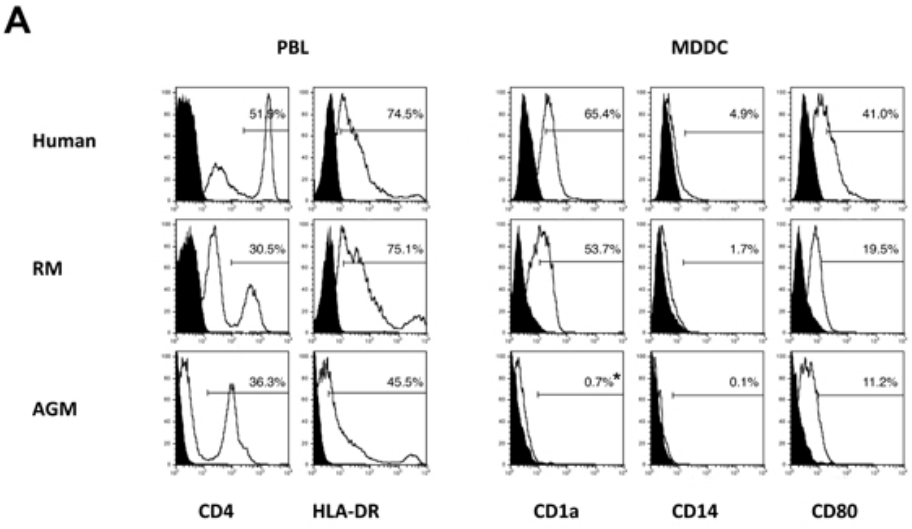
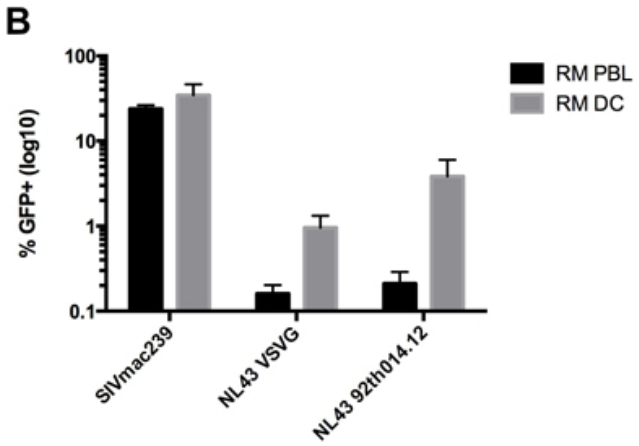
Cell Reports

Supplemental Information

Endogenous TRIM5α Function Is Regulated by SUMOylation and Nuclear Sequestration for Efficient Innate Sensing in Dendritic Cells

Débora M. Portilho, Juliette Fernandez, Mathieu Ringeard, Anthony K. Machado, Aude Boulay, Martha Mayer, Michaela Müller-Trutwin, Anne-Sophie Beignon, Frank Kirchhoff, Sébastien Nisole, and Nathalie J. Arhel


A
PBL
MDDC
Human
RM
AGM
51.9%
74.5%
65.4%
4.9%
41.0%
30.5%
75.1%
53.7%
1.7%
19.5%
36.3%
45.5%
0.7%*
0.1%
11.2%
CD4
HLA-DR
CD1a
CD14
CD80


B
% GFP+ (log10)
100
10
1
0.1
RM PBL
RM DC
SIVmac239
NL43 VSVG
NL43 92th014.12

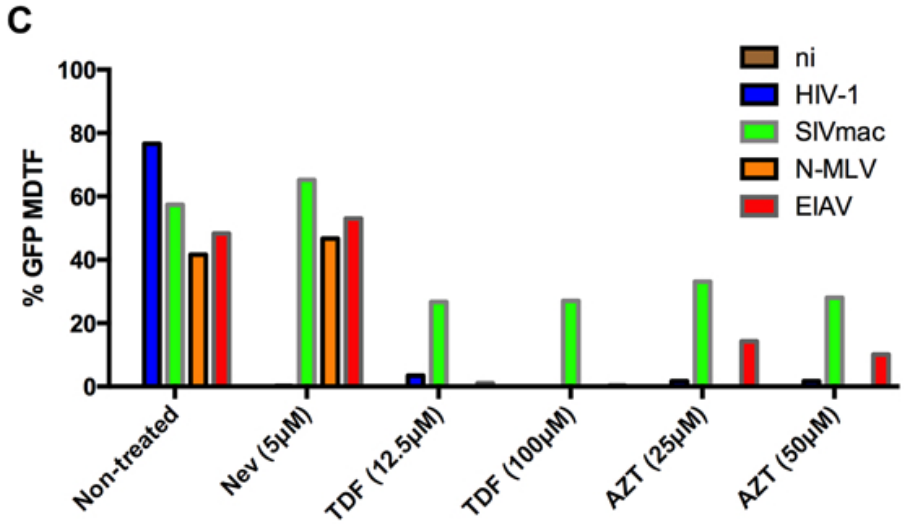
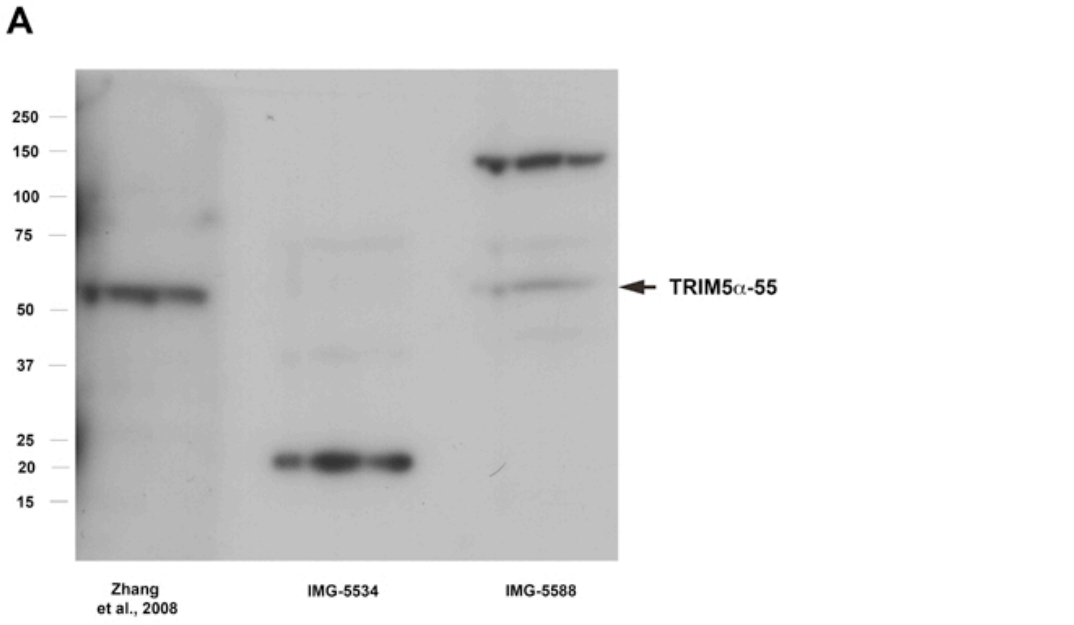
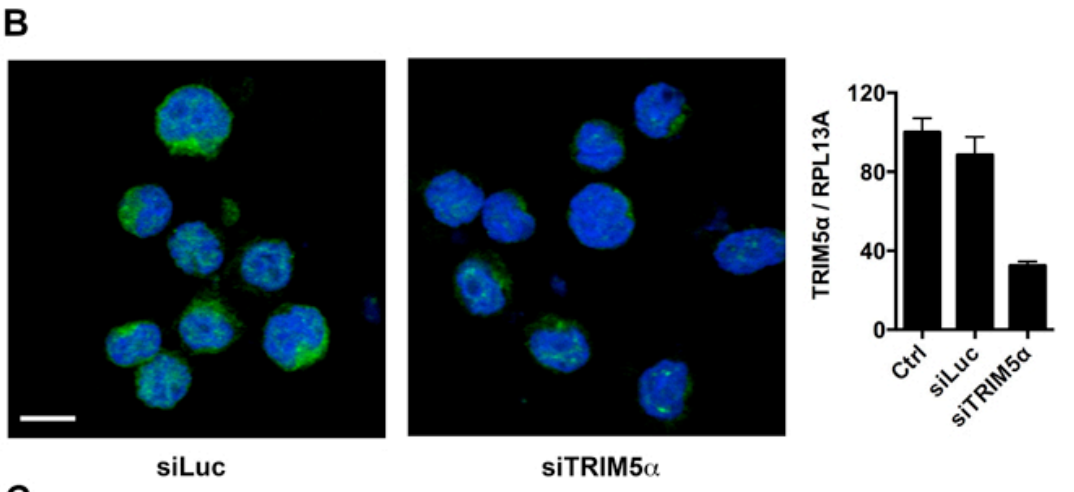

C
% GFP MDTF
100
80
60
40
20
0
ni
HIV-1
SIVmac
N-MLV
EIAV
Non-treated
Nev (5μM)
TDF (12.5μM)
TDF (100μM)
AZT (25μM)
AZT (50μM)

Fig. S1


A
250
150
100
75
50
37
25
20
15
TRIM5α-55
Zhang et al., 2008
IMG-5534
IMG-5588


B
siLuc
siTRIM5α
TRIM5α / RPL13A
120
80
40
0
Ctrl
siLuc
siTRIM5α

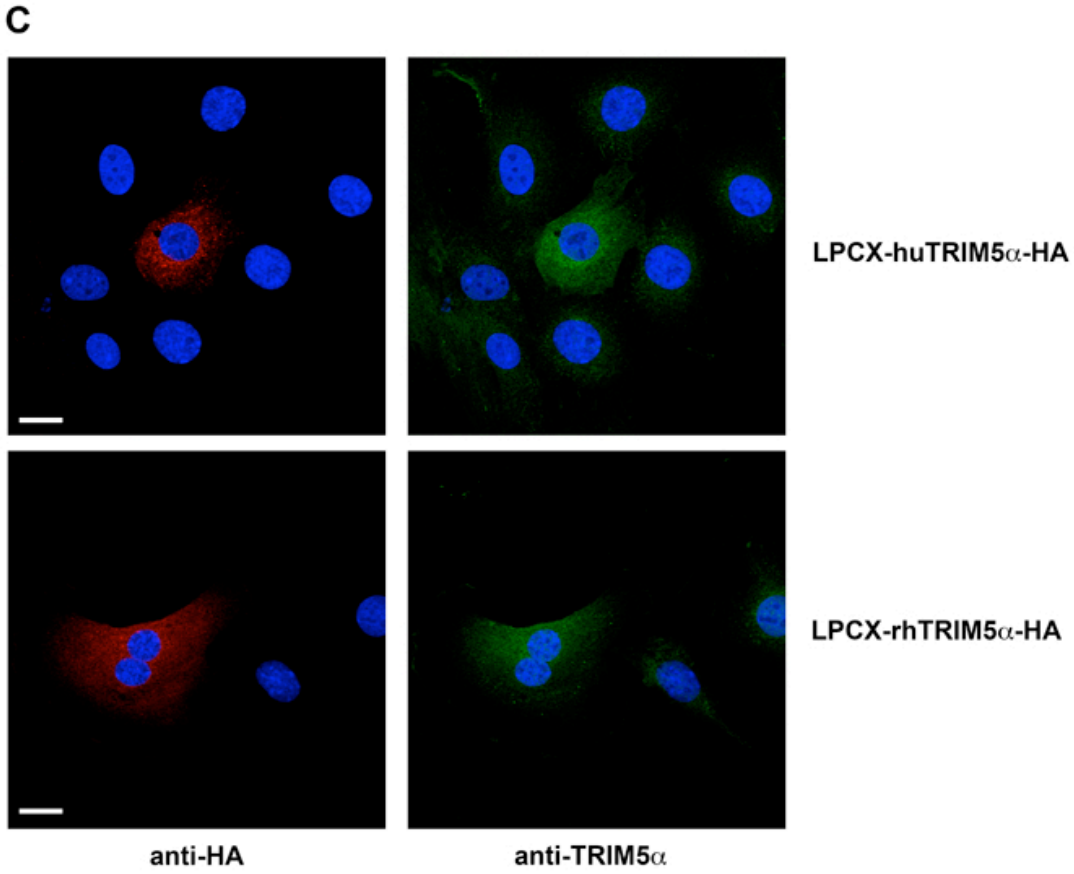
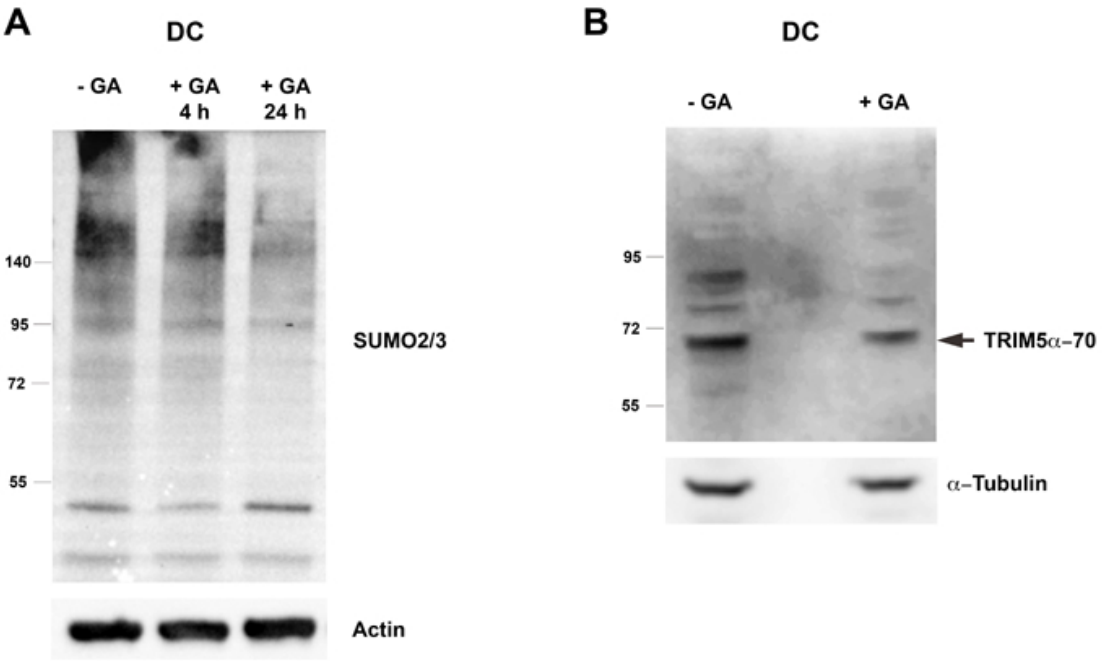

C
LPCX-huTRIM5α-HA
LPCX-rhTRIM5α-HA
anti-HA
anti-TRIM5α

Fig. S2


A
DC
- GA
+ GA 4 h
+ GA 24 h
140
95
72
55
SUMO2/3
Actin
B
DC
- GA
+ GA
95
72
55
TRIM5α-70
α-Tubulin

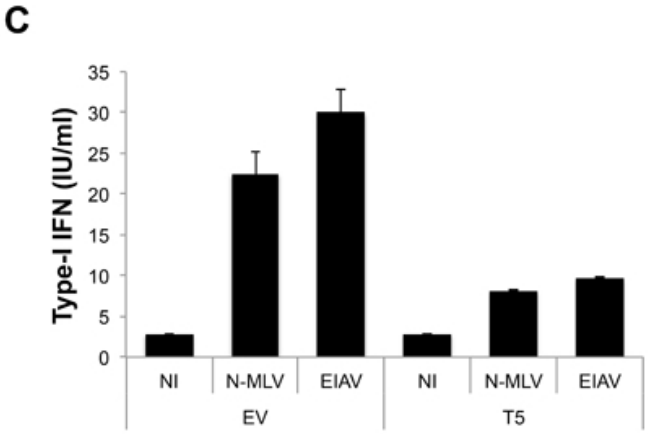

C
Type-I IFN (IU/ml)
35
30
25
20
15
10
5
0
NI
N-MLV
EIAV
NI
N-MLV
EIAV
EV
T5

Fig. S3

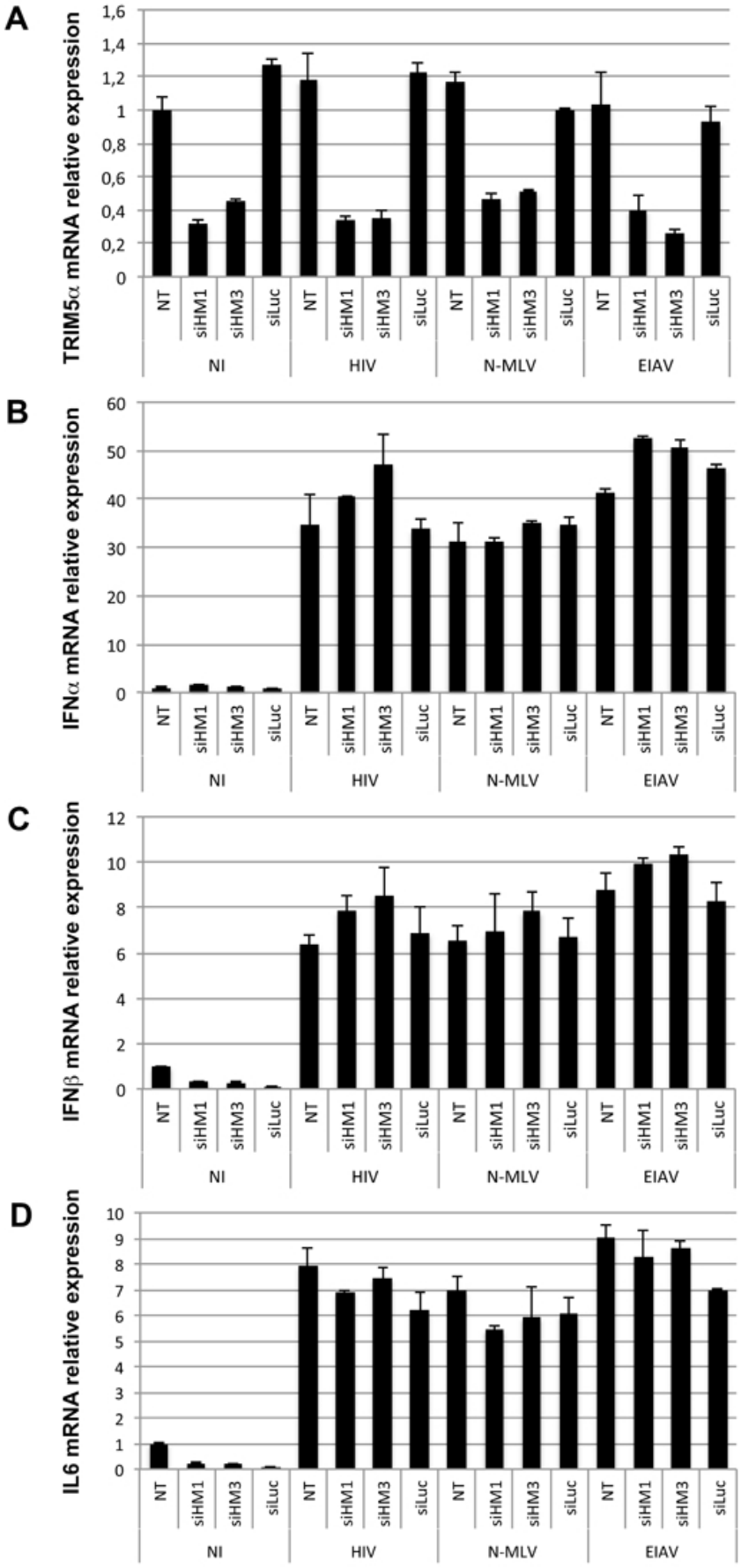

A
TRIM5α mRNA relative expression
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
NT
siHM1
siHM3
siLuc
NI
HIV
N-MLV
EIAV
B
IFNα mRNA relative expression
60
50
40
30
20
10
0
C
IFNβ mRNA relative expression
12
10
8
6
4
2
0
D
IL6 mRNA relative expression
10
9
8
7
6
5
4
3
2
1
0

Fig. S4

Supplemental Figure Legends

Fig. S1 related to Fig. 1: Primary cell phenotypic characterisation, susceptibility to replicative virus, and sensitivity to reverse transcription inhibitors. (A) Representative phenotypic marker characterisation of human, RM, and AGM PBL and DC. Cells were labelled with marker antibodies and analysed by flow cytometry using a BD FACSCalibur. HLA-DR labels activated PBL. Monocyte-derived DC are CD14−CD1a+. MHC class II HLA-DR and co-stimulatory molecule CD80 labelling indicate PBL activation and DC maturation, respectively. * The CD1a antibody did not cross-react with AGM CD1a surface antigen, as previously shown (Mortara et al., 2006). **(B)** PBL and DC were isolated and activated/differentiated from rhesus macaque blood. Cells (3×10^5) were infected in 12-well plates with 100 ng of p24/p27 antigen of SIVmac239-IRES-eGFP, env-defective NL43-IRES-eGFP pseudotyped with VSV-G, or R5-tropic NL43-92th014.12 IRES-eGFP. Infectivity was assessed 3 dpi by flow cytometry. The graph shows triplicate values ± SEM of a representative experiment. **(C)** Reverse transcription inhibitors were tested for efficiency against different retroviruses. MDTF cells, which lack TRIM5α-mediated restriction, were transduced with the indicated retroviruses at MOI 1 in the presence of antiretroviral drugs. Transduction efficiency was measured at 3 dpt by flow cytometry detection of GFP.

Fig. S2 related to Fig.2: Specificity of antibody-mediated detection of endogenous TRIM5α. (A) Human PBMC lysates were probed for endogenous TRIM5α using a home-made rabbit polyclonal antibody raised against a C-terminal fragment of TRIM5α (Zhang et al., 2008), comparing it with two commercial antibodies: polyclonal rabbit anti-TRIM5α antibody (IMG-5534) and peptide-affinity purified polyclonal antibody to TRIM5α (IN1; IMG-5588). Samples were run as triplicates on the same gel, and after transfer the membrane was cut in three to probe with each antibody separately. All three membranes were then developed simultaneously on the same radiograph. **(B)** Human PBL were lipofected with a siRNA against TRIM5α (HM3) and labelled with the rabbit polyclonal anti-TRIM5α antibody followed by anti-rabbit Alexa 488 at 24 h post-transfection. Knockdown efficiency was assessed in parallel by quantitative PCR. **(C)** MDTF cells were transduced with retroviral vector LPCX encoding human or RM TRIM5α-HA and labelled with anti-HA and anti-TRIM5α antibodies. Scale bars = 10 μm.

Fig. S3 related for Fig. 5: Effect of GA treatment on TRIM5α SUMOylation, and quantification of produced type I IFN upon retroviral infection. (A) Human DC were treated with GA for 4 and 24 h, or left untreated. Total cellular SUMOylation was assessed by probing for SUMO2/3. SUMOylated proteins appear as high molecular weight smear that disappears upon 24 h GA treatment. Actin was used to control for loading. **(B)** Human DC were treated with GA for 24 h or left untreated. Extracts were prepared in the presence of IAA. The lower Western blot shows α-tubulin reactivity of the same samples. TRIM5α labelling reveals high molecular weight bands that disappear upon GA treatment. **(C)** HL116 cells, which carry the luciferase gene under the control of the IFN-inducible 6–16 promoter (Uze et al., 1994), were incubated for 8 h with a standard containing titrated human IFNβ and the supernatants from DC transfected with pLPCX TRIM5α-HA (T5) or empty pLPCX (EV), and either uninfected (NI) or infected with N-MLV or EIAV. Cells were then lysed and luciferase activity measured. IFN levels are expressed as equivalent of IFNβ concentration, in IU/ml. Results show the mean of quadruplicates from a representative experiment ± SD.

Fig. S4 related to Fig. 6: **Assessment of TRIM5α contribution to viral sensing by DC.** Human DC were DOTAP transfected with two different TRIM5α specific siRNA (HM1 and HM3), siLuc, or left untransfected. After 48 h cells were transduced with HIV-1, N-MLV, or EIAV at MOI 5. RNA extracts were prepared at 24 hpi to quantify **(A)** TRIM5α, **(B)** IFNα1, **(C)** IFNβ, and **(D)** IL6. Copy numbers were normalised for housekeeping transcript RPL13A (60S ribosomal protein L13a).

Supplemental Experimental Procedures

Drugs. Ginkgolic acid (GA), iodocetamide (IAA), leptomycin B (LMB), nevirapine (Nev) were obtained from Sigma and Tenofovir (TDF) from Alsachim. Interferon alpha was purchased from R&D Systems (Recombinant Human IFNα2b).

siRNA/shRNA. siRNA directed against human (H) and rhesus macaque (M) TRIM5α, HM1 (GCUCAGGGAGGUCAAGUUG), HM3 (GCACUGUCUCAUUCUUCAA), and non-targeting siLuc control (GCCAUUCUAUCCUCUAGAGGAUG) siRNAs were purchased from Santa Cruz Biotechnology. shRNA against cGAS and LacZ were described previously (Lahaye et al., 2013).

Antibodies. Primary antibodies were rabbit polyclonal antibody raised against a C-terminal fragment of TRIM5α (Zhang et al., 2008), mouse monoclonal anti-coilin (Pdelta, Abcam), anti-SUMO1 (21C7, Developmental Studies Hybridoma Bank), anti-PML (PG-M3, Santa Cruz Biotechnology), anti-GM-130 (35/GM130, BD Transduction Laboratories), anti-lamin A/C (636, Leica Biosystems), anti-beta-tubulin (TUB2.1, Sigma), anti-β-actin (AC-74, Sigma) rat monoclonal anti-HA (3F10, Roche), and rabbit polyclonal anti-SUMO2/3 (Invitrogen). Conjugated antibodies were HLA-DR-PE, CD4-APC, CD14-FITC, and CD1a-PE from BD Biosciences, CD80-PE from Caltag and HRP-conjugated antibodies from GE Healthcare. Secondary antibodies were anti-rabbit Alexa 488 and anti-mouse Alexa 647 (Invitrogen).

Quantitative RT-PCR. Total RNAs were extracted using RNeasy Mini Kit (Qiagen) and cDNAs prepared using RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific). Real-time PCR reactions were performed in duplicate using Takyon ROX SYBR Mastermix dTTP blue (Eurogentec) following manufacturer's instructions. Real-time PCR reactions were performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems). Transcripts were quantified using the following program: 3 min at 95°C followed by forty cycles of 15 s at 95°C, 20 s at 60°C and 20 s at 72°C. RPL13A (ribosomal protein L13A) and beta-2-microglobulin (β2M) were used as housekeeping genes to normalize mRNA expression. The ratio of gene of interest versus housekeeping gene was calculated using the $2^{-\Delta\Delta Ct}$ method. Primers used for quantification of transcripts are as follows: RPL13A-F: CCTGGAGGAGAAGAGGAAAGAGA, RPL13A-R: TTGAGGACCTCTGTGTATTTGTCAA, β2M-F: TGCTGTCTCCATGTTTGATGTATCT, β2M-R: TCTCTGCTCCCCACCTCTAAGT, SUMO1-F: TCAAAGACAGGGTGTTCCAA, SUMO1-R: CCCCGTTTGTTCCTGATAAA, Ubc9-F: GGACCTGTGGCTGGAGAGGGAC, Ubc9-R: TTCCTCTCCTGGGCGAGTCTGC, IFNα1/13-F: CCAGTTCCAGAAGGCTCCAG, IFNα1/13-R: TCCTCCTGCATCACACAGGC (Primers amplify both IFNα1 and IFNα13 transcripts), IL6-F: TAACCACCCCTGACCCAACC and IL6-R: ATTTGCCGAAGAGCCCTCAG. Primers for DNA quantification are the following: GAPDH-F: TGCACCACCAACTGCTTAGC, GAPDH-R: GGCATGGACTGTGGTCATGAG, eGFP/eYFP-F: TAAACGGCCACAAGTTCAGCG, eGFP/eYFP-R: TGGTGCAGATGAACTTCAGGG.

IFN quantification. IFN secretion was quantified using the reporter cell line HL116 that carries the luciferase gene under the control of the IFN-inducible 6–16 promoter (Uze et al., 1994). HL116 cells (2×10^4) were plated in 96-well plates and incubated for 8 h with the DC culture supernatants or a standard of human IFNβ reference (Gb-23-902-531). Cells were then lysed (Luciferase Cell Culture Lysis Reagent, Promega) and luciferase activity measured using a luminometer. IFN titers are expressed in international unit/ml relative to the human IFNβ reference (Gb-23-902-531) of the NIH.

Supplemental References

Lahaye, X., Satoh, T., Gentili, M., Cerboni, S., Conrad, C., Hurbain, I., El Marjou, A., Lacabaratz, C., Lelievre, J.D., and Manel, N. (2013). The capsids of HIV-1 and HIV-2 determine immune detection of the viral cDNA by the innate sensor cGAS in dendritic cells. Immunity *39*, 1132-1142.

Mortara, L., Ploquin, M.J., Faye, A., Scott-Algara, D., Vaslin, B., Butor, C., Hosmalin, A., Barre-Sinoussi, F., Diop, O.M., and Muller-Trutwin, M.C. (2006). Phenotype and function of myeloid dendritic cells derived from African green monkey blood monocytes. J Immunol Methods *308*, 138-155.

Uze, G., Di Marco, S., Mouchel-Vielh, E., Monneron, D., Bandu, M.T., Horisberger, M.A., Dorques, A., Lutfalla, G., and Mogensen, K.E. (1994). Domains of interaction between alpha interferon and its receptor components. J Mol Biol *243*, 245-257.

Zhang, F., Perez-Caballero, D., Hatziioannou, T., and Bieniasz, P.D. (2008). No effect of endogenous TRIM5alpha on HIV-1 production. Nat Med *14*, 235-236; author reply 236-238.