $\beta$ -Galactosidase  
15  
16 coding sequence; 2. Upon viral infection, the viral transactivator protein Tas activates the  
17  
18 synthesis of  $\beta$ -galactosidase mRNA; 3. After 72 h of infection, the  $\beta$ -galactosidase protein is  
19  
20 detected through the addition of its chromogenic substrate, X-gal, into the wells, leading to  
21  
22 the blue **staining** of SFV-infected cells; 4. Infectious units (blue cells or syncytia) are  
23  
24 numerated by an image analyser: photographs show a stained well and an analyzed  
25  
26 photograph where counted cells are surrounded by an orange line. B. GFAB cells were  
27  
28 infected by two gorilla SFV isolates or mock infected, as indicated in the above scheme. After  
29  
30 72 h of infection, the cells were fixed and stained as described in the materials and methods  
31  
32 section. Pictures from light microscope, magnification 250X.

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34  
35 **Figure 2. GFAB and FAB cells detect both chimpanzee and gorilla SFV infections.** Viral stocks  
36  
37 from chimpanzee and gorilla SFVs were titrated on the GFAB cell line. Then, GFAB and FAB  
38  
39 cells were infected at a moi of 0.02. After 72 h of infection, the cells were fixed, stained and  
40  
41 counted. An undiluted stock of SFVcni-hu.AG16 was used as a negative control. Pictures  
42  
43 from the Ultimate UV Image analyzer are shown side by side for GFAB (left) and FAB cell line  
44  
45 (right) for one representative experiment. The viral strain and the number of stained cells  
46  
47 are indicated above the photographs.

**Figure 3. Tas protein and LTR functional motifs from SFVggo-hu.BAK74 and SFVpsc-hu.PFV.**

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2  
3 A. The Tas central DNA binding domain (BD) and the nuclear localization signal (NLS) from  
4  
5 SFVpsc-hu.PFV (aa 89-223) and SFVggo-hu.BAK74 (aa 88-222) were aligned. Functional  
6  
7 motifs are superimposed on the sequences: the consensus DNA binding sequence proposed  
8  
9 for primate and nonprimate foamy viruses (Löchelt, 2003) and the R<sub>199</sub>H<sub>200</sub> motif (Ma et al.,  
10  
11 2014). Amino acid changes in these motifs are indicated with red characters. Identical  
12  
13 residues are shown as dots.  
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18 B. The 5'LTR U3 and beginning of R regions from SFVpsc-hu.PFV(HSRV13) (Genbank  
19  
20 U21247.1) and SFVggo-hu.BAK74 (GenBank JQ867464) were aligned. Sequences transduced  
21  
22 into FAB and GFAB cells are indicated by the blue arrows. Dots indicate identity, gaps are  
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24 represented in yellow, and the numbers used for motif description are those from SFVpsc-  
25  
26 hu.PFV(HSRV13). The transcription initiation start and TATA box are highlighted in red. In the  
27  
28 U3 region proximal to the transcription initiation site, the Tas binding element (TBE) at  
29  
30 position -65 to -35 (He et al., 1996) is highlighted in grey. Within this TBE, the heptamer  
31  
32 sequence conserved in the LTR and internal promoter from SFVpsc-hu.PFV (He et al., 1996),  
33  
34 and the -58 to -47 nucleotide sequence conserved with SFVggo-Gg (Schulze et al., 2011) are  
35  
36 highlighted. Several, more distant and longer Tas responsive elements, and one negative  
37  
38 regulatory element (NRE), are highlighted in purple and named according to their original  
39  
40 description (BRE stands for bel-1-responsive elements, (Erlwein and Rethwilm, 1993; Lee et  
41  
42 al.)). Binding sites for cellular transcription factors are highlighted in green: three AP-1  
43  
44 binding sites (Maurer et al., 1991) and the first of the two putative Ets-1 binding sites  
45  
46 (Schmidt et al., 1997). The second Ets-1 site was absent in the studied viruses. Alignments  
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48 were performed using CLC Main Workbench software.  
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**Table 1: Viral strains used in the study.**

<b>Virus<sup>1</sup></b>	<b>Nonhuman primate host species</b>	<b>Isolated from human sample</b>	<b>Genbank accession number</b>	<b>First description</b>
SFVpsc-hu.PFV	<i>Pan troglodytes schweinfurthii</i>	Yes	Y07725.1	(Achong et al., 1971)
SFVpsc-hu.PFV(HSRV13)	<i>Pan troglodytes schweinfurthii</i> (Molecular clone HSRV13)	--	U21247.1	(Löchelt et al., 1991)
SFVpve-7.Pan2	<i>Pan troglodytes verus</i>	No	U04327.1	(Rogers et al., 1967)
SFVptr-hu.BAD327	<i>Pan troglodytes troglodytes</i>	Yes	JQ867463.1	(Rua et al., 2012)
SFVptr-hu.AG15	<i>Pan troglodytes troglodytes</i>	Yes	JQ867462.1	(Calattini et al., 2007)
SFVggo-hu.BAK74	<i>Gorilla gorilla gorilla</i>	Yes	JQ867464.1	(Rua et al., 2012)
SFVggo-hu.BAD468	<i>Gorilla gorilla gorilla</i>	Yes	JQ867465.1	(Rua et al., 2012)
SFVcni-hu.AG16	<i>Cercopithecus nictitans</i>	Yes	JQ867466.1	(Calattini et al., 2007)

<sup>1</sup> According to the last update of FV taxonomy and development of a consensus nomenclature of virus isolates that was discussed at the 10<sup>th</sup> International Foamy Virus Conference (2014) in Pulawy, Poland (Materniak et al., 2015).



Supplemental material presented in the pdf file

Lambert\_Virology\_Supplementaldata.pdf

**Supplementary figure 1: Percent amino acid identity and functional motifs in SFV Tas proteins.**

We aligned Tas protein sequences from isolates tested in our study or related to them: SFVpsc-hu.PFV, SFVpve (as the SFVpve-7.Pan2 Tas sequence was not available), SFVptr-hu.AG15, SFVptr-hu.BAD327, SFVggo-Gg, SFVggo-hu.BAK74, SFVggo-hu.BAD468, and SFVcni-hu.AG16 that we used as a control in several experiments. Percent amino acid identity are shown for full length Tas proteins (panel A) and its 3 functional domains (B: shared domain, C: DNA binding domain, D: activation domain). The background color indicates levels of identity, where red corresponds to the most highly conserved sequences and blue to the least conserved sequences. Panel E presents known functional motifs superimposed on the sequences. Gap residues are shown in yellow, identical residues are shown as dots, and positions of motifs are indicated relative to the SFVpsc-hu.PFV sequence. The consensus DNA binding sequence proposed for primate and nonprimate foamy viruses (DNA BD, (Löchelt, 2003)), highlighted in red, was fully conserved across the eight isolates, except at three positions, where conservative changes were found in the three gorilla SFVs: at positions 96 (L to F), 144 (L to D), and 156 (I to V). The *Cercopithecus* SFVcni-hu.AG16 strain had conservative changes at positions 144 and 156. The RH motif at position 199-200, highlighted in blue, was proposed to be the first part of a bipartite nuclear localization signal (Chang et al., 1995) and subsequently shown to be involved in DNA binding (Ma et al., 2014). This motif was conserved in all isolates except SFVcni-hu.AG16. The three R/K residues at position 221 to 223, highlighted in green, were proposed to be the second part of the

1 nuclear localization signal (Chang et al., 1995). These residues were conserved in  
2 chimpanzee SFVs. The primary sequences were different in gorilla and *Cercopithecus* SFVs,  
3  
4 but this region contained two stretches of basic residues that constitute a putative nuclear  
5  
6 localization signal, as previously described (Rua et al., 2012). The minimal conserved  
7  
8 activation motif at position 273 to 287 (He et al., 1993; Venkatesh and Chinnadurai, 1993),  
9  
10 highlighted in grey, was highly conserved, with one nonconservative change (E to K) in  
11  
12 SFVpve at position 284, one conservative change (I to Y) at position 287 in the 3 gorilla SFVs,  
13  
14 and two nonconservative changes in the *Cercopithecus* SFV isolate: E to Q at position 281  
15  
16 and G to N at position 284. Alignments were performed using CLC Main Workbench  
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18 software.  
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### 26 **Supplementary figure 2: Percent identity and functional motifs in SFV LTRs.**

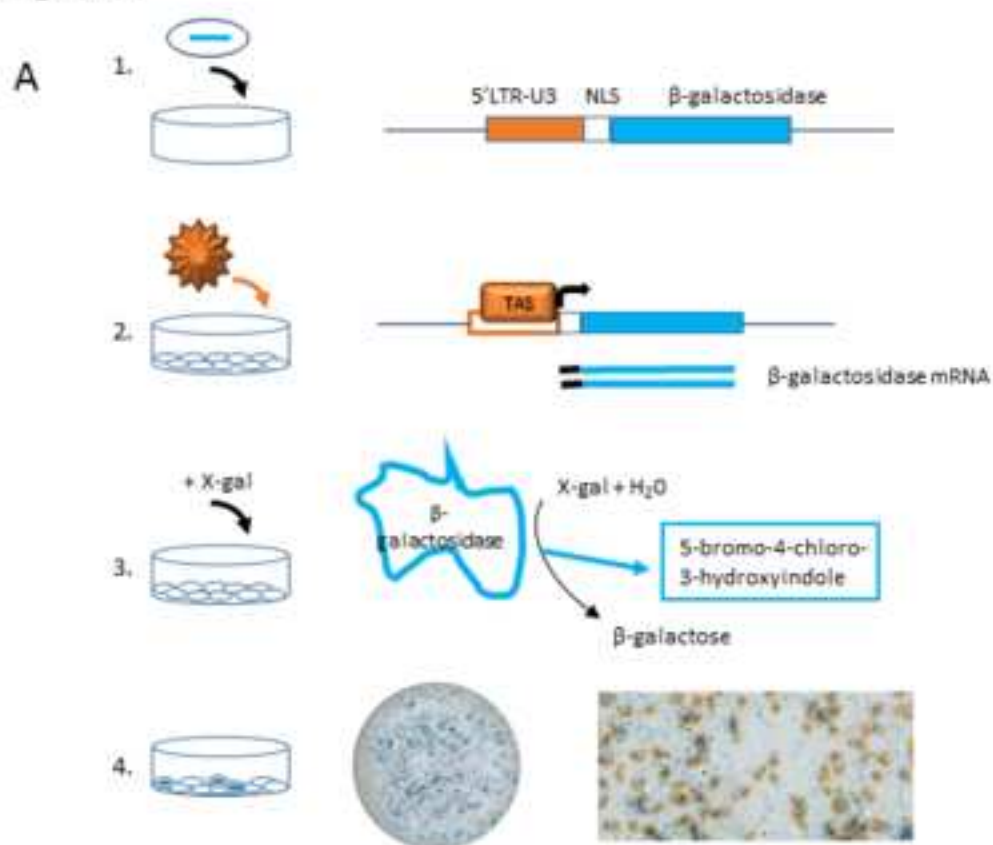
27  
28 We aligned LTR sequences from isolates tested in our study or related to them: SFVpsc-  
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30 hu.PFV (full length LTR), the HSRV13 molecular clone of SFVpsc-hu.PFV (SFVpsc-hu.HSRV13,  
31  
32 with a short U3 form, inserted in the FAB cell line (Yu and Linial, 1993)), from SFVpve (as the  
33  
34 SFVpve-7.Pan2 LTR sequence was not available), SFVptr-hu.AG15, SFVptr-hu.BAD327,  
35  
36 SFVggo-Gg, SFVggo-hu.BAK74, SFVggo-hu.BAD468, and SFVcni-hu.AG16 that we used as a  
37  
38 control in cross-transactivation experiments. The percent nucleotide identity is shown for  
39  
40 the whole U3, the last 70 nt of the U3, R, and U5 regions (panels A to D) of the nine SFV  
41  
42 sequences. The background color indicates the level of identity, where red corresponds to  
43  
44 the most highly conserved sequences and blue to the least conserved sequences. In panel E,  
45  
46 we superimposed regions shown or predicted to interact with viral Tas protein or cellular  
47  
48 factors. The blue arrows indicate the beginning of the LTR fragments inserted in the FAB and  
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50 GFAB cell lines. The transcription initiation start and TATA box are highlighted in red (Rua et  
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1 al., 2012). In the U3 region proximal to the transcription initiation site, we show the Tas  
2 binding element (TBE) at position -65 to -35, highlighted in grey (He et al., 1996), that  
3  
4 contained an heptamer sequence conserved in LTR and internal promoter from SFVpsc-  
5 hu.PFV (He et al., 1996), and the conserved sequence at position -58 to -47 proposed at the  
6  
7 time of the SFVggo-Gg sequence description (Schulze et al., 2011). These sequences were  
8  
9 partially conserved across chimpanzee and gorilla SFV LTRs. Several, more distant and longer  
10  
11 Tas responding elements (named BRE for bel-1 responsive elements) and one negative  
12  
13 regulatory element (NRE) are highlighted in purple (Erlwein and Rethwilm, 1993; Lee et al.).  
14  
15 Overall, primary nucleotide sequences in these regions differed considerably between  
16  
17 chimpanzee and gorilla SFVs. Cellular transcription factors are highlighted in green. The  
18  
19 three AP-1 binding sites described in SFVpsc-hu.PFV (Maurer et al., 1991) were conserved in  
20  
21 all chimpanzee SFVs. Only the third AP-1 binding site was present in the gorilla SFVs, and  
22  
23 none were present in SFVcni-hu.AG16. Two putative Ets-1 binding sites have been proposed  
24  
25 by Schmidt et al. (Schmidt et al., 1997). The first one was conserved in the chimpanzee SFV  
26  
27 only. The second Ets-1 site was deleted in the three viruses with a short U3 (SFVpsc-  
28  
29 hu.HSRV13, SFVggo-Gg and SFVggo-hu.Bak74), in the SFVcni-hu.AG16 strain, and was  
30  
31 mutated in the other viruses. Alignments were performed using CLC Main Workbench  
32  
33 software.  
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# Figure

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## Figure 1

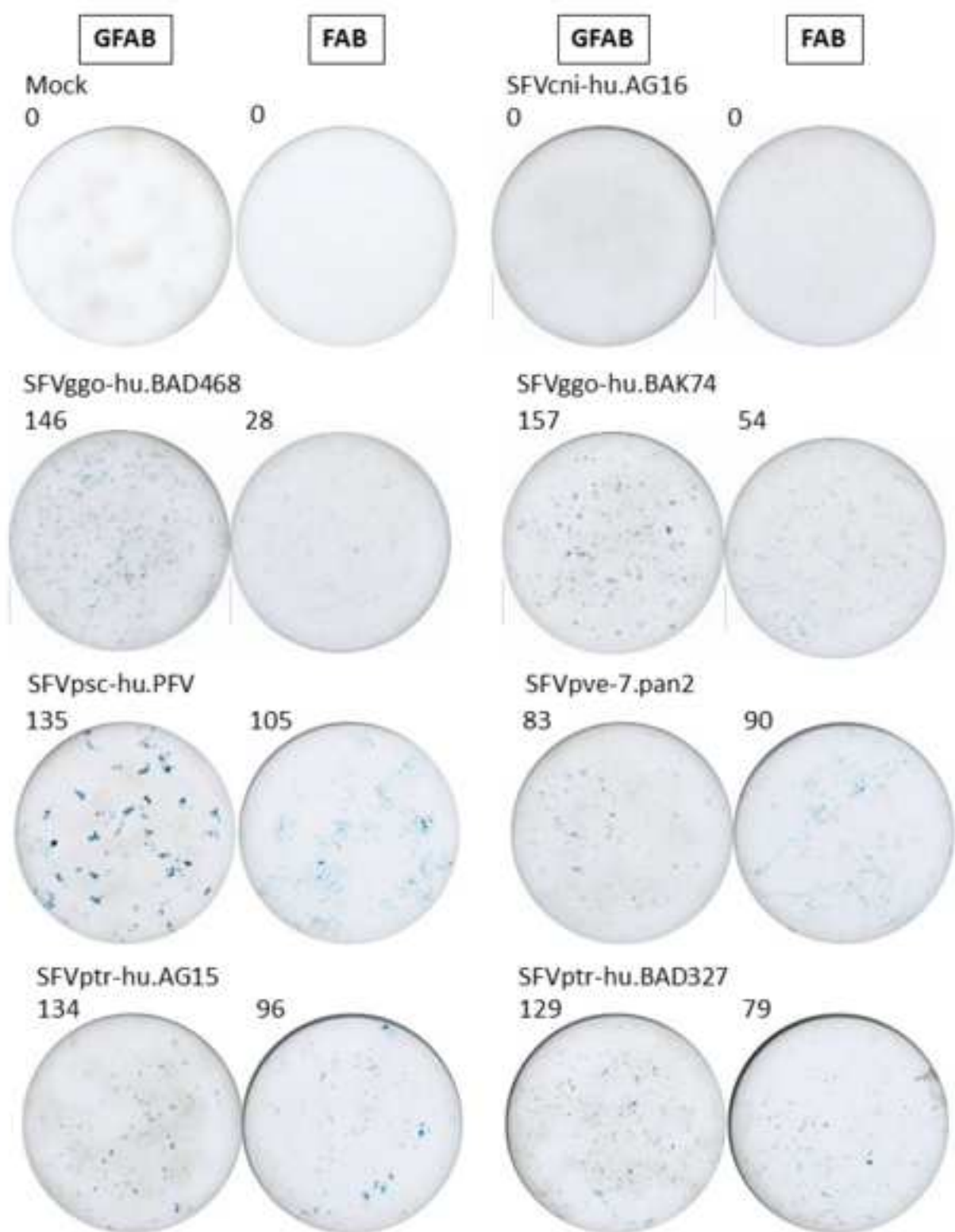


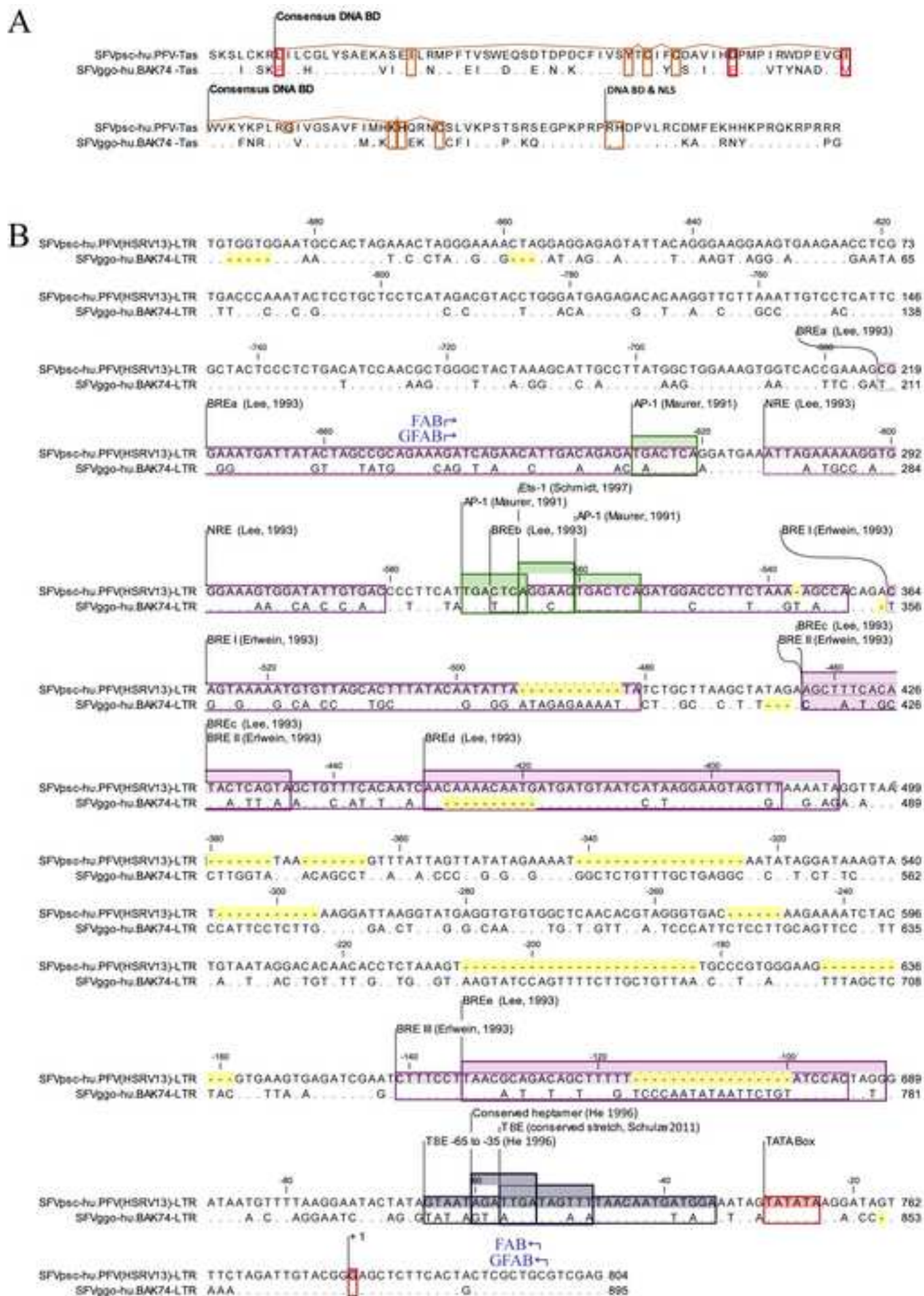
**B**

Mock      SFVggo-hu.BAK74      SFVggo-hu.BAD468



Figure 2







Supplementary figure 1: Percent amino acid identity and functional motifs in SFV Tas proteins.

A. Tas protein (full length)

		1	2	3	4	5	6	7	8
SFVpsc-hu.PFV-Tas	1		86,00	91,67	88,00	66,00	65,33	64,67	37,83
SFVpve-Tas	2	86,00		83,67	80,67	62,33	61,67	61,67	36,51
SFVptr-hu.BAD327-Tas	3	91,67	83,67		96,33	64,67	64,33	64,67	37,83
SFVptr-hu.AG15-Tas	4	88,00	80,67	96,33		62,00	61,67	62,00	37,50
SFVggo-Tas	5	66,00	62,33	64,67	62,00		97,99	97,32	33,55
SFVggo-hu.BAK74 -Tas	6	65,33	61,67	64,33	61,67	97,99		97,32	32,89
SFVggo-hu.BAD468-Tas	7	64,67	61,67	64,67	62,00	97,32	97,32		33,55
SFVcni-hu.AG16-Tas	8	37,83	36,51	37,83	37,50	33,55	32,89	33,55	

B. Tas protein, shared domain (aa 1-88)

		1	2	3	4	5	6	7	8
SFVpsc-hu.PFV-Tas	1		88,64	90,91	82,95	70,45	69,32	68,18	21,98
SFVpve-Tas	2	88,64		88,64	81,82	63,64	64,77	63,64	20,88
SFVptr-hu.BAD327-Tas	3	90,91	88,64		92,05	63,64	64,77	65,91	23,08
SFVptr-hu.AG15-Tas	4	82,95	81,82	92,05		57,95	59,09	60,23	21,98
SFVggo-Tas	5	70,45	63,64	63,64	57,95		97,70	97,70	24,18
SFVggo-hu.BAK74 -Tas	6	69,32	64,77	64,77	59,09	97,70		97,70	23,08
SFVggo-hu.BAD468-Tas	7	68,18	63,64	65,91	60,23	97,70	97,70		24,18
SFVcni-hu.AG16-Tas	8	21,98	20,88	23,08	21,98	24,18	23,08	24,18	

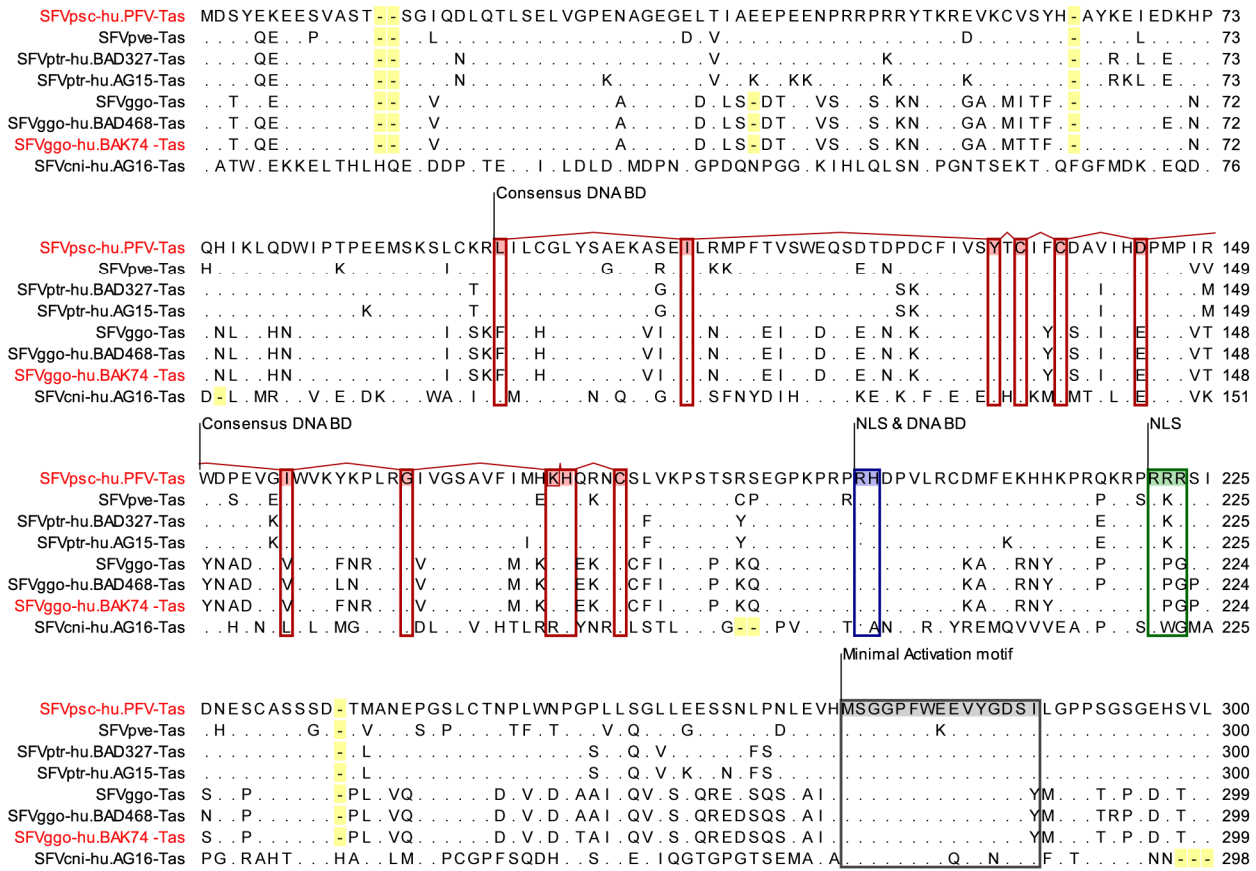
C. Tas Protein, DNA binding domain (aa 89-200)

		1	2	3	4	5	6	7	8
SFVpsc-hu.PFV-Tas	1		85,71	91,96	91,07	65,18	65,18	66,07	49,11
SFVpve-Tas	2	85,71		81,25	80,36	66,07	66,07	66,96	47,32
SFVptr-hu.BAD327-Tas	3	91,96	81,25		99,11	66,07	66,07	66,96	47,32
SFVptr-hu.AG15-Tas	4	91,07	80,36	99,11		65,18	65,18	66,07	47,32
SFVggo-Tas	5	65,18	66,07	66,07	65,18		100,00	98,21	40,18
SFVggo-hu.BAK74 -Tas	6	65,18	66,07	66,07	65,18	100,00		98,21	40,18
SFVggo-hu.BAD468-Tas	7	66,07	66,96	66,96	66,07	98,21	98,21		41,07
SFVcni-hu.AG16-Tas	8	49,11	47,32	47,32	47,32	40,18	40,18	41,07	

D. Tas protein, C terminal domain (aa 201-300)

		1	2	3	4	5	6	7	8
SFVpsc-hu.PFV-Tas	1		84,00	92,00	89,00	63,00	62,00	60,00	39,60
SFVpve-Tas	2	84,00		82,00	80,00	57,00	54,00	54,00	38,61
SFVptr-hu.BAD327-Tas	3	92,00	82,00		97,00	64,00	62,00	61,00	40,59
SFVptr-hu.AG15-Tas	4	89,00	80,00	97,00		62,00	60,00	59,00	40,59
SFVggo-Tas	5	63,00	57,00	64,00	62,00		96,00	96,00	34,65
SFVggo-hu.BAK74 -Tas	6	62,00	54,00	62,00	60,00	96,00		96,00	33,66
SFVggo-hu.BAD468-Tas	7	60,00	54,00	61,00	59,00	96,00	96,00		33,66
SFVcni-hu.AG16-Tas	8	39,60	38,61	40,59	40,59	34,65	33,66	33,66	

Supplementary figure 1 (continued)





Supplementary figure 2: Percent identity and functional motifs in SFV LTRs.

A. 5'LTR-U3 region: percent identity

		1	2	3	4	5	6	7	8	9
SFVpsc.hu-PFV-LTR	1		54,18	87,98	90,63	90,41	43,82	63,21	38,26	43,97
SFVpsc.hu-HSRV13	2	54,18		46,47	48,22	48,00	54,97	36,24	47,26	27,39
SFVpve-LTR	3	87,98	46,47		88,81	88,66	43,79	62,79	38,19	43,64
SFVptr.hu-AG15-LTR	4	90,63	48,22	88,81		99,16	44,21	63,68	38,46	43,60
SFVptr.hu-BAD327-LTR	5	90,41	48,00	88,66	99,16		44,07	63,27	38,37	44,01
SFVggo-LTR	6	43,82	54,97	43,79	44,21	44,07		64,05	82,31	30,63
SFVggo.hu-BAD468-LTR	7	63,21	36,24	62,79	63,68	63,27	64,05		56,39	40,42
SFVggo.hu-BAK74-LTR	8	38,26	47,26	38,19	38,46	38,37	82,31	56,39		28,33
SFVcni.hu-AG16-LTR	9	43,97	27,39	43,64	43,60	44,01	30,63	40,42	28,33	

B. 5'LTR-U3 region (last 70 nt): percent identity

		1	2	3	4	5	6	7	8	9
SFVpsc.hu-PFV-LTR	1		95,71	83,10	87,14	85,71	66,20	66,20	67,61	45,07
SFVpsc.hu-HSRV13	2	95,71		81,69	85,71	84,29	67,61	67,61	69,01	43,66
SFVpve-LTR	3	83,10	81,69		87,32	87,32	67,61	67,61	69,01	45,83
SFVptr.hu-AG15-LTR	4	87,14	85,71	87,32		97,14	67,61	67,61	69,01	43,66
SFVptr.hu-BAD327-LTR	5	85,71	84,29	87,32	97,14		67,61	67,61	69,01	46,48
SFVggo-LTR	6	66,20	67,61	67,61	67,61	67,61		97,10	92,75	44,44
SFVggo.hu-BAD468-LTR	7	66,20	67,61	67,61	67,61	67,61	97,10		92,75	47,22
SFVggo.hu-BAK74-LTR	8	67,61	69,01	69,01	69,01	69,01	92,75	92,75		44,44
SFVcni.hu-AG16-LTR	9	45,07	43,66	45,83	43,66	46,48	44,44	47,22	44,44	

C. 5'LTR-R region: percent identity

		1	2	3	4	5	6	7	8	9
SFVpsc.hu-PFV-LTR	1		100,00	93,26	91,19	91,19	82,99	82,99	81,54	69,37
SFVpsc.hu-HSRV13	2	100,00		93,26	91,19	91,19	82,99	82,99	81,54	69,37
SFVpve-LTR	3	93,26	93,26		97,30	97,30	88,71	88,71	87,17	68,47
SFVptr.hu-AG15-LTR	4	91,19	91,19	97,30		98,91	89,13	89,13	88,65	66,22
SFVptr.hu-BAD327-LTR	5	91,19	91,19	97,30	98,91		89,13	89,13	88,65	66,67
SFVggo-LTR	6	82,99	82,99	88,71	89,13	89,13		98,91	93,48	66,22
SFVggo.hu-BAD468-LTR	7	82,99	82,99	88,71	89,13	89,13	98,91		93,48	66,22
SFVggo.hu-BAK74-LTR	8	81,54	81,54	87,17	88,65	88,65	93,48	93,48		66,22
SFVcni.hu-AG16-LTR	9	69,37	69,37	68,47	66,22	66,67	66,22	66,22	66,22	

D. 5'LTR-U5 region: percent identity

		1	2	3	4	5	6	7	8	9
SFVpsc.hu-PFV-LTR	1		98,69	95,45	91,56	90,91	66,88	77,71	78,98	76,10
SFVpsc.hu-HSRV13	2	98,69		96,71	92,76	92,11	67,74	78,71	80,00	77,07
SFVpve-LTR	3	95,45	96,71		94,08	93,42	68,39	79,35	80,00	78,98
SFVptr.hu-AG15-LTR	4	91,56	92,76	94,08		99,34	67,74	78,71	78,06	79,62
SFVptr.hu-BAD327-LTR	5	90,91	92,11	93,42	99,34		67,10	78,06	77,42	78,98
SFVggo-LTR	6	66,88	67,74	68,39	67,74	67,10		85,81	82,58	67,09
SFVggo.hu-BAD468-LTR	7	77,71	78,71	79,35	78,71	78,06	85,81		93,55	77,22
SFVggo.hu-BAK74-LTR	8	78,98	80,00	80,00	78,06	77,42	82,58	93,55		76,58
SFVcni.hu-AG16-LTR	9	76,10	77,07	78,98	79,62	78,98	67,09	77,22	76,58	

# Supplementary figure 2 -continued

