

# The Antiviral Spectra of TRIM5 $\alpha$ Orthologues and Human TRIM Family Proteins against Lentiviral Production

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## Abstract

**Background:** Rhesus monkey TRIM5 $\alpha$  (TRIM5 $\alpha$ rh) recognizes the incoming HIV-1 core through its C-terminal B30.2(PRYSPRY) domain and promotes its premature disassembly or degradation before reverse transcription. Previously, we have shown that TRIM5 $\alpha$ rh blocks HIV-1 production through the N-terminal RBCC domain by the recognition of Gag polyproteins. Although all TRIM family proteins have RBCC domains, it remains elusive whether they possess similar late-restriction activities.

**Methodology/Principal Findings:** We examined the antiviral spectra of TRIM5 $\alpha$  orthologues and human TRIM family members which have a genetic locus proximal to human TRIM5 $\alpha$  (TRIM5 $\alpha$ hu), against primate lentiviral production. When HIV-1 virus-like particles (VLPs) were generated in the presence of TRIM5 $\alpha$  proteins, rhesus, African green and cynomolgus monkey TRIM5 $\alpha$  (TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy), but not TRIM5 $\alpha$ hu, were efficiently incorporated into VLPs, suggesting an interaction between HIV-1 Gag and TRIM5 $\alpha$  proteins. TRIM5 $\alpha$ rh potently restricted the viral production of HIV-1 groups M and O and HIV-2, but not simian lentiviruses including SIV<sub>MAC</sub>1A11, SIV<sub>AGM</sub>Tan-1 or SIV<sub>AGM</sub>SAB-1. TRIM5 $\alpha$ hu did not show notable late restriction activities against these lentiviruses. TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy showed intermediate restriction phenotypes against HIV-1 and HIV-2, but showed no restriction activity against SIV production. A series of chimeric TRIM5 $\alpha$  constructs indicated that the N-terminal region of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy are essential for the late restriction activity, while the C-terminal region of TRIM5 $\alpha$ cy negatively regulates the late restriction activity against HIV-1. When select human TRIM family proteins were examined, TRIM21 and 22 were efficiently incorporated into HIV-1 VLPs, while only TRIM22 reduced HIV-1 titers up to 5-fold. The antiviral activities and encapsidation efficiencies did not correlate with their relative expression levels in the producer cells.

**Conclusions/Significance:** Our results demonstrated the variations in the late restriction activities among closely related TRIM5 $\alpha$  orthologues and a subset of human TRIM family proteins, providing further insights into the late restriction activities of TRIM proteins.

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## Introduction

Approximately 8% of the human genome is comprised of retroviral elements, implicating an extensive history of competition between hosts and retroviruses [1,2]. To counteract these viruses, primates have developed defensive measures which target various aspects of the retroviral life cycle. Cellular restriction factor TRIM5 $\alpha$  is one such contributing element in this antiviral defense against retroviruses [3,4,5,6]. TRIM5 $\alpha$  belongs to the TRIM family of proteins, which are characterized by sequential domains in the N-terminal half of the protein, RING, with one or two b-boxes followed by a coiled-coil motif and its  $\alpha$  isoform includes a C-terminal B30.2(PRYSPRY) domain. The rhesus monkey TRIM5 $\alpha$  (TRIM5 $\alpha$ rh) recognizes the incoming HIV-1 core through its C-terminal B30.2(PRYSPRY) domain and promotes its premature disassembly or degradation before reverse transcription [7,8,9,10]. Primate TRIM5 $\alpha$  orthologues have distinct post-

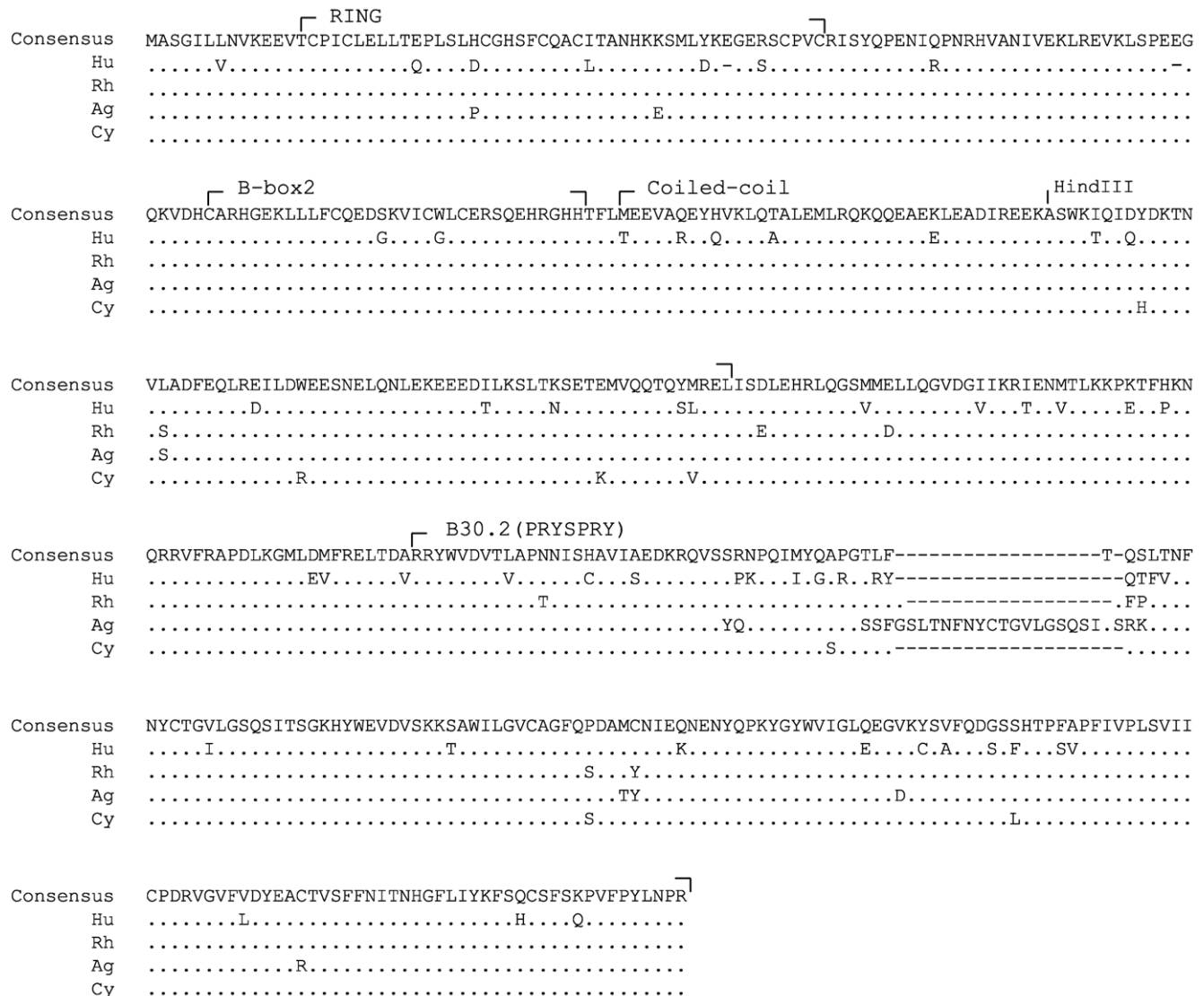
entry restriction activities against a range of retro- and lentiviruses; however, they generally lack strong restriction activity against their own host-specific viruses. For instance, human TRIM5 $\alpha$  (TRIM5 $\alpha$ hu) restricts N-tropic murine leukemia virus (N-MLV) as well as equine infectious anemia virus (EIAV), but not human immunodeficiency virus type-1 (HIV-1) or simian immunodeficiency virus (SIV) [3,4,6,11]. In contrast, TRIM5 $\alpha$ rh expression in HIV-1-permissive cells confers strong antiviral activity against HIV-1, EIAV, N-MLV and SIV from African green monkeys (SIV<sub>AGM</sub>), but not against SIV from rhesus macaques (SIV<sub>MAC</sub>) [3,4,6,9,11,12]. The African green monkey TRIM5 $\alpha$  orthologue (TRIM5 $\alpha$ ag) restricts HIV-1, SIV<sub>MAC</sub>, EIAV and N-MLV, but not SIV<sub>AGM</sub> [3,11], while the cynomolgus monkey orthologue (TRIM5 $\alpha$ cy) restricts HIV-1 and HIV-2, but not SIV<sub>MAC</sub> infection [13]. These post-entry restriction patterns of TRIM5 $\alpha$  orthologues suggest that lentiviruses have evolved to evade TRIM5 $\alpha$ -mediated post-entry restriction when colonizing respec-

tive species. In response, host species also appear to have evolved their TRIM5 $\alpha$  proteins, especially the coiled-coil and B30.2(PRYSPRY) domains, against retro- and lentiviruses [14,15].

TRIM5 $\alpha$ rh also exhibits an additional antiviral activity against HIV-1 production, independently of the well-characterized post-entry restriction, to block the late phase of HIV-1 replication [16,17]. High levels of TRIM5 $\alpha$ rh expression blocks HIV-1 production predominantly by reducing the number of HIV-1 virions, while modest TRIM5 $\alpha$ rh expression blocks the late phase of HIV-1 replication by reducing virion infectivity as well as virion numbers [16,18]. When HIV-1 virus-like-particles (VLPs) are produced in the presence of TRIM5 $\alpha$ rh, TRIM5 $\alpha$ rh is efficiently incorporated into VLPs, implicating the interaction between cellular and viral components during viral assembly [16]. This TRIM5 $\alpha$ rh-mediated restriction of HIV-1 production is mediated by the N-terminal RBCC domain, but not the C-terminal B30.2(PRYSPRY) domain [16]. Further studies have identified several determinants for this late restriction. A RING structure is essential for the efficient interaction with HIV-1 Gag, while two

amino acid residues in TRIM5 $\alpha$ rh coiled-coil domain (M133 and T146) are critical for the late restriction activity [19]. Our data suggest that the TRIM5 $\alpha$ rh-mediated late restriction involves at least two distinct activities: (i) interaction with HIV-1 Gag polypeptide through the N-terminal, RING and b-box 2 regions of a TRIM5 $\alpha$ rh monomer, and (ii) an effector function(s) that depends upon the coiled-coil and linker 2 domains of TRIM5 $\alpha$ rh [19]. Although TRIM5 $\alpha$ hu does not show strong late restriction activities against HIV-1 group M viruses [16], it remains to be determined if the human orthologue has any late restriction activity against other human and non-human primate lentiviruses.

Previous studies have shown that TRIM5 $\alpha$  is interferon-responsive [20,21]. Recent study identified TRIM6, 21, 22 and 34, which are located on chromosome 11p together with TRIM5, are also interferon responsive [22]. Given that the late restriction of TRIM5 $\alpha$  is dependent on the RBCC domain and these TRIM family proteins possess an N-terminal RBCC, it is possible that TRIM5 $\alpha$ hu paralogues may have similar late-restriction activities. In the present study, we determined the antiviral spectra and



**Figure 1. Amino acid sequence alignment of TRIM5 $\alpha$  orthologues examined in this study.** Consensus amino acid sequences of TRIM5 $\alpha$ , as well as TRIM5 $\alpha$ hu (Hu), TRIM5 $\alpha$ rh (Rh), TRIM5 $\alpha$ ag (Ag) and TRIM5 $\alpha$ cy (Cy) amino acid sequences are shown. Identical residues are indicated by dots. Gaps are indicated by dashes. HindIII site in the coiled-coil domain was used as the junction for the chimeras used in this study. doi:10.1371/journal.pone.0016121.g001

encapsidation efficiency of TRIM5 $\alpha$  orthologues and paralogues. We found that TRIM5 $\alpha$  orthologues from African green (TRIM5 $\alpha$ ag) and cynomolgus (TRIM5 $\alpha$ cy) monkeys have similar, but weaker late restriction activities against HIV-1 production. Similar to TRIM5 $\alpha$ rh, TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy proteins were efficiently incorporated in HIV-1 VLPs, and their RBCC domains were essential for the late restriction activities. Intriguingly, the C-terminal regions of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy proteins negatively regulated the late restriction activities. Studies using human TRIM5 paralogues with conserved RBCC domains demonstrated that TRIM21 and TRIM22 specifically incorporated into HIV-1 VLPs, while only TRIM22 mildly restricted HIV-1 production. Our results therefore demonstrate the variable late restriction activities of TRIM5 $\alpha$  orthologues and paralogues. The involvement of the C-terminal sequences of TRIM5 $\alpha$  proteins in determining the potency of late restriction activities suggests more complex mechanisms underlying TRIM protein-mediated late restriction activities than previously reported.

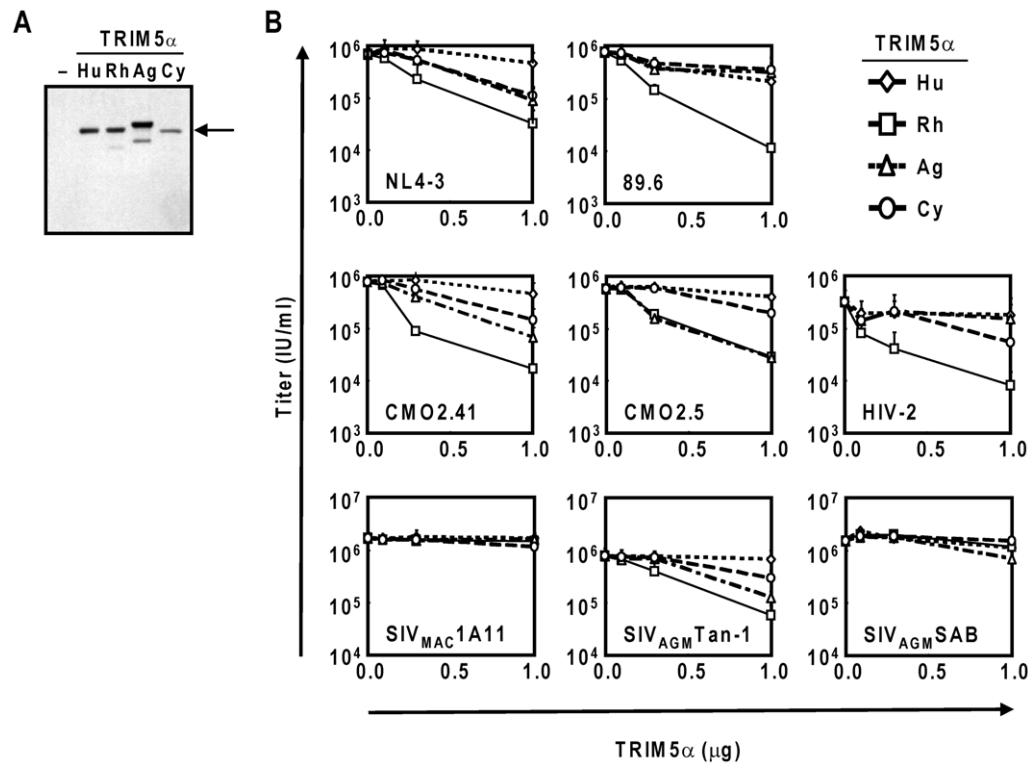
## Methods

### Cell culture

293T and GHOST(3)R3/X4/R5 [23] cells were maintained in Dulbecco's Modified Eagle's Medium with 4.5 g/L glucose, supplemented with 10% fetal bovine serum (FBS) and antibiotics.

### Plasmids

TRIM5 $\alpha$ rh- or TRIM5 $\alpha$ hu-expressing plasmids with a C-terminal HA tag, pRhT5 $\alpha$  and pHuT5 $\alpha$ , respectively, and codon-optimized HIV-1 GagPol expression construct pH-G/P were described previously [16]. pcDNA3.1-based TRIM5 $\alpha$ ag expression plasmid (pAgmT5 $\alpha$ ) was generated from pDON-aT5 [18] and TRIM5 $\alpha$ cy cDNA was amplified from total RNA isolated from cynomolgus monkey lymphoid line, HSC-F cells (kindly provided by Dr. Hirofumi Akari) and cloned into pcDNA3.1 to generate pCynT5 $\alpha$ . Proviral lentivirus plasmids pNL4-3, p89.6, p94UG114.1, pSIVmac1A11, pSIVagmTan-1 and pSAB-1 were obtained from the NIH AIDS Research and Reference Reagent Program [24,25,26,27,28,29,30,31]. pROD10 was described previously [32]. pCMO2.41 and pCMO2.5 were generously provided by Dr. Hans-Georg Kräusslich. Chimeric TRIM5 $\alpha$ -expression plasmids were generated using the conserved *HindIII* sites in the coiled coil domain of TRIM5 $\alpha$  sequences, and cloned into pcDNA3.1. N-terminally tagged TRIM1, 6, 18, 21, 22 and 34 were kindly provided by Dr. Paul Bieniasz [33]. Since modifications in the N-terminal region of TRIM5 $\alpha$ rh ablated its late restriction activity [16], we generated C-terminally HA-tagged human TRIM expression plasmids based on pcDNA3.1(+) (Invitrogen) and verified their sequences. TRIM5 $\alpha$ rh point mutants were generated using the QuikChange II XL site-directed mutagenesis kit (Stratagene, Cedar Creek, TX).



**Figure 2. TRIM5 $\alpha$  orthologue expression in 293T cells and antiviral spectrum of TRIM5 $\alpha$  orthologues against lentiviral production.** (A) Verification of the proper expression of TRIM5 $\alpha$  orthologues. Control plasmid (-), TRIM5 $\alpha$ hu (Hu), TRIM5 $\alpha$ rh (Rh), TRIM5 $\alpha$ ag (Ag) and TRIM5 $\alpha$ cy (Cy) expression in transfected 293T cells were verified via immunoblot analysis. Arrow depicts approximate TRIM5 $\alpha$  band size. (B) 293T cells were co-transfected with a primate lentivirus proviral plasmid and increasing amounts of human (Hu), rhesus monkey (Rh), African green monkey (Ag) or cynomolgus monkey (Cy) TRIM5 $\alpha$ -expressing plasmids. As infectious proviral plasmids, HIV-1 Group M (pNL4-3 and p89.6), HIV-1 Group O (pCMO2.41, pCMO2.5), HIV-2 (pROD10), and SIV (pSIV<sub>MAC</sub>1A11, pSIV<sub>AGM</sub>Tan-1 and pSIV<sub>AGM</sub>SAB-1) were used. Viral titers were determined in GHOST(3)R3X4R5 indicator cells and described as infectious units per ml (IU/ml). Error bars indicate one standard deviation. doi:10.1371/journal.pone.0016121.g002

### Confocal microscopy analysis

293T cells were transfected with 1.0  $\mu$ g TRIM5 $\alpha$ -expression plasmid using FuGene 6 (Roche, Madison, WI) in a 6-well culture plate. 6-h post-transfection, 293T cells were seeded into LabTek II 8-well chamber slides (Nunc, Rochester, NY) at approximately  $1.5 \times 10^5$  cells/ml. 36 h post-transfection, cells were fixed in 4% paraformaldehyde, permeabilized on ice using 0.1% saponin after fixation then blocked with 5% FBS PBS solution for 30 min at room temperature. Primary antibody (rat anti-HA, 1:250, Roche) and secondary antibody (FITC-conjugated goat anti-rat IgG, 1:250, Thermo Scientific, Waltham, MA) were used to visualize HA signals. Images were obtained using the Zeiss LSM 510 confocal microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY) and analyzed with the Zeiss LSM Image Browser software.

### Western Blotting

Proteins were subjected to SDS-PAGE in a 4–15% Tris-HCl gel and then transferred onto a PVDF membrane at 0.7 mA/cm<sup>2</sup> for 40 min. Membranes were blocked in 5% milk PBS overnight prior to application of antibodies. Antibodies were used in the following concentrations: rat anti-HA (1:1000, Roche), and mouse anti- $\beta$ -actin (1:1000). HIV-1 Gag proteins were detected using a mixture of anti-p24 antibodies (183-H12-5C, 1:1000 and AG3.0, 1:500) [34,35]. Peroxidase-conjugated secondary antibodies (goat anti-rat IgG and goat anti-mouse IgG, Thermo Scientific) were used at a 1:2000 concentration.

### Virus-like-particle incorporation assay

1.0  $\mu$ g of TRIM5 $\alpha$ - or TRIM-expressing plasmid and 0.2  $\mu$ g of a codon-optimized HIV-1 Gag-Pol expression plasmid, pH-GP [36], were co-transfected into 293T cells using FuGene 6. Two days post-transfection, transfected cells were harvested in RIPA buffer to assess TRIM5 $\alpha$  and HIV-1 GagPol expression. Culture supernatants were also harvested, and passed through a 0.45  $\mu$ m-pore syringe filter for VLP purification. The filtered supernatants were then centrifuged at 18,000 $\times$ g for 90 min through a 20% sucrose cushion, resuspended in PBS and centrifuged at 18,000 $\times$ g for 90 min. Pelleted VLPs were lysed in 5  $\mu$ l of RIPA buffer and to which 5  $\mu$ l of sample buffer was added, heat-denatured and subjected to immunoblot analysis.

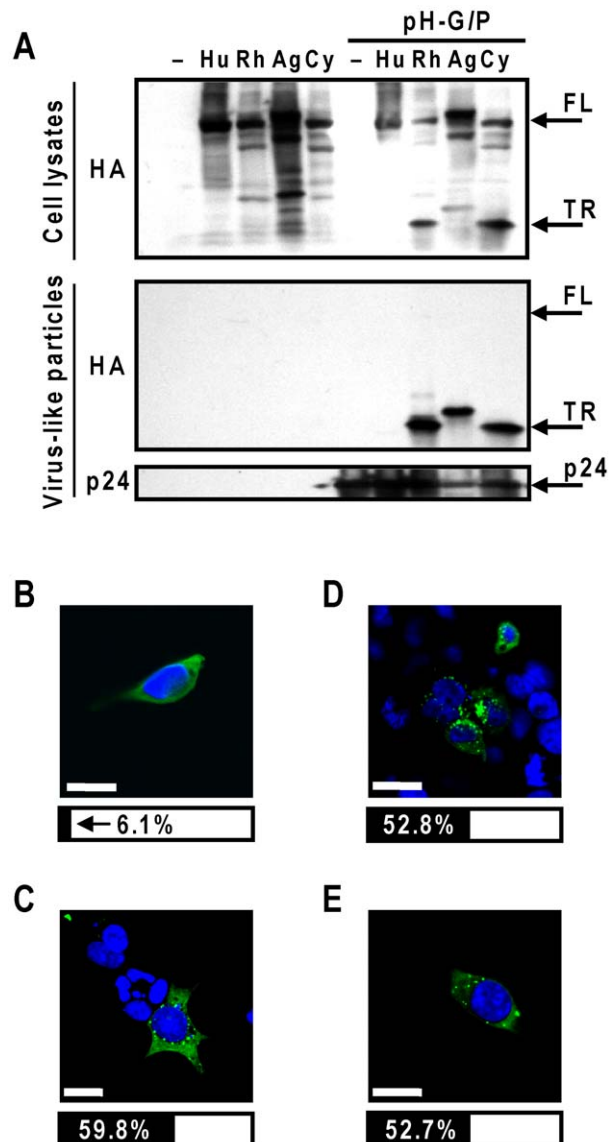
### Viral production assay

For the assessment of TRIM5 $\alpha$  orthologue antiviral activities, increasing amounts of pHuT5 $\alpha$ , pRhT5 $\alpha$ , pAgt5 $\alpha$  and pCynT5 $\alpha$  (0.1, 0.3 and 1.0  $\mu$ g) were co-transfected with 0.1  $\mu$ g of infectious lentiviral proviral plasmid into 293T cells ( $1.0 \times 10^6$  cells) using FuGene 6 (Roche). A control plasmid (pBlueScript-III(+), Stratagene) was added to each transfection reaction to bring the final plasmid concentrations to 1.2  $\mu$ g per transfection. Human TRIM family protein antiviral activities were examined similarly, except 1.0  $\mu$ g of TRIM-expression plasmid was used in each reaction. Two days post-transfection, cellular supernatants were passed through a 0.45  $\mu$ m-pore syringe filter and the viral titers in the supernatants were determined as infectious units/ml (IU/ml) in GHOST(3)R3/X4/R5 indicator cells.

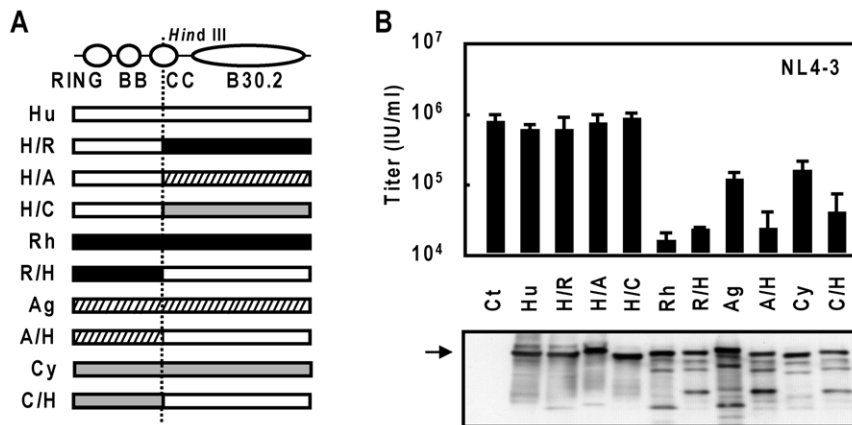
## Results

### Late restriction activities of TRIM5 $\alpha$ rh, TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy against primate lentiviruses

In the well-characterized TRIM5 $\alpha$  post-entry restriction, the TRIM5 $\alpha$  B30.2(PRYSPRY) determines the potency and specificity of restriction. In contrast, the late restriction of HIV-1 depends



**Figure 3. TRIM5 $\alpha$  orthologue incorporation into HIV-1 virus-like particles and subcellular localization in 293T cells.** (A) HIV-1 VLP encapsidation assays using human and simian TRIM5 $\alpha$  orthologues. 293T cells were co-transfected with 1.0  $\mu$ g of TRIM5 $\alpha$ -expressing plasmid and 0.2  $\mu$ g of a codon-optimized HIV-1 Gag-Pol expression plasmid (pH-GP). Producer cell proteins and supernatant containing VLPs were harvested two days post-transfection. Upper panel shows immunoblot analysis of producer cell lysates probed against TRIM5 $\alpha$  (anti-HA). Lower two panels show immunoblot analysis of VLPs probed against TRIM (anti-HA), HIV-1 (anti-p24; 183-5C-H12, 1:1000 and AG3.0, 1:500). Full length (FL), truncated (TR) TRIM5 $\alpha$  bands and HIV-1 CA (p24) are indicated by the arrows. (B–E) 293T cells were transfected with TRIM5 $\alpha$ -expressing plasmid and seeded onto 8-well chamber slides 6h post-transfection. Cells were fixed with paraformaldehyde and permeabilized with 0.1% saponin, then stained against TRIM5 $\alpha$ HA tag using rat anti-HA antibody 3F10 and a FITC conjugated anti-rat IgG secondary antibody. Representative TRIM5 $\alpha$ hu (B), TRIM5 $\alpha$ rh (C), TRIM5 $\alpha$ ag (D) and TRIM5 $\alpha$ cy (E) images are shown. Bar under the respective images represent the percentages of cells exhibiting more than three discrete cytoplasmic bodies (black) or a diffuse signal void of cytoplasmic bodies (white). Scale bars represent 20  $\mu$ m. doi:10.1371/journal.pone.0016121.g003



**Figure 4. Antiviral activities of chimeric TRIM5 $\alpha$  proteins against HIV-1 production.** (A) Schematic representation of the chimeric TRIM5 $\alpha$  constructs between human (Hu), rhesus monkey (Rh), African green monkey (Ag) and cynomolgus monkey (Cy) TRIM5 $\alpha$  proteins. (B) Upper panel: late-restriction activities of chimeric TRIM5 $\alpha$  proteins upon co-transfection with pNL4-3 into 293T cells. Viral titers were determined in GHOST(3)R3X4R5 indicator cells and reported as infectious units per ml (IU/ml). Error bars indicate one standard deviation. Lower panel: western blot analysis of chimeric TRIM5 $\alpha$  proteins following transfection into 293T cells. Arrow indicates approximate full-length TRIM5 $\alpha$  size. doi:10.1371/journal.pone.0016121.g004

on the TRIM5 $\alpha$  RBCC domain [16,19]. The highly conserved RBCC sequences among simian TRIM5 $\alpha$  orthologues suggest the possible late restriction activities in other primate TRIM5 $\alpha$  proteins (Fig. 1). We therefore examined the late-restriction activities of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy against a series of human and non-human primate lentiviruses. Human lentiviruses, HIV-1 group M viruses, HIV-1 group O and HIV-2 viruses were examined for their sensitivity to TRIM5 $\alpha$ -mediated late restriction. HIV-1 group O viruses are divergent from group M viruses [37] and show different sensitivities to TRIM5 $\alpha$ rh-mediated Lvl1 restriction [38], partly due to their cyclophilin A-independence [38,39]. Since group O and HIV-2 viruses have been less successful in colonizing human population than the more-prevalent HIV-1 group M viruses, we hypothesized that TRIM5 $\alpha$ zhu might be able to block the late phase of group O HIV-1 or HIV-2 replication. SIV<sub>MAC</sub> and SIV<sub>AGM</sub> were also included to test the late restriction activities of TRIM5 $\alpha$  proteins against non-human primate lentiviruses.

Immunoblot analysis was performed to verify the proper expression of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy proteins following transfection into 293T cells (Fig. 2A). TRIM5 $\alpha$ rh reduced the titers of two HIV-1 group M clones (NL4-3, 89.6) and HIV-1 group O clones (CMO2.41 and CMO2.5) by up to 60-fold in a dose-dependent manner (Fig. 2B). TRIM5 $\alpha$ rh also reduced the titers of HIV-2 up to 40-fold. Although SIV<sub>MAC</sub> and SIV<sub>AGM</sub> SAB-1 titers were largely unaffected by TRIM5 $\alpha$ rh, we observed a 10-fold decrease in the titers of SIV<sub>AGM</sub>Tan-1 in the presence of TRIM5 $\alpha$ rh (Fig. 2B). TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy showed similar patterns of late restriction activity to its rhesus monkey orthologue, although the reductions in viral titers were modest. NL4-3 titers were reduced by 7-fold in cells expressing TRIM5 $\alpha$ ag or TRIM5 $\alpha$ cy, while 89.6 titers were only reduced by 2-fold. Although group O isolate CMO2.41 virus titers were reduced by up to 7-fold when producer cells expressed TRIM5 $\alpha$ ag or TRIM5 $\alpha$ cy, the titers of another Group O clone CMO2.5, which is based on the CMO2.41 isolate but containing the 5'LTR to *vpr* sequences from the MVP2171 O-type isolate [40], were strongly affected by TRIM5 $\alpha$ ag but not TRIM5 $\alpha$ cy. TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy showed little late restriction activity on SIV<sub>MAC</sub>1A11, SIV<sub>AGM</sub>Tan-1, SIV<sub>AGM</sub>SAB-1 and HIV-2 production. Intriguingly, TRIM5 $\alpha$ ag reduced SIV<sub>AGM</sub>Tan-1 titers by 6-fold (Fig. 2B),

which may be explained by the difference in the host species of SIV<sub>AGM</sub> and TRIM5 $\alpha$ ag: SIV<sub>AGM</sub>Tan-1 is isolated from the *Chlorocebus tantalus* [31] but the TRIM5 $\alpha$ ag protein used in these experiments were derived from *Chlorocebus aethiops*-derived CV1 cells [41]. TRIM5 $\alpha$ zhu marginally reduced HIV titers, while it did not affect SIV titers (Fig. 2B).

#### Efficient encapsidation of rhesus monkey, African green monkey and cynomolgus monkey TRIM5 $\alpha$ into HIV-1 virus-like particles

Previously, we have shown that when HIV-1 proteins were produced in the presence of TRIM5 $\alpha$ rh, HIV-1 Gag polyproteins were rapidly degraded [16]. In contrast, over-expression of codon-optimized HIV-1 GagPol was able to saturate the late restriction activity, leading to production of sufficient amounts of VLPs in the presence of TRIM5 $\alpha$ rh and efficient incorporation of TRIM5 $\alpha$ rh in the VLPs [16,17]. Efficient encapsidation of TRIM5 $\alpha$ rh into VLPs generated without HIV-1 protease suggests specific interaction between TRIM5 $\alpha$ rh and HIV-1 Gag before or during HIV-1 assembly [16]. HIV-1 protease appears to cleave TRIM5 $\alpha$ rh in the B30.2(PRYSPRY) domain to produce the truncated, 20 kDa form of TRIM5 $\alpha$ rh in the VLPs, because formation of the 20 kDa form was not seen in the VLPs made without HIV-1 Pol, or in the VLPs treated with HIV-1 protease inhibitors [16]. TRIM5 $\alpha$ rh and TRIM5 $\alpha$ zhu chimeric constructs demonstrated that processing of TRIM5 $\alpha$  proteins was not necessary for their late restriction activities [16,17]. Since three simian TRIM5 $\alpha$  proteins showed different late restriction activities (Fig. 2B), we examined the differences in their HIV-1 Gag-association efficiencies by the VLP encapsidation assay. When high levels of HIV-1 VLPs were generated in the presence of TRIM5 $\alpha$  proteins, efficient incorporation of truncated forms of TRIM5 $\alpha$ rh, TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy, but not TRIM5 $\alpha$ zhu, were observed (Fig. 3A). The truncated forms of TRIM5 $\alpha$  likely resulted from the cleavage of TRIM5 $\alpha$  proteins in the B30.2(PRYSPRY) domain by the HIV-1 protease. The efficient incorporation of the simian TRIM5 $\alpha$  proteins into VLPs suggests a specific interaction between simian TRIM5 $\alpha$  proteins and HIV-1 Gag proteins. Since this experiment was performed under conditions where the late restriction activities were saturated by

the over-expressed HIV-1 Gag, the slight reduction of p24 in the TRIM5 $\alpha$ ag lane (Fig 3A) did not reflect the level of restriction.

### Simian TRIM5 $\alpha$ orthologues form prominent cytoplasmic bodies

TRIM5 $\alpha$  proteins self-associate to form cytoplasmic bodies, while dimerization is also required for efficiently binding to retroviral capsid [42,43]. Immunohistochemistry studies in 293T cells suggest that TRIM5 $\alpha$ rh efficiently form cytoplasmic bodies, while TRIM5 $\alpha$ hu primarily displayed more diffused cytoplasmic localizations [19]. In order to address whether the varying late restriction activities of three simian TRIM5 $\alpha$  proteins were due to their different subcellular localizations, we determine the localizations of simian TRIM proteins by immunostaining. 293T cells were transfected with 1.0  $\mu$ g of TRIM5 $\alpha$ -expressing plasmid. TRIM5 $\alpha$  proteins were then detected with anti-HA antibodies and analyzed by confocal microscope. TRIM5 $\alpha$ hu, which does not strongly affect HIV-1 production, showed predominantly diffuse cytoplasmic distribution with little discernible cytoplasmic bodies (Fig. 3B). In contrast, 59.8%, 52.8% and 52.7% of 293T cells expressing TRIM5 $\alpha$ rh, TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy, respectively, showed discrete cytoplasmic bodies (>3 discrete cytoplasmic body formations per cell) throughout the cytoplasm (Fig. 3 C–E). Our data therefore demonstrated similar subcellular localizations of TRIM5 $\alpha$ rh, TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy, despite their varying late restriction activities.

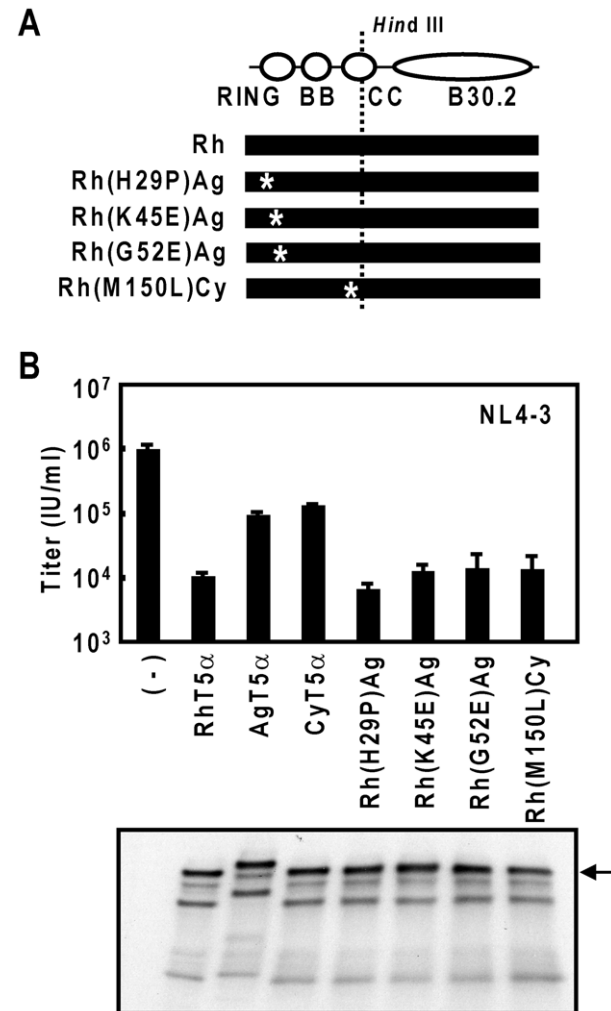
### The RBCC sequences of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy are essential for the late restriction activity

TRIM5 $\alpha$ hu amino acid sequences are the most divergent and showed the weakest late restriction activity among the tested TRIM5 $\alpha$  orthologues (Fig. 2B). We therefore assessed whether the substitution with TRIM5 $\alpha$ hu N- or C-terminal sequences can relieve the late restriction activities of the simian TRIM5 $\alpha$  orthologues. Chimeric TRIM5 $\alpha$  constructs were generated as depicted in Figure 4A. Simian TRIM5 $\alpha$  proteins with N-terminal TRIM5 $\alpha$ hu sequences showed no effect on HIV-1 (Fig. 4B, upper panel) or SIV<sub>MAC</sub> production (data not shown), underscoring the importance of the RBCC sequences in the late restriction activity against HIV-1. Although the TRIM5 $\alpha$ rh-TRIM5 $\alpha$ hu chimera, R/H, showed a late restriction activity as potent as wild-type TRIM5 $\alpha$ rh, the two simian TRIM5 $\alpha$  chimeras with human N-terminal sequences, A/H and C/H, showed stronger late restriction activities than wild-type TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy (Fig. 4B, upper panel). No prominent differences in protein expression levels were observed between the chimeric TRIM5 $\alpha$  proteins (Fig. 4B, lower panel). These data indicate that the RBCC sequences of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy are essential for the late restriction activity and suggest the possibility that the C-terminal regions of simian TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy proteins negatively regulate TRIM5 $\alpha$  late restriction activities against HIV-1.

### The influence of variations in the RBCC sequences of simian TRIM5 $\alpha$ proteins on the late restriction activities

Previously, we demonstrated that introduction of two TRIM5 $\alpha$ hu-specific amino acid residues into TRIM5 $\alpha$ rh (M133T and T146A in the coiled-coil region) abrogates the late restriction activity of TRIM5 $\alpha$ rh [19]. In the RING, b-box 2 and partial coiled-coil domains, there are three amino acid differences between TRIM5 $\alpha$ rh and TRIM5 $\alpha$ ag, while only one residue separates TRIM5 $\alpha$ rh and TRIM5 $\alpha$ cy. In order to address the possible contributions of these residues on the modest late restriction activities of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy, we introduced

single amino-acid substitutions into the N-terminal half of TRIM5 $\alpha$ rh using corresponding TRIM5 $\alpha$ ag or TRIM5 $\alpha$ cy sequences (Fig. 5A). After verification of similar chimeric TRIM5 $\alpha$  expression levels by immunoblot (Fig. 5B, lower panel), we examined respective late restriction activities against HIV-1. When HIV-1 NL4-3 was produced in the presence of TRIM5 $\alpha$ rh, TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy, HIV-1 titers were reduced approximately 60-, 7- and 6-folds, respectively, while the TRIM5 $\alpha$ rh mutants with single amino acid substitutions, Rh(H29P)Ag, Rh(K45E)Ag, Rh(G52E)Ag and Rh(M150L)Cy, showed potent late restriction activities, comparable to that of wild-type



**Figure 5. Antiviral activities of TRIM5 $\alpha$ rh proteins with single-amino acid substitutions in the RBCC region against HIV-1.** (A) Schematic representation of TRIM5 $\alpha$ rh constructs carrying single amino acid residue substitutions. Single residue substitutions corresponding to TRIM5 $\alpha$ ag sequences were introduced into C-terminally HA-tagged TRIM5 $\alpha$ rh (Rh) to generate Rh(H29P)Ag, Rh(K45E)Ag and Rh(G52E)Ag. 150L TRIM5 $\alpha$ cy sequence was introduced into TRIM5 $\alpha$ rh to generate Rh(M150L)Cy. (B) Upper panel: viral titers of HIV-1 NL4-3 generated in the presence of TRIM5 $\alpha$  proteins carrying single amino acid substitutions. Viral production in the presence of TRIM5 $\alpha$ rh (RhT5 $\alpha$ ), TRIM5 $\alpha$ ag (AgT5 $\alpha$ ) and TRIM5 $\alpha$ cy (CyT5 $\alpha$ ) are shown for comparison. Viral titers were determined in GHOST(3)R3X4R5 indicator cells and reported as infectious units per ml (IU/ml). Error bars indicate one standard deviation. Lower panel: western blot analysis of single-residue-substituted TRIM5 $\alpha$ rh proteins following transfection into 293T cells. Arrow indicates approximate full-length TRIM5 $\alpha$  size. doi:10.1371/journal.pone.0016121.g005

TRIM5 $\alpha$ rh (Fig. 5B, upper panel). These data indicate that variations in the single amino acid residues alone (29P, 45E, 52E or 150L) cannot explain the modest late restriction activities of TRIM5 $\alpha$ cy and TRIM5 $\alpha$ ag.

### C-terminal sequences of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy negatively regulates the potency of late restriction activity

To test whether TRIM5 $\alpha$ ag or TRIM5 $\alpha$ cy C-terminal sequences can impair the late restriction activity against HIV-1, we generated chimeric TRIM5 $\alpha$  constructs as depicted in Figure 6A. Similar levels of TRIM5 $\alpha$  expression were confirmed via immunoblot (Fig. 6B). Although the restriction activity of the TRIM5 $\alpha$ rh protein with N-terminal TRIM5 $\alpha$ cy sequence (RhM150LCy) did not notably differ from wild-type TRIM5 $\alpha$ rh restriction activities (Fig. 4B), the restriction against HIV-1 was relieved when C-terminal TRIM5 $\alpha$ cy sequences were fused with the N-terminal region of TRIM5 $\alpha$ rh (Fig. 6C). TRIM5 $\alpha$ ag C-terminal sequences in TRIM5 $\alpha$ rh (R/A) impaired the late restriction against HIV-1 by 3-fold when compared to wild-type TRIM5 $\alpha$ rh (Fig. 6C). These data suggest that C-terminal sequences of TRIM5 $\alpha$  proteins can negatively regulate the late restriction activities, offering partial explanation to the modest late restriction activities of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy against HIV-1.

### Encapsidation and late restriction activities of human TRIM proteins

The 5 $\alpha$  isoform of human TRIM protein had very little effect on HIV-1 production; however TRIM5 $\alpha$ hu is one of over 80 members of the RBCC family of TRIM proteins. We therefore sought to examine whether other human TRIM family proteins may (1) be incorporated into HIV-1 VLPs and (2) restrict HIV-1 production. From the vast numbers of TRIM family proteins, we examined TRIM6, TRIM34 and TRIM22 since they are located in a paralogous cluster which includes TRIM5 $\alpha$  [44]. We also examined the influence of TRIM1, TRIM18 and TRIM21 expression on HIV-1 VLP incorporation. TRIM1 has antiviral activity against N-tropic murine leukemia virus infection [6] and TRIM18 is its paralogue. TRIM21 can modulate TRIM5 $\alpha$  ubiquitination [45] as well as the interferon-mediated antiviral response [46].

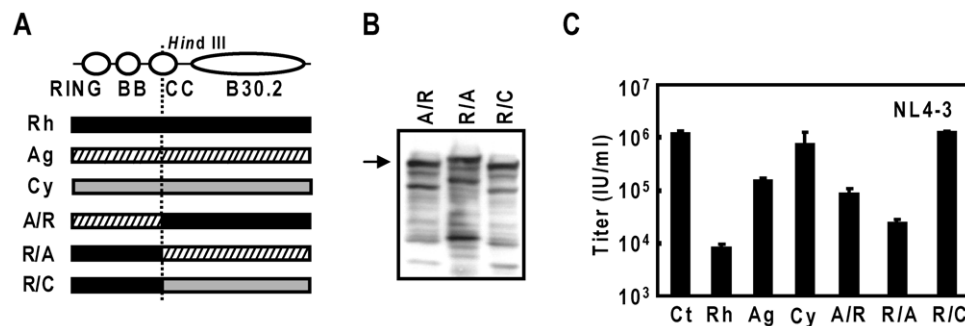
To obtain sufficient amounts of VLPs in the presence of TRIM proteins, we used pH-GP, which generates high levels of HIV-1

GagPol and abrogates the late restriction activities of TRIM5 $\alpha$ rh [16]. 293T cells were co-transfected with 1.0  $\mu$ g of TRIM-expressing plasmids and 0.25  $\mu$ g of codon-optimized HIV-1 GagPol-expression plasmid, pH-GP. Cell lysates and HIV-1 VLPs were harvested as described in materials and methods. Immunoblot analysis was performed to detect HA-tagged TRIM proteins as well as HIV-1 Gag proteins. All TRIM proteins were detected in the producer cells (Fig. 7A). Efficient VLP incorporation was evident with TRIM5 $\alpha$ rh, human TRIM21 and TRIM22 (Fig. 7A). Previously, we have demonstrated that HIV-1 Gag maturation delays in the presence of TRIM5 $\alpha$ , resulting in accumulation of premature Gag proteins in producer cells and VLPs [16,17]. This was also true with human TRIM21 and TRIM22, where VLPs made in the presence of TRIM21 and TRIM22 showed notable accumulation of premature Gag proteins, particularly in VLPs (Fig. 7A). No HA signal was detected in the VLPs made in the presence of TRIM1, 6 and 34 (Fig. 7A). Intracellular expression levels of the TRIM proteins were not necessarily correlated with their incorporation into VLPs, indicating that the incorporation of TRIM proteins is not due to non-specific packaging of RBCC proteins into VLPs. These observations suggest the direct or indirect interaction of TRIM21 and TRIM22 with HIV-1 Gag proteins in producer cells. No effects on HIV-1 Gag levels in the presence of TRIM5 $\alpha$  or TRIM22 are likely due to the saturation of late restriction activities by over-expression of codon optimized HIV-1 Gag.

Next, we examined the correlation between VLP incorporation status of human TRIM proteins and late restriction activities. To assess the antiviral activities of these TRIM proteins, 1.0  $\mu$ g of TRIM-expressing plasmids were co-transfected with 0.1  $\mu$ g of pNL4-3, p89.6, pROD10 or pSIVmac1A11 into 293T cells, and viral titers were determined in GHOST indicator cells. Of the human TRIM proteins that were assessed, only TRIM22 showed a slight reduction in NL4-3 and 89.6 titers, while the other TRIM proteins had no effect (Fig. 7B). None of the human TRIM proteins showed notable effects on HIV-2 or SIV<sub>MAC1A11</sub> production (Fig. 7B).

### Discussion

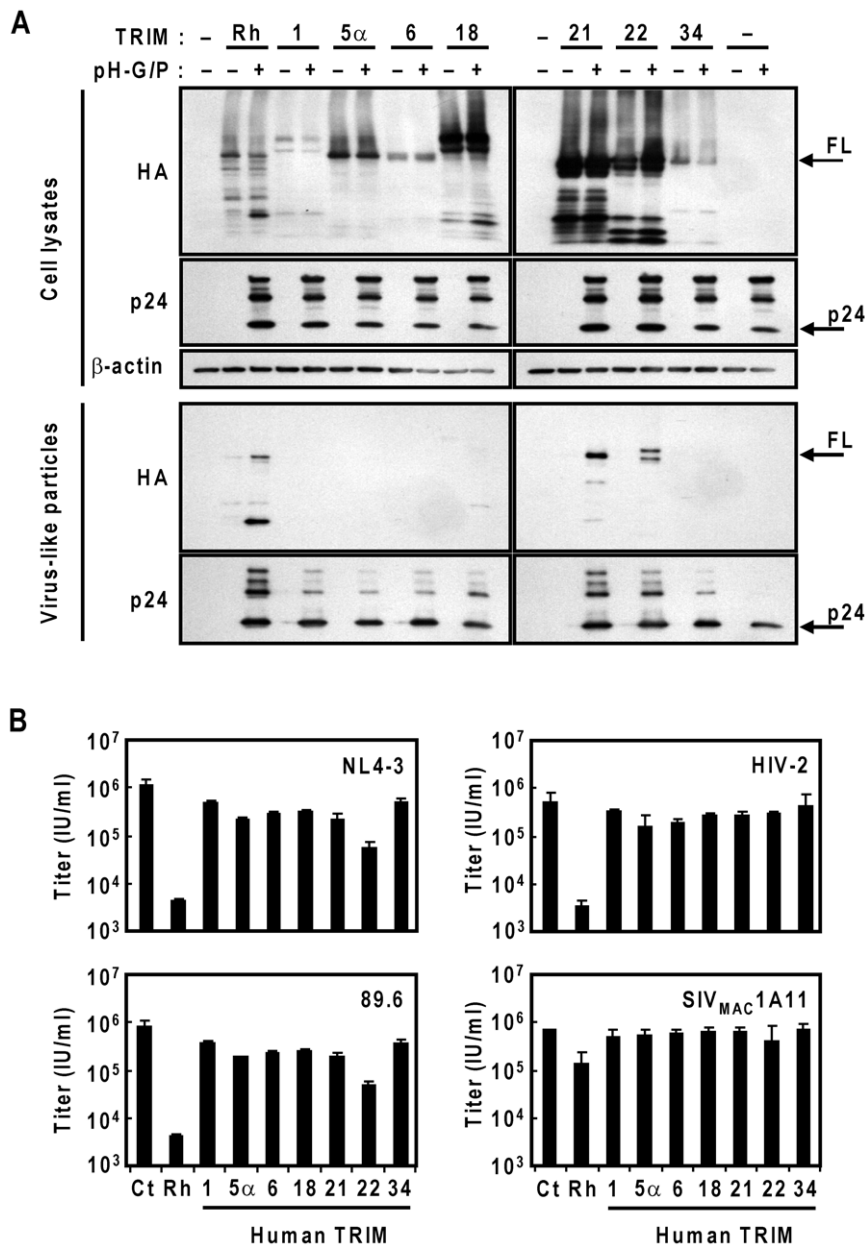
In this report, we examined the late restriction activities and VLP encapsidation efficiencies of simian TRIM5 $\alpha$  orthologues and related human TRIM proteins, and their late restriction activities against a panel of lentiviruses. Our results revealed the



**Figure 6. The effects of African green and cynomolgus monkey TRIM5 $\alpha$  C-terminal sequences on TRIM5 $\alpha$ rh-mediated HIV-1 late restriction activities.** (A) Schematic representation of the chimeric TRIM5 $\alpha$  constructs between rhesus monkey (Rh, filled), African green monkey (Ag, hatched) and cynomolgus (Cy, dotted) TRIM5 $\alpha$  proteins. (B) Western blot analysis of chimeric TRIM5 $\alpha$  proteins A/R, R/A and R/C following transfection into 293T cells. Arrow indicates approximate full-length TRIM5 $\alpha$  size. (C) Late-restriction activities of chimeric TRIM5 $\alpha$  proteins upon co-transfection with pNL4-3 into 293T cells. Viral titers were determined in GHOST(3)R3X4R5 indicator cells and reported as infectious units per ml (IU/ml). Error bars indicate one standard deviation.

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**Figure 7. HIV-1 VLP encapsidation and late restriction activities of human TRIM proteins.** (A) Immunoblot analyses of C-terminally HA-tagged human TRIM proteins (1, 5 $\alpha$ , 6, 18, 21, 22 and 34) with or without codon-optimized HIV-1 Gag-Pol expression plasmid (pH-G/P) are shown. TRIM5 $\alpha$ rh was used as a positive control for incorporation. Top three panels show proteins harvested from producer cell lysates, immunoblotted against TRIM (anti-HA), HIV-1 (anti-p24; 183-5C-H12, 1:1000 and AG3.0, 1:500) or actin (anti- $\beta$ -actin), while the bottom two panels show immunoblots of VLPs harvested from respective producer cells against TRIM (anti-HA) or HIV-1 (anti-p24; 183-5C-H12, 1:1000 and AG3.0, 1:500). FL designates approximate full-length TRIM size, and p24 designates HIV-1 p24 capsid size. (B) 293T cells were co-transfected with 1.0  $\mu$ g of TRIM-expressing plasmid: TRIM5 $\alpha$ rh, TRIM1, TRIM5 $\alpha$ hu, TRIM6, TRIM18, TRIM21, TRIM22 and TRIM34, with 0.1  $\mu$ g of lentivirus proviral plasmid: pNL4-3, p89.6, pROD10 or pSIV<sub>MAC</sub>1A11. Viral titers were determined in GHOST(3)R3X4R5 indicator cells and reported as infectious units per ml (IU/ml). Error bars indicate one standard deviation. doi:10.1371/journal.pone.0016121.g007

antiviral spectra and varying restriction activities of these TRIM proteins against lentiviral production. The relative expression levels or subcellular localizations of TRIM5 $\alpha$  could not explain the encapsidation efficiency or the potency of late restriction activity. The RBCC domains of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy were essential for the late restriction, while the C-terminal regions of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy negatively regulated the restriction activities. Similar antiviral spectra between simian TRIM5 $\alpha$  orthologues may suggest a conserved restriction mechanism among these

proteins. Of the examined human TRIM5 paralogues, TRIM21 and TRIM22 were efficiently incorporated into HIV-1 VLP, while only TRIM22 showed marginal late restriction activity.

Among primate lentiviruses, HIV-2 and SIV<sub>MAC</sub> are closely related, as sooty mangabey SIV has transferred to humans and rhesus monkeys as HIV-2 and SIV<sub>MAC</sub> [47]. However, TRIM5 $\alpha$ rh blocks the infection of HIV-2, but not SIV<sub>MAC</sub>, mainly due to the difference in the structure in the capsid protein that recruits cyclophilin A into HIV-1 virions [48]. Examination of HIV-2 and



SIV<sub>MAC</sub> production in the presence of simian TRIM5 $\alpha$  proteins demonstrated the most remarkable differences between the two closely related lentiviruses. Although SIV<sub>MAC</sub> was resistant to all four TRIM5 $\alpha$  orthologues in the late-restriction, up to a 40-fold reduction in HIV-2 production was observed in the presence of TRIM5 $\alpha$ rh. It is possible that the variations in the CA loop regions of SIV<sub>MAC</sub> and HIV-2, which correspond to the HIV-1 CA cyclophilin-binding loop, may in part contribute to the differential late restriction phenotypes. However, the CA loop region cannot solely explain the resistance of SIV<sub>MAC</sub> to the TRIM5 $\alpha$ rh-mediated late restriction, because an HIV-1 cyclophilin-binding loop mutant with corresponding SIV<sub>MAC</sub> sequence was still sensitive to the late restriction [36]. Intriguingly, a recent study has shown that a single amino acid change in the HIV-1 CA cyclophilin-binding loop allowed the virus to escape from TRIM5 $\alpha$ rh-mediated post-entry restriction, suggesting the importance of the CA loop structure for restriction factor recognition [49]. Our previous study has also suggested that SIV<sub>MAC</sub> resists TRIM5 $\alpha$ rh-mediated late restriction by counteracting or saturating the TRIM5 $\alpha$  late restriction machinery, rather than escaping TRIM5 $\alpha$ rh recognition altogether [36]. We therefore speculate that the sensitivity of HIV-2 to TRIM5 $\alpha$ rh-mediated late restriction is partly due to the relatively inefficient HIV-2 production from pROD10, where production of progeny virions may not be sufficient to overcome the TRIM5 $\alpha$ rh-mediated late restriction. The different replication kinetics of CMO2.41 and CMO2.5 in PBMCs can be attributed to the *gag-pol* region of CMO2.5, which is derived from a separate primary type-O isolate [50]. The differences in viral gag-pol sequences offers partial explanation as to why CMO2.41 and CMO2.5 responded differently to the late restriction activities of TRIM5 $\alpha$ ag (Fig. 2B).

The incorporation of TRIM5 $\alpha$ rh into the VLPs made with HIV-1 Gag suggests a specific interaction between TRIM5 $\alpha$ rh and HIV-1 Gag polyproteins [16]. Determinants for this interaction lie in the RING and coiled-coil domains of TRIM5 $\alpha$ rh, and the B30.2(PRYSPRY) motif is not required for the interaction or the late restriction activity of TRIM5 $\alpha$ rh [19]. TRIM5 $\alpha$ rh mutants with the M133T and/or T146A amino acid substitutions in TRIM5 $\alpha$ rh coiled-coil domain showed efficient encapsidation but impaired late restriction activity [19]. Although the RBCC sequences, including the M133 and T146, of three simian TRIM5 $\alpha$  proteins are highly conserved among the three simian TRIM5 $\alpha$  proteins, we found that TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy showed weaker late restriction activities than TRIM5 $\alpha$ rh. Similar to our previous study, replacement of the N-terminal RBCC sequences of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy with the corresponding TRIM5 $\alpha$ hu sequences resulted in the loss of prominent late restriction effects of these two simian TRIM5 $\alpha$  proteins, indicating the essential roles of the RBCC domains in their late restriction activities (Fig. 4B). Unexpectedly, C-terminal

TRIM5 $\alpha$ hu sequences mildly strengthened the late restriction activities of TRIM5 $\alpha$ ag and TRIM5 $\alpha$ cy; while impaired late restriction activities were observed when the N-terminal region of TRIM5 $\alpha$ rh was fused with C-terminal TRIM5 $\alpha$ ag or TRIM5 $\alpha$ cy sequences (Fig. 6C). These results indicate that TRIM5 $\alpha$  RBCC sequences are required for the late restriction, and C-terminal amino acid sequences can modulate the potency of the late restriction against HIV-1, adding further complexity to the mechanisms of TRIM5 $\alpha$ -mediated late restriction. Further studies will determine the extent of inter-domain communication within the TRIM5 $\alpha$  protein and the amino acid residues which are responsible for the negative regulation.

Proteins that have a conserved RING, b-box 1 and/or b-box 2 and coiled-coil domains are included in the superfamily of TRIM genes, and many TRIM proteins have been implicated to be interferon-responsive and a contributing factor in the defense against infectious agents [42,51]. Located in the same genetic locus on chromosome 11p15, TRIM5, TRIM22, TRIM6 and TRIM34 were classified in the same clade [22]. Of the human TRIM proteins assessed in this study, TRIM21 and TRIM22 were efficiently incorporated into VLPs. A faint TRIM18 signal was also detected in the purified VLPs, suggesting weak interaction between human TRIM18 and HIV-1 Gag (Fig. 7A). In contrast to the efficient encapsidation of these proteins, only TRIM22 showed a modest late restriction activity against HIV-1 (Fig. 7B and C). These data suggest that encapsidation efficiency alone could not fully explain the differences in the late restriction activities of TRIM proteins. It is likely that additional determinant(s) controls the potency of the late restriction activity following initial binding of a TRIM protein with HIV-1 Gag.

In summary, we demonstrated the examples of TRIM protein-mediated late restriction activities and their potential to interact with viral proteins. Our data provide further insights into the complex host-pathogen interplay in TRIM protein-mediated late restriction.

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## Author Contributions

Conceived and designed the experiments: SO RS YI. Performed the experiments: SO RS. Analyzed the data: SO RS YI. Contributed reagents/materials/analysis tools: TS TT HT. Wrote the paper: SO YI.

## References

- Bannert N, Kurth R (2004) Retroelements and the human genome: new perspectives on an old relation. *Proc Natl Acad Sci U S A* 101 Suppl 2: 14572–14579.
- Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, et al. (2001) The sequence of the human genome. *Science* 291: 1304–1351.
- Hatzioannou T, Perez-Caballero D, Yang A, Cowan S, Bieniasz PD (2004) Retrovirus resistance factors Ref1 and Lvl1 are species-specific variants of TRIM5 $\alpha$ . *Proc Natl Acad Sci U S A* 101: 10774–10779.
- Perron MJ, Stremlau M, Song B, Ulm W, Mulligan RC, et al. (2004) TRIM5 $\alpha$  mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proc Natl Acad Sci U S A* 101: 11827–11832.
- Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, et al. (2004) The cytoplasmic body component TRIM5 $\alpha$  restricts HIV-1 infection in Old World monkeys. *Nature* 427: 848–853.
- Yap MW, Nisole S, Lynch C, Stoye JP (2004) Trim5 $\alpha$  protein restricts both HIV-1 and murine leukemia virus. *Proc Natl Acad Sci U S A* 101: 10786–10791.
- Perez-Caballero D, Hatzioannou T, Yang A, Cowan S, Bieniasz PD (2005) Human tripartite motif 5 $\alpha$  domains responsible for retrovirus restriction activity and specificity. *J Virol* 79: 8969–8978.
- Song B, Javanbakht H, Perron M, Park DH, Stremlau M, et al. (2005) Retrovirus restriction by TRIM5 $\alpha$  variants from Old World and New World primates. *J Virol* 79: 3930–3937.
- Stremlau M, Perron M, Lee M, Li Y, Song B, et al. (2006) Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5 $\alpha$  restriction factor. *Proc Natl Acad Sci U S A* 103: 5514–5519.
- Yap MW, Nisole S, Stoye JP (2005) A Single Amino Acid Change in the SPRY Domain of Human Trim5 $\alpha$  Leads to HIV-1 Restriction. *Current Biology* 15: 73–78.

11. Keckesova Z, Ylinen LM, Towers GJ (2004) The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proc Natl Acad Sci U S A* 101: 10780–10785.
12. Diaz-Griffero F, Kar A, Perron M, Xiang SH, Javanbakht H, et al. (2007) Modulation of retroviral restriction and proteasome inhibitor-resistant turnover by changes in the TRIM5alpha B-box 2 domain. *J Virol* 81: 10362–10378.
13. Nakayama EE, Miyoshi H, Nagai Y, Shioda T (2005) A specific region of 37 amino acid residues in the SPRY (B30.2) domain of African green monkey TRIM5alpha determines species-specific restriction of simian immunodeficiency virus SIVmac infection. *J Virol* 79: 8870–8877.
14. Maillard PV, Ecco G, Ortiz M, Trono D (2010) The specificity of TRIM5{alpha}-mediated restriction is influenced by its coiled-coil domain. *J Virol* 84: 5970–5801.
15. Sawyer SL, Wu LI, Emerman M, Malik HS (2005) Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. *Proc Natl Acad Sci U S A* 102: 2832–2837.
16. Sakuma R, Noser JA, Ohmine S, Ikeda Y (2007) Rhesus monkey TRIM5alpha restricts HIV-1 production through rapid degradation of viral Gag polyproteins. *Nat Med* 13: 631–635.
17. Sakuma R, Ohmine S, Mael AA, Noser JA, Ikeda Y (2008) Reply to: No effect of endogenous TRIM5alpha on HIV-1 production. *Nat Med* 14: 236–238.
18. Sakuma R, Noser JA, Ohmine S, Ikeda Y (2007) Inhibition of HIV-1 replication by simian restriction factors, TRIM5alpha and APOBEC3G. *Gene Ther* 14: 185–189.
19. Sakuma R, Ohmine S, Ikeda Y (2009) Determinants for the rhesus monkey TRIM5{alpha}-mediated block of the late phase of HIV-1 replication. *J Biol Chem*.
20. Asaoka K, Ikeda K, Hishinuma T, Horie-Inoue K, Takeda S, et al. (2005) A retrovirus restriction factor TRIM5a is transcriptionally regulated by interferons. *Biochem and Biophysical Research Communications* 338: 1950–1956.
21. Sakuma R, Mael AA, Ikeda Y (2007) Alpha interferon enhances TRIM5alpha-mediated antiviral activities in human and rhesus monkey cells. *J Virol* 81: 10201–10206.
22. Li X, Gold B, O'Huigin C, Diaz-Griffero F, Song B, et al. (2007) Unique features of TRIM5alpha among closely related human TRIM family members. *Virology* 360: 419–433.
23. Morner A, Bjorndal A, Albert J, Kewalramani VN, Littman DR, et al. (1999) Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. *J Virol* 73: 2343–2349.
24. Adachi A, Gendelman HE, Koenig S, Folks T, Willey R, et al. (1986) Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J Virol* 59: 284–291.
25. Collman R, Balliet JW, Gregory SA, Friedman H, Kolson DL, et al. (1992) An infectious molecular clone of an unusual macrophage-tropic and highly cytopathic strain of human immunodeficiency virus type 1. *J Virol* 66: 7517–7521.
26. Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, et al. (1996) A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* 85: 1149–1158.
27. Gao F, Robertson DL, Carruthers CD, Morrison SG, Jian B, et al. (1998) A comprehensive panel of near-full-length clones and reference sequences for non-subtype B isolates of human immunodeficiency virus type 1. *J Virol* 72: 5680–5698.
28. Jin MJ, Hui H, Robertson DL, Muller MC, Barre-Sinoussi F, et al. (1994) Mosaic genome structure of simian immunodeficiency virus from west African green monkeys. *Embo J* 13: 2935–2947.
29. Kim FM, Kolson DL, Balliet JW, Srinivasan A, Collman RG (1995) V3-independent determinants of macrophage tropism in a primary human immunodeficiency virus type 1 isolate. *J Virol* 69: 1755–1761.
30. Luciw PA, Shaw KE, Unger RE, Planclles V, Stout MW, et al. (1992) Genetic and biological comparisons of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIVmac). *AIDS Res Hum Retroviruses* 8: 395–402.
31. Soares MA, Robertson DL, Hui H, Allan JS, Shaw GM, et al. (1997) A full-length and replication-competent proviral clone of SIVAGM from tantalus monkeys. *Virology* 228: 394–399.
32. Bour S, Strebel K (1996) The human immunodeficiency virus (HIV) type 2 envelope protein is a functional complement to HIV type 1 Vpu that enhances particle release of heterologous retroviruses. *J Virol* 70: 8285–8300.
33. Zhang F, Hatzioannou T, Perez-Caballero D, Derse D, Bieniasz PD (2006) Antiretroviral potential of human tripartite motif-5 and related proteins. *Virology* 353: 396–409.
34. Chesebro B, Wehrly K, Nishio J, Perryman S (1992) Macrophage-tropic human immunodeficiency virus isolates from different patients exhibit unusual V3 envelope sequence homogeneity in comparison with T-cell-tropic isolates: definition of critical amino acids involved in cell tropism. *J Virol* 66: 6547–6554.
35. Simm M, Shahabuddin M, Chao W, Allan JS, Volsky DJ (1995) Aberrant Gag protein composition of a human immunodeficiency virus type 1 vif mutant produced in primary lymphocytes. *J Virol* 69: 4582–4586.
36. Zhang F, Perez-Caballero D, Hatzioannou T, Bieniasz PD (2008) No effect of endogenous TRIM5alpha on HIV-1 production. *Nat Med* 14: 235–236; author reply 236–238.
37. Charneau P, Borman AM, Quillent C, Guetard D, Chamaret S, et al. (1994) Isolation and envelope sequence of a highly divergent HIV-1 isolate: definition of a new HIV-1 group. *Virology* 205: 247–253.
38. Ikeda Y, Ylinen LM, Kahar-Bador M, Towers GJ (2004) Influence of gag on human immunodeficiency virus type 1 species-specific tropism. *J Virol* 78: 11816–11822.
39. Braaten D, Ansari H, Luban J (1997) The hydrophobic pocket of cyclophilin is the binding site for the human immunodeficiency virus type 1 Gag polyprotein. *J Virol* 71: 2107–2113.
40. Tebit DM, Zekeng L, Kaptue L, Gurtler L, Fackler OT, et al. (2004) Construction and characterization of an HIV-1 group O infectious molecular clone and analysis of vpr- and nef-negative derivatives. *Virology* 326: 329–339.
41. Jensen FC, Girardi AJ, Gilden RV, Koprowski H (1964) Infection of Human and Simian Tissue Cultures with Rous Sarcoma Virus. *Proc Natl Acad Sci U S A* 52: 53–59.
42. Reymond A, Meroni G, Fantozzi A, Merla G, Cairo S, et al. (2001) The tripartite motif family identifies cell compartments. *Embo J* 20: 2140–2151.
43. Li X, Sodroski J (2008) The TRIM5alpha B-box 2 domain promotes cooperative binding to the retroviral capsid by mediating higher-order self-association. *J Virol* 82: 11495–11502.
44. Li X, Song B, Xiang SH, Sodroski J (2007) Functional interplay between the B-box 2 and the B30.2(SPRY) domains of TRIM5alpha. *Virology* 366: 234–244.
45. Yamauchi K, Wada K, Tanji K, Tanaka M, Kamitani T (2008) Ubiquitination of E3 ubiquitin ligase TRIM5 alpha and its potential role. *Febs J* 275: 1540–1555.
46. Yang K, Shi HX, Liu XY, Shan YF, Wei B, et al. (2009) TRIM21 is essential to sustain IFN regulatory factor 3 activation during antiviral response. *J Immunol* 182: 3782–3792.
47. Hirsch VM, Olmsted RA, Murphey-Corb M, Purcell RH, Johnson PR (1989) An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339: 389–392.
48. Ylinen LM, Keckesova Z, Wilson SJ, Ranasinghe S, Towers GJ (2005) Differential restriction of human immunodeficiency virus type 2 and simian immunodeficiency virus SIVmac by TRIM5alpha alleles. *J Virol* 79: 11580–11587.
49. Pacheco B, Finzi A, Stremlau M, Sodroski J (2010) Adaptation of HIV-1 to cells expressing rhesus monkey TRIM5alpha. *Virology* 408: 204–212.
50. Dittmar MT, Zekeng L, Kaptue L, Eberle J, Krausslich HG, et al. (1999) Coreceptor requirements of primary HIV type 1 group O isolates from Cameroon. *AIDS Res Hum Retroviruses* 15: 707–712.
51. Rajsbaum R, Stoye JP, O'Garra A (2008) Type I interferon-dependent and -independent expression of tripartite motif proteins in immune cells. *Eur J Immunol* 38: 619–630.