

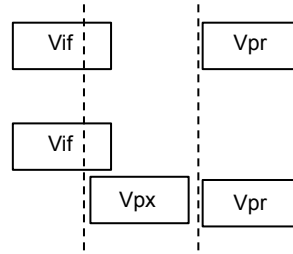
# Figure S1. (related to Figure 1)

**A.**

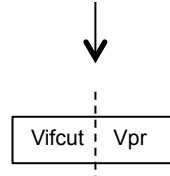
**1/ One alignment of Vif-(Vpx)-Vpr from SIVs**

that do not have Vpx (i.e. SIVcpz, SIVver, SIVlst, SIVmus)

that encode for Vpx (i.e. SIVsmm, SIVrcm)



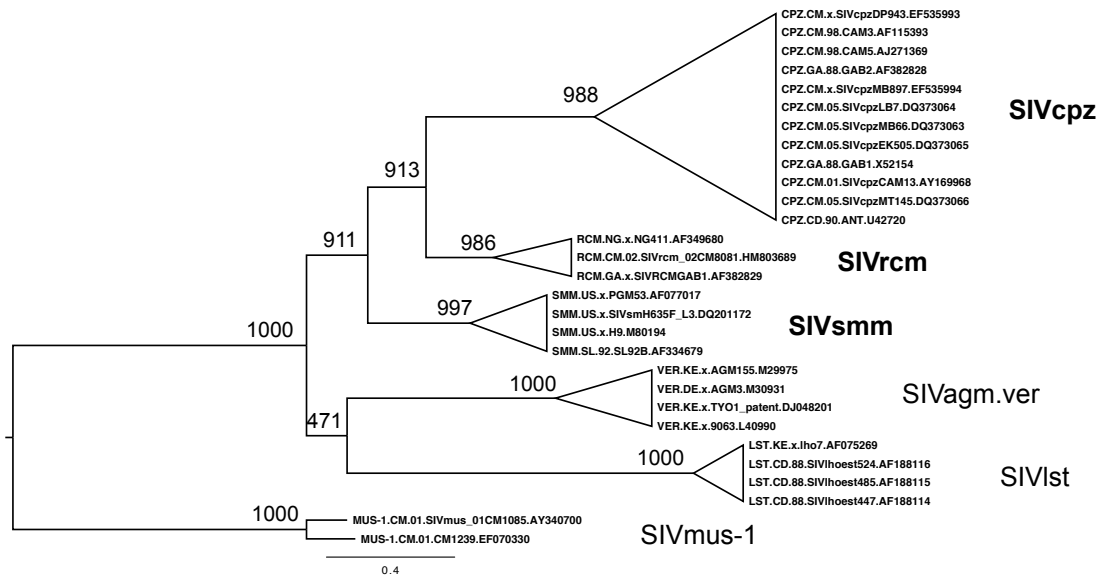
**2/ Alignment trimmed and merged**



**B.**

	If <i>vpx</i> was acquired in the ancestor of SIVsmm and SIVrcm, prior to the jump of SIVrcm to chimpanzee	If <i>vpx</i> was transferred between SIVrcm and SIVsmm	
		before the jump of SIVrcm to chimpanzee	after the jump of SIVrcm to chimpanzee
Tree topology for the trimmed and merged alignment	✓	✓	✗
Evidence of recombination	No recombination between SIVrcm and SIVsmm ✓	Recombination breakpoints identified between SIVrcm and SIVsmm ✗	Recombination breakpoints identified between SIVrcm and SIVsmm ✗

**C.**



## Figure S2. (related to Figure 2)

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RCM.CM.02.SIVrcm\_02CM8081.HM803689  
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CPZ.CD.90.ANT.U42720  
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CPZ.CM.05.SIVcpzEK505.DQ373065  
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CPZ.CM.98.CAM5.AJ271369  
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CPZ.GA.88.GAB2.AF382828  
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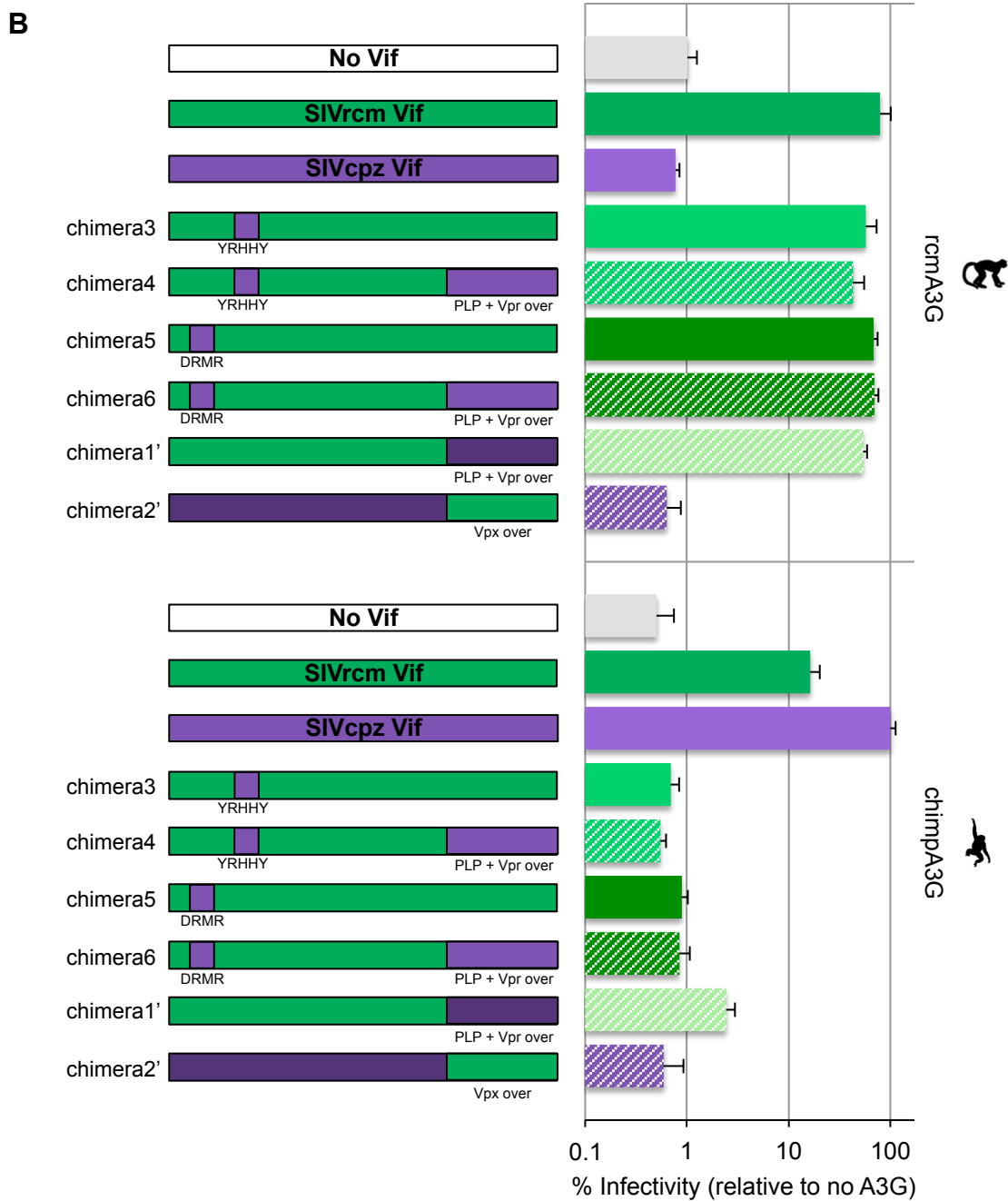
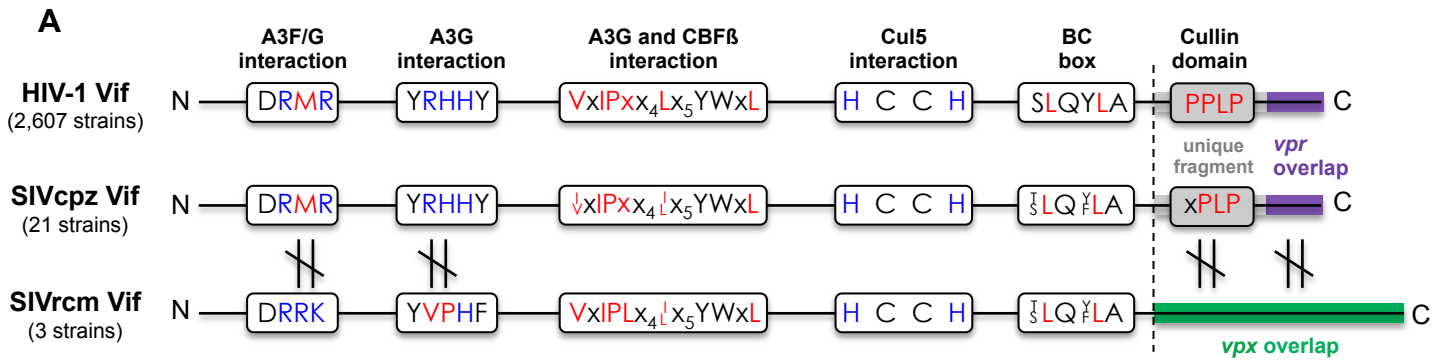
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CPZ.TZ.01.TAN2.EF394357  
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CPZ.US.85.US\_Marilyn.AF103818  
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Figure S3. (related to Figure 3B)



## Supplemental information

### Supplementary figure legends

**Figure S1, related to Figure 1. Phylogenetic and recombination analyses of the region spanning *vif*, *vpx*, and *vpr* show that the *vpx* gene was acquired before the divergence of SIVrcm and SIVsmm lineages, i.e. before the jump of SIVrcm to chimpanzee.**

While *vpx* was acquired only once during primate lentiviral evolution, this acquisition could have happened in multiple ways: either *vpx* was acquired in the ancestor of SIVsmm and SIVrcm (before their divergence), or *vpx* was acquired in one lineage (after the divergence of SIVsmm and SIVrcm) and was later transferred to the other one. Thus, to determine if SIVrcm had a *vpx* gene when it transferred to chimpanzee, we analyzed an alignment of the surrounding regions of Vpx (panel A) to look for any evidence of non-shared ancestry or of gene transfer between SIVrcm and SIVsmm. The alignment was made with sequences from SIVrcm, SIVsmm, and related SIV lineages spanning the *vif-vpx-vpr* region, but where the portion coding for *vpx* was taken out (panel A). The hypotheses and the methods to decipher between the scenarios are depicted in panel B. The results of the analyses are shown in the table with the “check” (true) or “x” (false) signs. More specifically, we performed a phylogenetic analysis on this amino acid alignment with the JTT model using maximum likelihood with 1,000 bootstrap replicates and found that SIVsmm and SIVrcm share a common ancestry in this fragment (panel C).

Next, to rule out any *vpx* gene transfer between the two lineages, we performed recombination analyses with GARD and PHI-test on both the nucleotide and the amino acid alignments and we did not find any evidence of recombination between SIVrcm and SIVsmm in this region with a 0.1 cut-off. This shows that no horizontal *vpx* gene transfer occurred between these lineages. Overall, these analyses show that the acquisition of *vpx* occurred prior to the divergence of SIVsmm and SIVrcm, which implies that SIVrcm had a *vpx* gene when it transferred to chimpanzee.

**Figure S2, related to Figure 2. Alignment of nucleotide sequences from the 5' end of the *vpr* gene of SIVcpz and SIVrcm.**

Nucleotide sequences of the 5' end of *vpr* were aligned with FSA. For each nucleotide sequence, the two important reading frames are shown: for SIVrcm sequences, the first translation corresponds to frame 2 in Figure 2A and codes for Vpr (N-ter, green), and the second corresponds to frame 3 and is not an ORF but the residues highlighted in purple correspond to the region used to reconstruct SIVcpz Vif (purple fragment in Figure 2A); for SIVcpz sequences, the first translation corresponds to frame 3 in Figure 2A and codes for Vpr (N-ter, green), and the second corresponds to frame 1 and codes for Vif (C-ter, overprinting region, purple).

**Figure S3, related to Figure 3B. The gain of function of SIVrcm Vif upon adaptation to the chimpanzee A3G involved both the N- and the C- terminal domains.**

A- Representations of HIV-1 Vif, SIVcpz Vif and SIVrcm Vif. The conserved domains shown to be important for A3G degradation by HIV-1 are depicted (blue, basic residues; red, hydrophobic). Each letter corresponds to the one-letter code amino acid and

shows that more than 85% of the strains had this residue at this particular position. If two residues were seen at high rates at one position in the dataset, then two letters are shown. On the left, the number of strains compared for this analysis is indicated for each lentiviral Vif (dataset from the HIV database, [www.hiv.lanl.gov](http://www.hiv.lanl.gov)). The differences in major motifs are shown by the “≠” signs, and the differences in the C-terminal domain are further highlighted.

B- Single round infectivity assay. On the left side, the Vif constructs (no Vif, SIVcpz or SIVrcm Vifs, and mutants or chimeras) are represented. Green corresponds to SIVrcm Vif fragments and purple corresponds to SIVcpz Vif regions (purple for SIVcpzTan3 strain, chimeras 3 to 6, and dark purple for SIVcpzUG38 strain, chimera1’ and chimera2’). Major motifs are highlighted: <sup>40</sup>YRHHY, <sup>14</sup>DRMR, PPLP. Regions of *vif* overlapping with *vpr* or *vpx* (“vpr over” or “vpx over”) are also depicted. The same order can be found for the top and the bottom panel. On the right side, the corresponding infectivity assays are shown, as previously described in Figure 3B. Infectivity values in percentage are the average of six infections; error bars indicate the standard deviation from the mean of these replicates. Top panel, infections tested against red-capped mangabey A3G; bottom panel, infections tested against chimpanzee A3G. Grey bars, HIV-1ΔVif; purple, SIVcpz Vif or chimera made in the context of SIVcpz Vif; green, SIVrcm Vif or chimeras made in the context of SIVrcm Vif. Striped bars are for chimeras modified in the C-ter region. All Vifs were expressed at a level sufficient for their anti-A3G activity.

## Extended experimental procedures

### Plasmids

SAMHD1 expression plasmids. The SAMHD1 plasmids are as previously reported (Lim et al., 2012): chimpanzee and red-capped mangabey SAMHD1 (GenBank accession numbers: JQ231123 and JQ231133) were cloned and ligated into a pLPCX construct with a 3' end hemagglutinin (HA) tag.

Vpr and Vpx constructs. The following viral *vpr* and *vpx* genes were ligated into pCDNA3.1 with a 5' end 3xFLAG epitope tag as previously described (Lim et al., 2012): SIVrcmNG411 Vpr and Vpx (AF349680) and SIVmus1CM1239 Vpr (EF070330).

A3 expression plasmids. The following *Apobec3Gs* were cloned into the mammalian expression vector pcDNA3.1 with a 5' end HA epitope tag: chimpanzee A3G (AY331715), red-capped mangabeys A3G (PR00485), human A3G (NM\_021822). The chimpanzee *Apobec3F* (XM\_525658.2) and *Apobec3D* (JN247642) were cloned with a 5' end HA epitope tag into the expression plasmids pcDNA3.1 and pCS2 (Duggal et al., 2011), respectively.

Recombinant HIV-1 Proviral Plasmids. HIV-1 and SIV Vif sequences were cloned into a HIV-1 $\Delta$ Vif molecular clone (pLai3 $\Delta$ EnvLuc2 $\Delta$ Vif, generated after NdeI-StuI deletion in pLai3 $\Delta$ EnvLuc2). The resulting proviral plasmids lack *env*, have a firefly luciferase gene inserted into *nef*, and encode HIV-1 or SIV Vif in the context of the HIV-1 backbone. The following genes were cloned from provirus plasmids: HIV-1 LAI, SIVcpzPtsTAN3 (DQ374658) (proviral plasmid obtained from the NIH AIDS Research and Reference Reagent Program, (Takehisa et al., 2007)), SIVcpzPtsUG38 (JN091690) and SIVcpzPtsTAN13 (JQ768416); and the following from synthesized genes:

SIVrcmCM8081 (HM803689), SIVmusCM1085 (AY340700), SIVcpzPttMB66 (DQ373063), and SIVcpzPttGab1 (X52154). The chimeric and mutant Vif proteins were generated by overlapping PCR and/or site-directed mutagenesis (QuikChange Site-directed mutagenesis kit, Stratagene). To control for Vif expression, constructs were also made with a 3' end 3xFLAG epitope tag.

### **Antibodies for western-blot analyses**

The following antibodies were used for the western-blot analyses: mouse HA-specific antibody (Balco), mouse anti-FLAG M2 antibody (Sigma-Aldrich), and mouse anti-tubulin (Sigma-Aldrich). The primary antibodies were detected with a goat anti-mouse horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich).

### **Supplemental references**

Takehisa, J., Kraus, M.H., Decker, J.M., Li, Y., Keele, B.F., Bibollet-Ruche, F., Zammit, K.P., Weng, Z., Santiago, M.L., Kamenya, S., *et al.* (2007). Generation of infectious molecular clones of simian immunodeficiency virus from fecal consensus sequences of wild chimpanzees. *J Virol* 81, 7463-7475.