[28] H. Kestler, T. Kodama, D. Ringler, M. Marthas, N. Pedersen, A. Lackner, D. Regier, P. Sehgal, M. Daniel, N. King, et al., Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus, Science 248 (1990) 1109-1112.

[29] R. Shibata, T. Miura, M. Hayami, H, Sakai, K. Ogawa, T. Kiyomasu, A. Ishimoto, A Adachi, Construction and Characterization of an Infectious DNA clone and of Mutants of Simian Immunodeficiency Virus Isolated from the African Green Monkey, J Virol 64 (1990) 307-312.

[30] K. Peden, M. Emerman, L. Montagnier, Changes in growth properties on passage in tissue culture of viruses derived from infectious molecular clones of HIV-1LAI, HIV-1MAL, and HIV-1ELI, Virology 185 (1991) 661-672.

[31] J. Katahira, T. Ishizaki, H. Sakai, A. Adachi, K. Yamamoto, H. Shida, Effects of translation initiation factor eIF-5A on the functioning of human T-cell leukemia virus type I Rex and human immunodeficiency virus Rev inhibited trans dominantly by a Rex mutant deficient in RNA binding, J Virol 69 (1995) 3125-3133.

[32] S. Tahara-Hanaoka, K. Sudo, H. Ema, H. Miyoshi, H. Nakauchi, Lentiviral vector-mediated transduction of murine CD34(-) hematopoietic stem cells, Exp Hematol 30 (2002) 11-17.

[33] M. Bock, K.N. Bishop, G. Towers, J.P. Stoye, Use of a transient assay for studying the genetic determinants of Fv1 restriction, J Virol 74 (2000) 7422-7430.

[34] K. Ikuta, C. Morita, S. Miyake, T. Ito, M. Okabayashi, K. Sano, M. Nakai, K. Hirai, S. Kato, Expression of human immunodeficiency virus type 1 (HIV-1) gag antigens on the surface of a cell line persistently infected with HIV-1 that highly expresses HIV-1 antigens, Virology 170 (1989) 408-417.

[35] X. Li, Y. Li, M. Stremlau, W. Yuan, B. Song, M. Perron, J. Sodroski, Functional replacement of the RING, B-box 2, and coiled-coil domains of tripartite motif 5alpha (TRIM5alpha) by heterologous TRIM domains, J Virol 80 (2006) 6198-6206.

[36] Z. Keckesova, L.M. Ylinen, G.J. Towers, The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities, Proc Natl Acad Sci U S A 101 (2004) 10780-10785.

[37] M.J. Perron, M. Stremlau, B. Song, W. Ulm, R.C. Mulligan, J. Sodroski, TRIM5alpha mediates the postentry block to N-tropic murine leukemia viruses in human cells, Proc Natl Acad Sci U S A 101 (2004) 11827-11832.

[38] M.W. Yap, S. Nisole, C. Lynch, J.P. Stoye, Trim5alpha protein restricts both HIV-1 and murine leukemia virus, Proc Natl Acad Sci U S A 101 (2004) 10786-10791.

[39] M.W. Yap, S. Nisole, J.P. Stoye, A single amino acid change in the SPRY domain of human Trim5alpha leads to HIV-1 restriction, Curr Biol 15 (2005) 73-78.

[40] Y. Li, X. Li, M. Stremlau, M. Lee, J. Sodroski, Removal of arginine 332 allows human TRIM5alpha to bind human immunodeficiency virus capsids and to restrict infection, J Virol 80 (2006) 6738-6744.

[41] F. Diaz-Griffero, M. Perron, K. McGee-Estrada, R. Hanna, P. V. Maillard, D. Trono, and J. Sodroski. A human TRIM5alpha B30.2/SPRY domain mutant gains the ability to restrict and prematurely uncoat B-tropic murine leukemia virus. Virology 378 (2008) 233-242.

[42] P.V. Maillard, S. Reynard, F. Serhan, P. Turelli, and D. Trono. Interfering residues narrow the spectrum of MLV restriction by human TRIM5alpha. PLoS Pathog 3 (2007) e200.

[43] E.C. Speelmon, D. Livingston-Rosanoff, S.S. Li, Q. Vu, J. Bui, D.E. Geraghty, L.P. Zhao, M.J. McElrath, Genetic association of the antiviral restriction factor

TRIM5alpha with human immunodeficiency virus type 1 infection, J Virol 80 (2006) 2463-2471.

[44] E.E. Nakayama, W. Carpentier, D. Costagliola, T. Shioda, A. Iwamoto, P. Debre, K. Yoshimura, B. Autran, S. Matsushita, I. Theodorou, Wild type and H43Y variant of human TRIM5alpha show similar anti-human immunodeficiency virus type 1 activity both in vivo and in vitro, Immunogenetics 59 (2007) 511-515.

[45] D.van Manen, M. A. Rits, C. Beugeling, K. van Dort, H. Schuitemaker, and N. A. Kootstra. The effect of Trim5 polymorphisms on the clinical course of HIV-1 infection. PLoS Pathog 4 (2008) e18.

[46] J.N. Torimiro, H. Javanbakht, F. Diaz-Griffero, J. Kim, J.K. Carr, M. Carrington, J. Sawitzke, D.S. Burke, N.D. Wolfe, M. Dean, J. Sodroski, A rare null allele potentially encoding a dominant-negative TRIM5alpha protein in Baka pygmies, Virology 391 (2009) 140-147.

[47] L. Carthagena, M.C. Parise, M. Ringeard, M.K. Chelbi-Alix, U. Hazan, S. Nisole, Implication of TRIM alpha and TRIMCyp in interferon-induced anti-retroviral restriction activities, Retrovirology 5 (2008) 59.

Figure legend

Fig 1. Effect of overexpressed $TRIM5_{hu}$ on HIV-1 production. (A) A schematic presentation of $TRIM5_{hu}$ protein with the domains labeled and domain boundaries numbered according to the amino acid residue. (B) 293T cells (2×10^5) were transfected with 0.01μg of pNLΔpolEGFP together with various amounts of $TRIM5\alpha_{hu}$-HA (hT5αWT or R437C) and $TRIM5\alpha_{rh}$-HA expression plasmid (rhT5α). Note that we used half amount of pRhT5α plasmid for transfection since we found half amount pRhT5α expressed an equal amount of TRIM5α-HA protein compared to pHuT5αWT and pHuT5αR437C. pCDM-β-gal (0.01μg) was also transfected as a control of transfection efficiency. The amount of released p24 in culture supernatant and β-gal activity in the cell lysate from pcDNA3.1-transfected cells was 38ng/ml and 8×10^{-1} unit, respectively. The ratio of p24 to β-gal activity was set as 1. (C) Lysates from 293T cells expressing the HA-tagged TRIM5 proteins were subjected to SDS-PAGE, and the expression of HIV-1 Gag proteins and TRIM5α was detected by immunoblotting. The order of the samples applied is the same as B. The results of a typical experiment are shown. Similar results were obtained in four independent experiments. (D) 293T cells were transfected with pYK-JRCSF, pNL4-3, p89.6 , pSIVmac239 or pSA212 together with various amounts of TRIM5α expression plasmids as in (B). After 2 days, the amount of released HIV-1 p24 in culture supernatant was measured by ELISA. The progeny viruses produced were infected to TZM-bl cells, and luciferase activity induced in the TZM-bl cells was evaluated to titrate the infectious viruses. Both luciferase activity and p24 amount were divided by β-gal activity in the 293T cell lysates to calculate relative viral titer and relative p24 amounts. The p24 concentration in the culture medium of pYK-JRCSF, pNL4-3, or p89.6-transfected cells that did not receive Trim5α expression plasmid was 193.2ng/ml, 102.7ng/ml or 10.16ng/ml, respectively. The luciferase activity in the TZM-bl cells infected

with the corresponding progeny viruses was 8.12×10^5, 3.56×10^5 or 2.03×10^4 relative light unit (RLU), respectively. The progeny viruses produced from 293T cells transfected with pSIVmac239 and pSA212 induced 9.62×10^3 RLU, and 1.3×10^3 RLU, respectively. The data represent a typical result of 2 independent experiments.

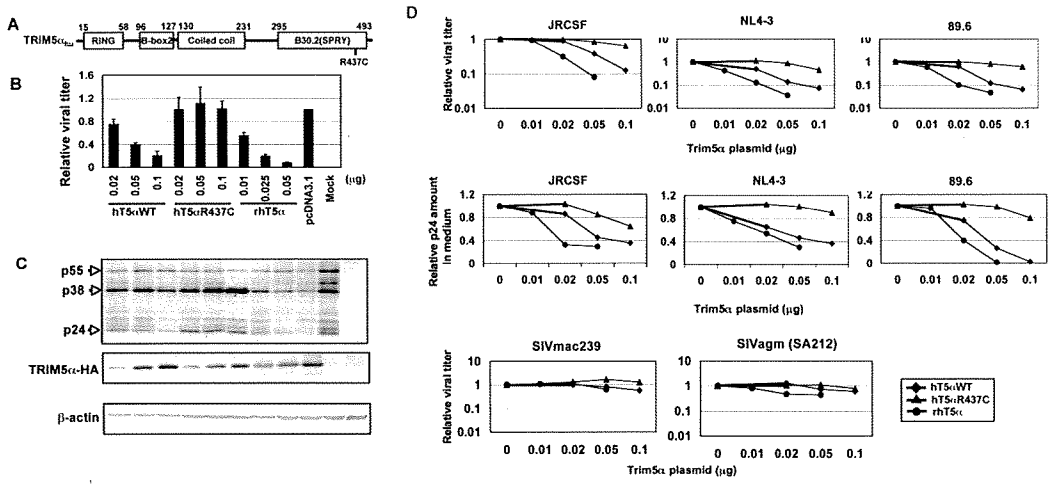
Fig 2. Incorporation of TRIM5α into HIV-1 virion. Culture supernatants of the 293T cells co-transfected with pNLΔpolEGFP and various TRIM5α plasmids were harvested and passed through 0.45μm filter followed by ultracentrifugation through 20% sucrose layer. The VLP fractions prepared from 1ml out of total 2ml culture medium were applied to immunoblotting to detect the HIV-1 Gag proteins and incorporated TRIM5α. (A) pNLΔpolEGFP (0.01μg) was transfected together with increasing amounts of $TRIM5\alpha_{hu}$-HA (hT5αWT or R437C) and $TRIM5\alpha_{rh}$-HA expression plasmid (rhT5α) as indicated in Fig.1B. (B) pNLΔpolEGFP (0.01μg) was transfected along with 0.1μg of $TRIM5\alpha_{hu}$-HA (hT5αWT or R437C) or 0.05μg $TRIM5\alpha_{rh}$-HA expression plasmid (rhT5αWT or R441C).

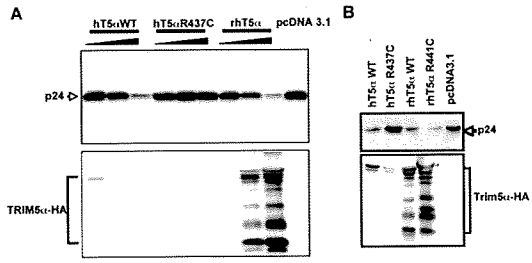
Fig 3. Effect of knockdown of TRIM5α in human cells on HIV-1 production. (A) HT1080 or 293T cells were co-transfected with pNLΔpolEGFP and control siRNA or siRNA against $Trim5\alpha_{hu}$. (B) Jurkat E6-1 cells were electroporated with the plasmid and siRNAs described above by nucleofection. pCDM-β-gal was also included in both A and B to monitor the electroporation efficiency. After 2 days, p24 levels in the supernatants and β-gal activity in the cell lysates were measured. The relative p24 production was calculated by dividing p24 amount by β-gal activity (right panel of A and B). The p24 levels in the culture media are indicated on the top of right panels of A and B. The level of TRIM5α expression was examined by usual and quantitative RT-PCR. Cells that were not subjected to any treatment (Nt) were used as blank controls. The pictures of RT-PCR in the left panels represent a typical

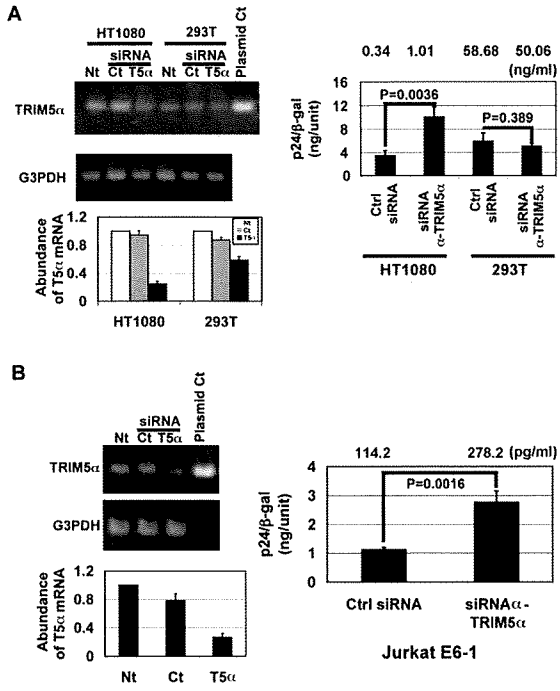
one of 3 independent experiments. The results of quantitative PCR represent the mean± S.D. of triplicate samples.

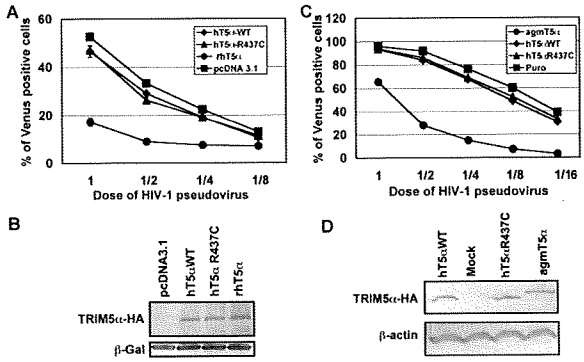
Fig 4. Effect of various TRIM5αs on HIV-1 entry. 293T cells were transfected with TRIM5α_{hu}-HA (hT5αWT or R437C) or TRIM5α_{rh}-HA (rhT5α) expression plasmid, and then infected with VSV-G pseudotyped HIV-1-Venus (A). HeLa cells that had been transduced with various TRIM5α encoding retrovectors were infected with VSV-G pseudotyped HIV-1-Venus (C). Forty-eight hours after infection, the cells were harvested and the Venus-positive cells were counted by FACS. (A and C) shows a typical result of three independent experiments. (B and D) The expression of TRIM5α was examined by immunoblot assay.

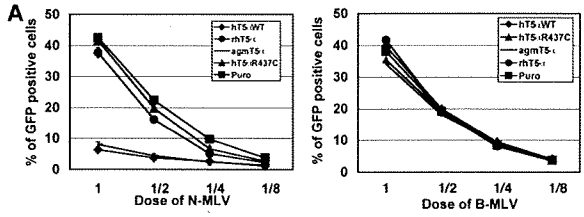
Fig 5. Effect of various TRIM5αs on N-MLV infection. The 293T cells transduced with various TRIM5α encoding retrovectors were infected with VSV-G-pseudotyped GFP encoding N and B tropic MLVs. Forty-eight hours after infection, the cells were harvested and GFP-positive cells were counted by FACS. (A) The left panel includes the means ± S.D, which was calculated based on three independent experiments. The right panel represents a typical result of 2 independent experiments. (B) The expression of TRIM5αs was examined by immunoblot assay.

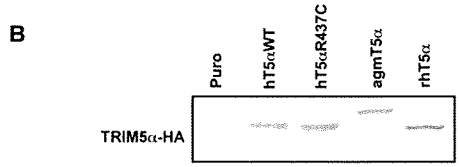



A
hT5αWT
hT5αR437C
rhT5α
pcDNA 3.1
p24
TRIM5α-HA
B
hT5α WT
hT5α R437C
rhT5α WT
rhT5α R441C
pcDNA3.1
p24
Trim5α-HA


A
HT1080
293T
siRNA
Nt Ct T5α Nt Ct T5α
Plasmid Ct
TRIM5α
G3PDH
Abundance of T5α mRNA
1.2
0.8
0.4
0
HT1080
293T
0.34 1.01 58.68 50.06
(ng/ml)
p24/β-gal (ng/unit)
16
12
8
4
0
P=0.0036
P=0.389
Ctrl siRNA
siRNA α-TRIM5α
Ctrl siRNA
siRNA α-TRIM5α
HT1080
293T
B
siRNA
Nt Ct T5α
Plasmid Ct
TRIM5α
G3PDH
Abundance of T5α mRNA
1.2
0.8
0.4
0
Nt
Ct
T5α
114.2
278.2 (pg/ml)
p24/β-gal (ng/unit)
4
3
2
1
0
P=0.0016
Ctrl siRNA
siRNAα-TRIM5α
Jurkat E6-1


A
% of Venus positive cells
60
40
20
0
hT5αWT
hT5αR437C
rhT5α
pcDNA 3.1
1
1/2
1/4
1/8
Dose of HIV-1 pseudovirus
C
% of Venus positive cells
120
100
80
60
40
20
0
agmT5α
hT5αWT
hT5αR437C
Puro
1
1/2
1/4
1/8
1/16
Dose of HIV-1 pseudovirus
B
pcDNA3.1
hT5αWT
hT5α R437C
rhT5α
TRIM5α-HA
β-Gal
D
hT5αWT
Mock
hT5αR437C
agmT5α
TRIM5α-HA
β-actin


A
% of GFP positive cells
50
40
30
20
10
0
1
1/2
1/4
1/8
Dose of N-MLV
hT5αWT
rhT5α
agmT5α
hT5αR437C
Puro
% of GFP positive cells
Dose of B-MLV
hT5αWT
hT5αR437C
agmT5α
rhT5α
Puro


B
Puro
hT5αWT
hT5αR437C
agmT5α
rhT5α
TRIM5α-HA