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Supplemental Information

IFI16 Targets the Transcription Factor Sp1 to Suppress HIV-1 Transcription and Latency Reactivation

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Supplemental Figures

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Seven supplemental figures
Four supplemental tables

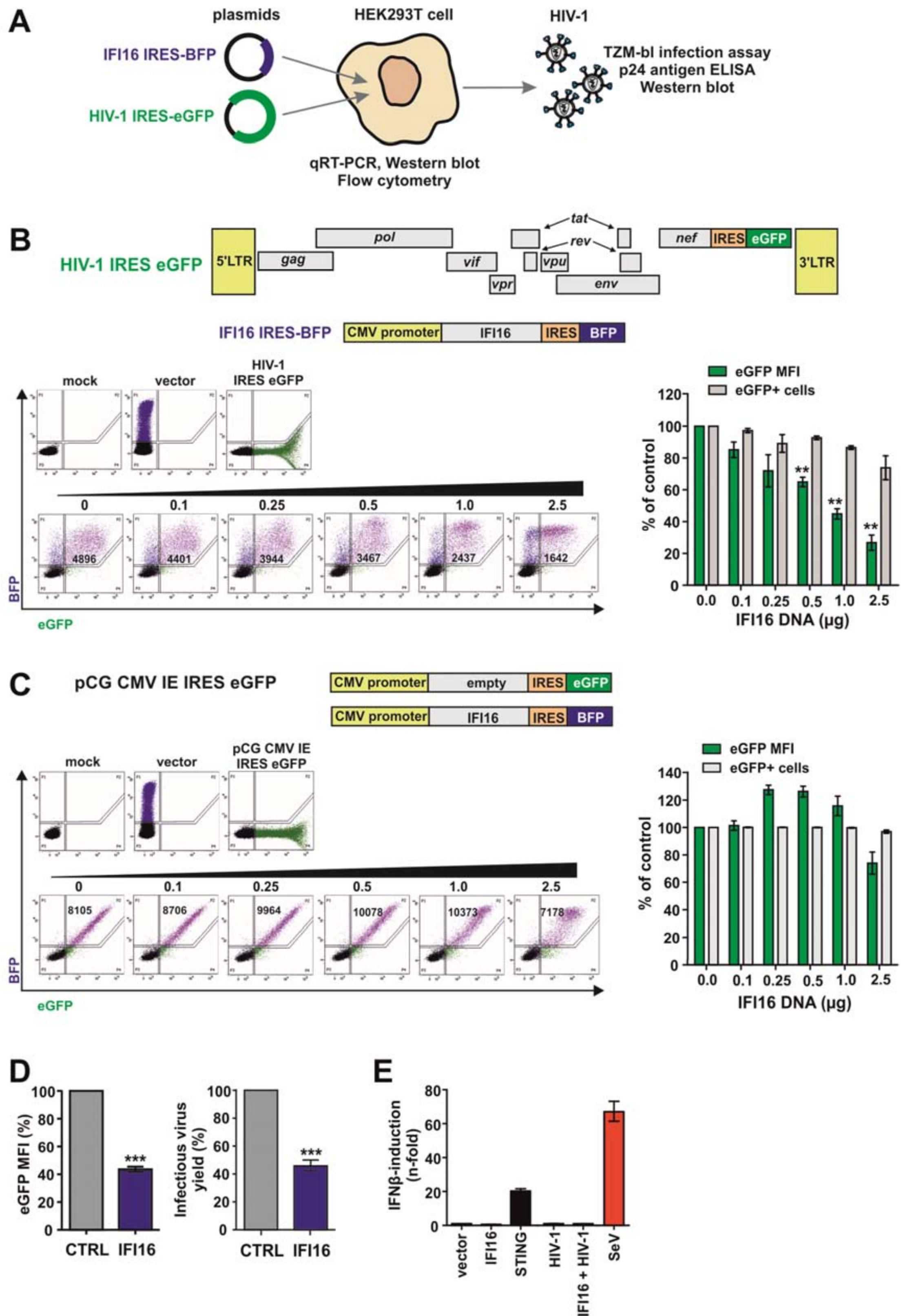
A
plasmids
HEK293T cell
HIV-1
IFI16 IRES-BFP
HIV-1 IRES-eGFP
TZM-bl infection assay
p24 antigen ELISA
Western blot
qRT-PCR, Western blot
Flow cytometry
B
HIV-1 IRES eGFP
5'LTR
gag
pol
vif
vpr
tat
rev
vpu
env
nef
IRES
eGFP
3'LTR
IFI16 IRES-BFP
CMV promoter
IFI16
IRES
BFP
mock
vector
HIV-1 IRES eGFP
0
0.1
0.25
0.5
1.0
2.5
4896
4401
3944
3467
2437
1642
BFP
eGFP
eGFP MFI
eGFP+ cells
% of control
IFI16 DNA (µg)
C
pCG CMV IE IRES eGFP
CMV promoter
empty
IRES
eGFP
CMV promoter
IFI16
IRES
BFP
mock
vector
pCG CMV IE IRES eGFP
8105
8706
9964
10078
10373
7178
BFP
eGFP
eGFP MFI
eGFP+ cells
% of control
IFI16 DNA (µg)
D
eGFP MFI (%)
Infectious virus yield (%)
CTRL
IFI16
***
E
IFNβ-induction (n-fold)
vector
IFI16
STING
HIV-1
IFI16 + HIV-1
SeV

Figure S1 (related to Figure 1). IFI16 inhibits HIV-1 independently of IFNβ induction. (A) Schematic overview on the experimental approach. **(B)** HEK293T cells were cotransfected with an NL4-3 IRES-eGFP construct (2.5 µg) (upper panel) and increasing doses of IFI16 IRES-BFP expression vector. Shown are primary FACS data (left) with mean fluorescence intensities (MFI) of eGFP in the eGFP+/BFP+ population and percentages of eGFP+ cells in BFP+ populations (right) from three experiments (±SEM) relative to the vector control (100%). **(C)** The experiment was performed as described in panel A, but instead of the proviral construct, a vector expressing eGFP via an IRES under the control of the CMV IE promoter was used. **(D)** HEK293T cells were cotransfected with plasmids expressing CD4, CXCR4 and IFI16 IRES-BFP or a vector control. 18 hours post-transfection, cells were infected with NL4-3 IRES-eGFP. 48 hours later, cells were used for flow cytometry and infectious virus yield was determined by infection of TZM-bl cells. eGFP MFI in the eGFP+/BFP+ population and infectious virus yield relative to the vector control (100%) are shown. n = 5 ±SEM. **(E)** HEK293T cells were cotransfected with a firefly luciferase reporter construct under the control of the IFNβ promoter and expression vectors for the indicted proteins or HIV-1 NL4-3 proviral constructs. A *Gaussia* luciferase construct under the control of a constitutively active pTAL promoter was cotransfected for normalization. Cells transfected with the luciferase reporters that were infected with SeV were included as control. 40 h post-transfection luciferase activities were determined. Shown is IFNβ-induction relative to the vector control measured in triplicate experiments ± SD.

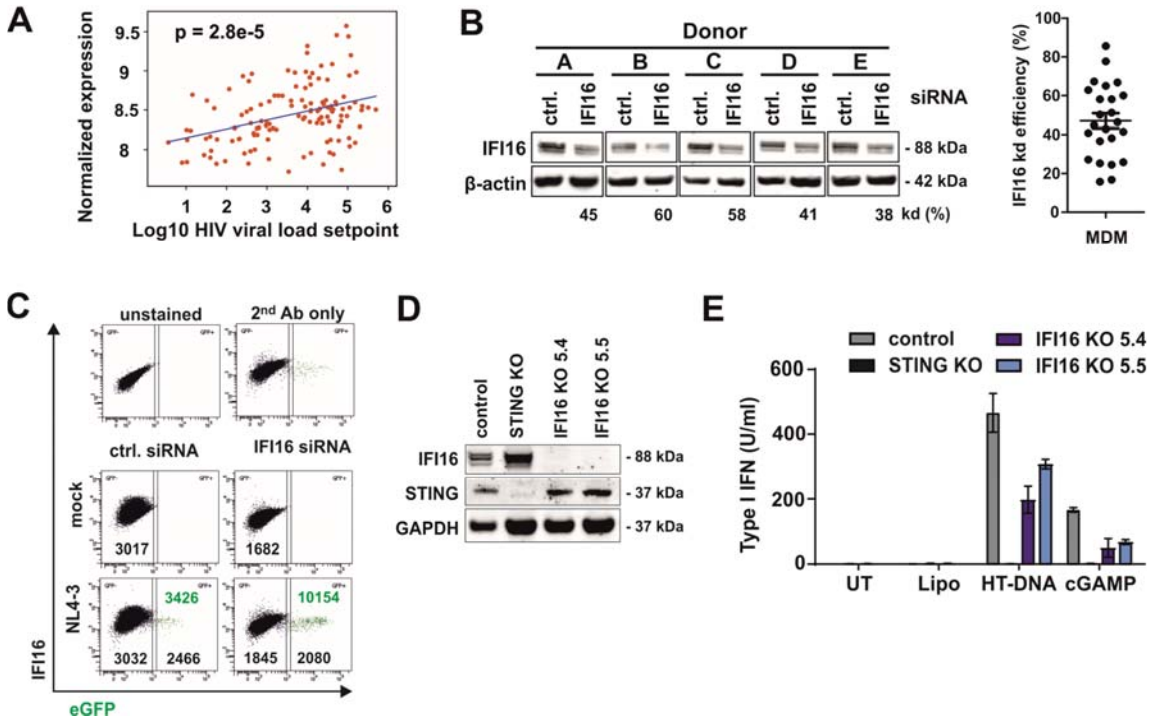

Figure S2 (related to Figure 2). Expression and silencing of IFI16 *in vitro* and *in vivo*. (**A**) Correlation between HIV-1 viral loads and *IFI16* mRNA levels in infected individuals taken from the GuavaH (Genomic Utility for Association and Viral Analyses in HIV) database. **(B)** Human monocyte-derived macrophages were treated with IFI16-specific or control siRNA and knockdown efficiencies were determined by Western blot analysis. Left panel: Representative Western blot examples. Right panel: Relative IFI16 knockdown efficiencies in all donors analyzed. **(C)** Human monocyte-derived macrophages were treated with control or IFI16-specific siRNAs before transduction with VSV-G pseudotyped NL4-3 IRES-eGFP. IFI16 and eGFP expression was determined by flow cytometry 3 days post-transduction. Mean fluorescence intensities of IFI16 (black) and eGFP (green) are shown in the representative flow cytometry dot plots for one donor. **(D)** Western blot analysis of PMA-differentiated parental, IFI16 or STING KO THP-1 cells lines. **(E)** PMA-differentiated parental, IFI16 or STING KO THP-1 cells were transfected with HT-DNA or cGAMP. 8 h post-transfection, secreted type I IFN was determined in a bioactivity assay using HEKblue cells. Mean values of three transfections ± SD are shown.

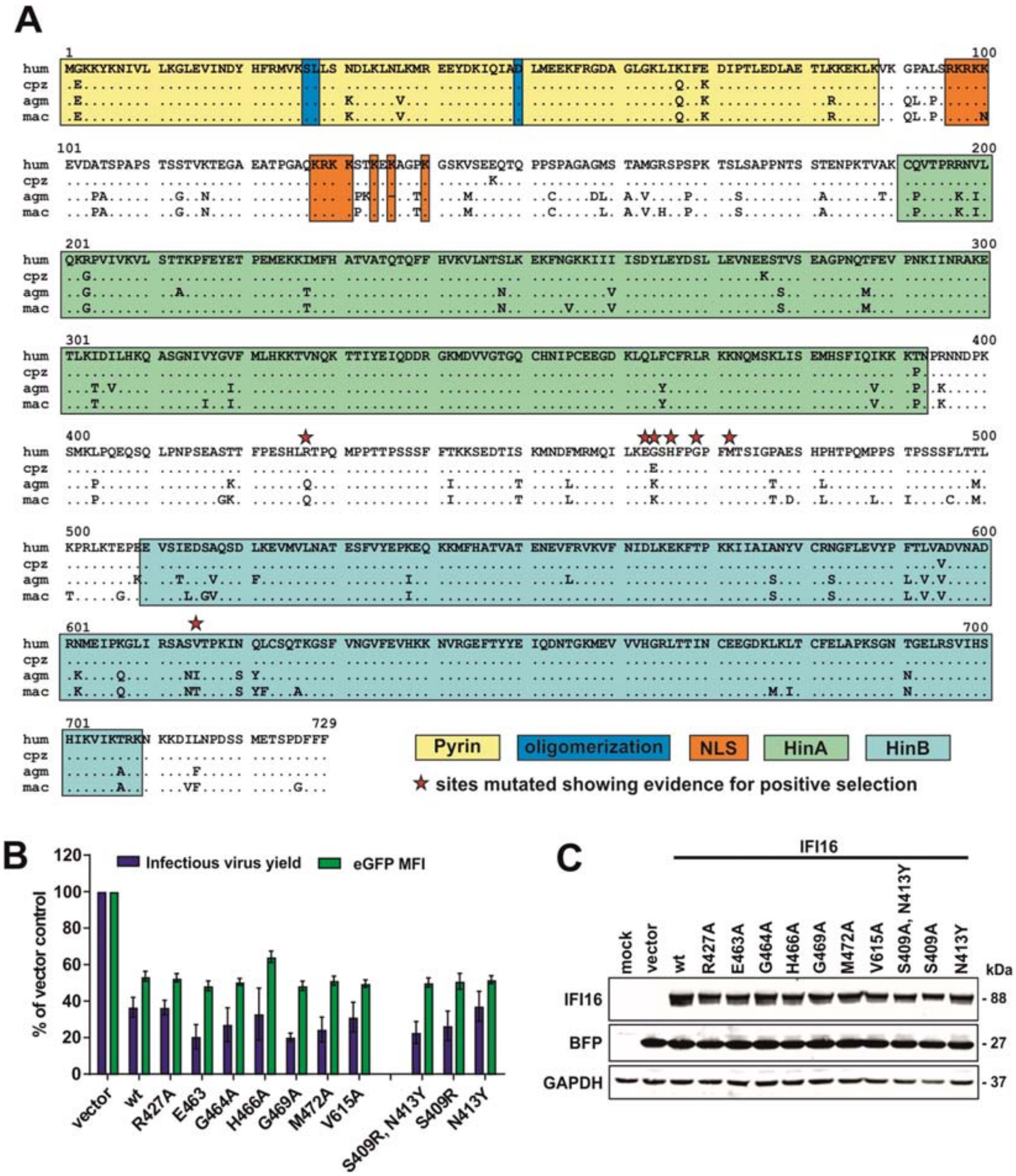

Figure S3 (related to Figure 3). Localization and functional relevance of sites showing evidence for positive selection in IFI16. (A) Alignment of IFI16 amino acid sequences from different species. The human (hum) IFI16 sequence is used as reference in the alignment. Points indicate sequence identity between human IFI16 and IFI16 of chimpanzees (cpz), African green monkeys (agm) or rhesus macaques (mac). Functional protein domains and sites showing evidence for positive selection are indicated. (**B**) HEK293T cells were cotransfected with constructs expressing the indicated IFI16 mutants or a vector control coexpressing BFP and proviral HIV-1 NL4-3 IRES-eGFP. 40 h post-transfection, infectious virus yield was determined by infection of TZM-bl cells and cells were analyzed by flow cytometry. Mean fluorescence intensities (MFI; ± SEM) of eGFP in the eGFP+/BFP+ population. Results were derived from three independent experiments each performed in triplicate. (**C**) Expression of wt and mutant IFI16 proteins determined by Western blot. BFP serves as control for transfection efficiencies.

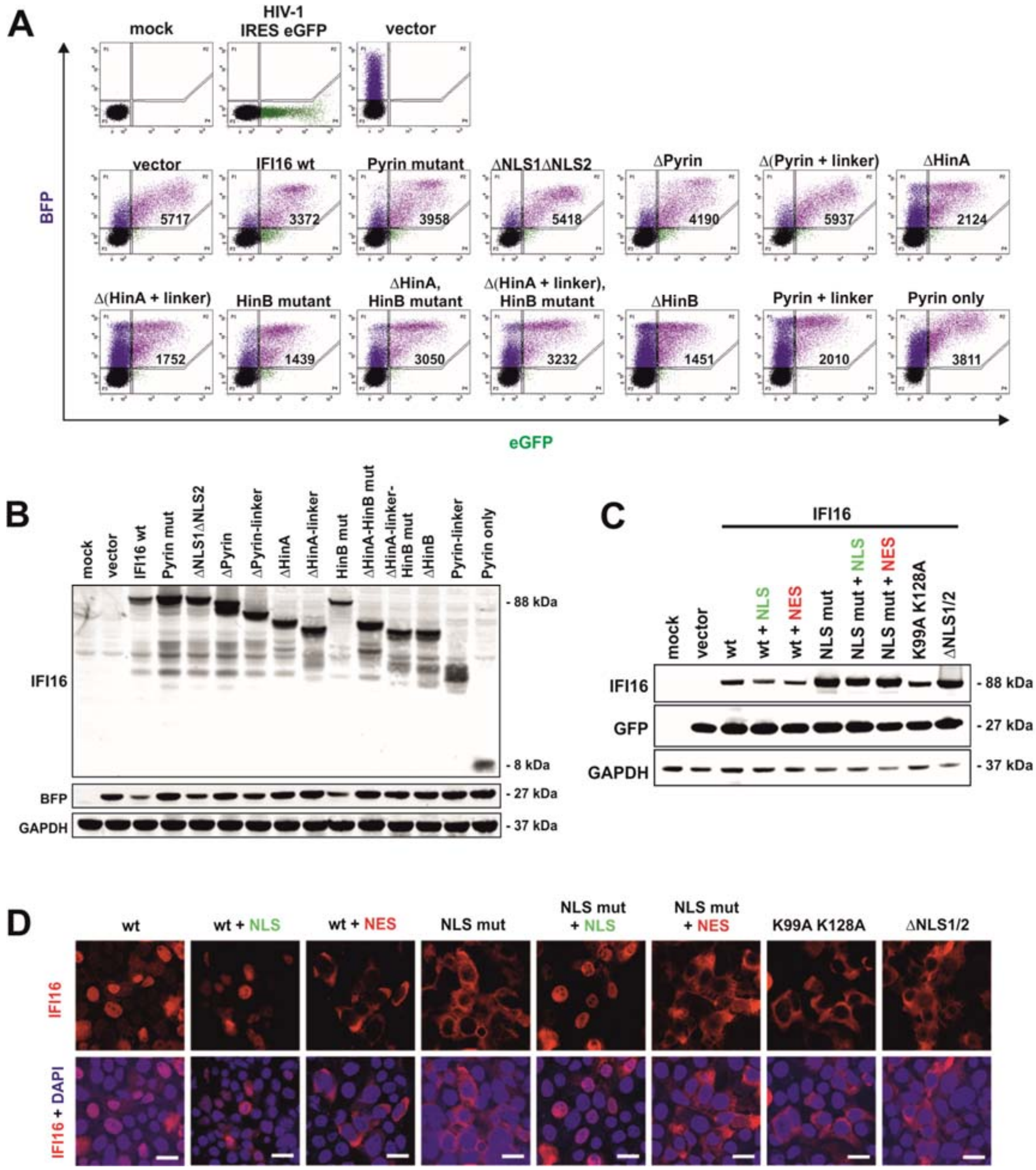

Figure S4 (related to Figure 4). Function, expression and subcellular localization of mutant forms of IFI16. (A) HEK293T cells were cotransfected with expression constructs for the indicated IFI16 mutant coexpressing BFP via an IRES (1 µg) and proviral constructs for NL4-3 coexpressing eGFP via an IRES (2.5 µg). 40 h post-transfection, BFP and eGFP expression was analyzed by flow cytometry. Mean eGFP fluorescence intensities are indicated in the representative flow cytometry dot plots. (**B, C**) Western blot analysis of HEK293T cells transfected with expression constructs for the indicated IFI16 mutant (1 µg). (**D**) 40 h post-transfection with the indicated IFI16 NLS mutant, HEK293T cells were analyzed by fluorescence microscopy.

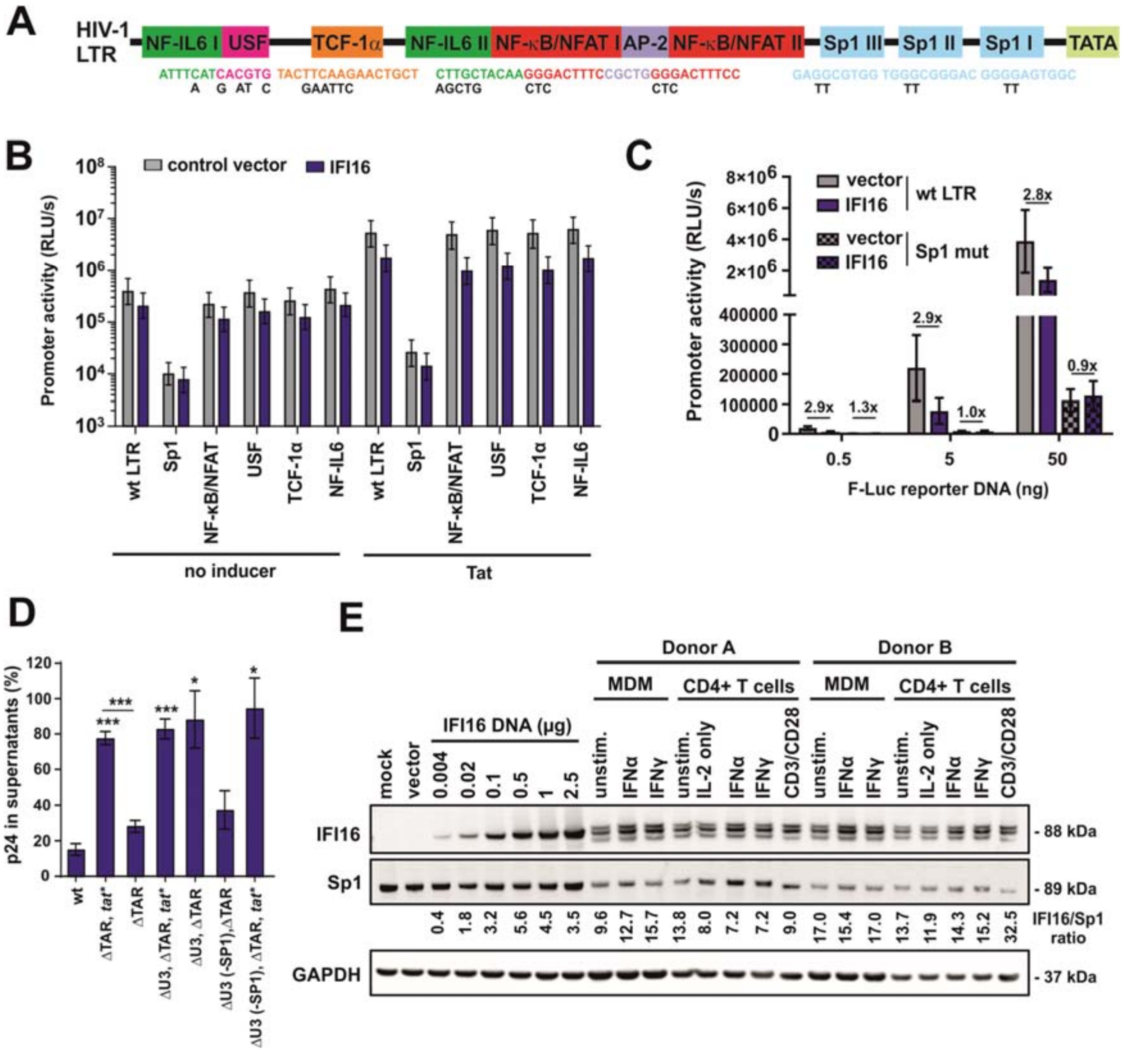

Figure S5. The antiviral effect of IFI16 depends on Sp1 binding sites (related to Figure 5). (**A**) Schematic presentation of binding sites for transcription factors in the HIV-1 LTR and specific mutations analyzed. (**B**) HEK293T cells were cotransfected with firefly luciferase reporter constructs under the control of the indicated HIV-1 LTR mutant and expression constructs for IFI16 or a vector control. A construct expressing NL4-3 Tat under the control of the CMV IE promoter was cotransfected to activate the LTR. 40 h post-transfection, luciferase activities were determined. Mean values (± SEM) were derived from four independent experiments each performed in triplicate. **(C)** HEK293T cells were transfected as in (B) using different concentrations of firefly luciferase reporter constructs under the control of the wt LTR or a mutant thereof lacking functional SP1 binding sites. n = 3 ± SEM. **(D)** HEK293T cells were cotransfected with expression constructs for IFI16 and mutant proviral constructs, carrying a doxycycline-responsive LTR promoter (Das et al., 2011). 40 h post-transfection, p24 in the culture supernatants was determined by ELISA. p24 yields relative to those obtained with the vector control (100%) are shown. n = 3 ± SEM. * $p < 0.05$, *** $p < 0.001$. **(E)** Western blot analysis was used to compare IFI16 and Sp1 expression levels in HEK293T cells, transfected with increasing does of expression constructs for IFI16, with those of monocyte-derived macrophages (MDM) and CD4+ T cells stimulated for 3 days with IL-2 [10 ng/ml] alone or together with IFNα2 [500 U/ml], IFNγ [200 U/ml] or anti-CD3/CD28 beads.

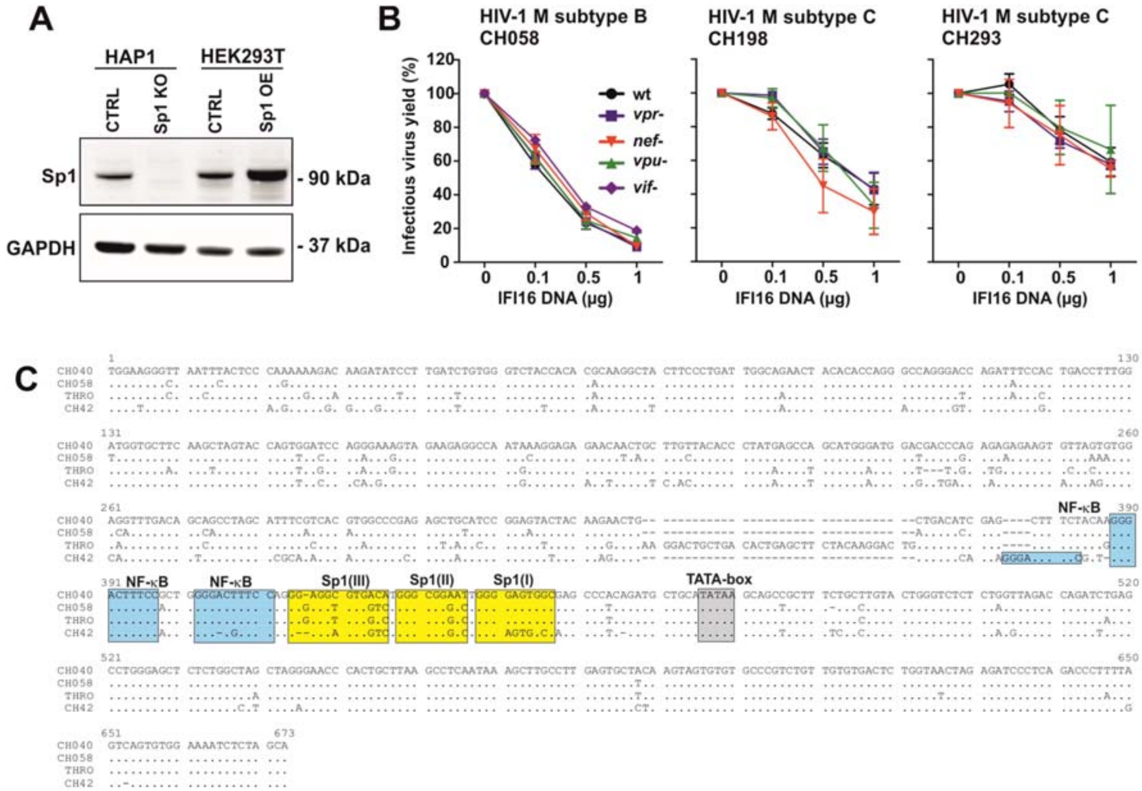

Figure S6. IFI16 sensitivity and Sp1 dependency of different HIV-1 strains (related to Figure 6). **(A)** Western blot analysis of Sp1 KO or ctrl. HAP1 cells and HEK293T cells transfected with a vector control or expression constructs for Sp1 (1 µg). (**B**) HEK293T cells were cotransfected with increasing doses of expression constructs for IFI16 and proviral constructs for the indicated mutants of HIV-1 CH058, CH198 or CH293 (2.5 µg). 40 h post-transfection, infectious virus yield was determined by infection of TZM-bl cells. Shown are mean values of three experiments ± SEM. (**C**) Alignment of LTRs from HIV-1 IMCs showing differential susceptibility to IFI16 inhibition. Dots indicate sequence identity and dashes indicate gaps introduced to optimize the alignment. The NF-κB and Sp1 binding sites and the TATA-box are indicated.

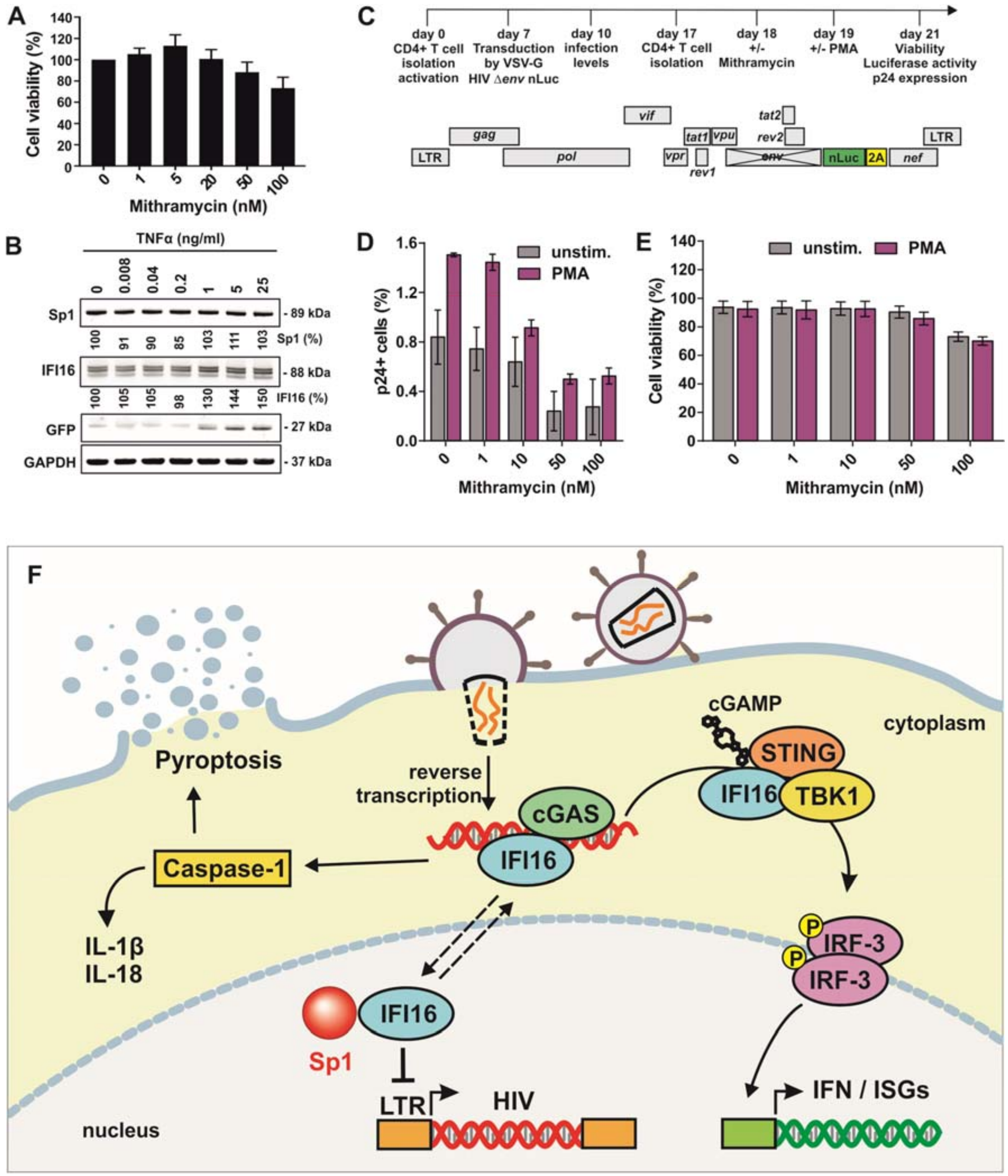

Figure S7. Role of IFI16 and Sp1 in HIV-1 latency and replication (related to Figure 7). (A) Two days after stimulation with increasing doses of Mithramycin A, viability of J-Lat 10.6 cells was determined by MTT assay. Shown are mean values (±SEM) derived from three independent experiments. **(B)** Sp1 and IFI16 expression levels in J-Lat 10.6 cells stimulated for two days with increasing doses of TNFα were determined by Western blot. **(C)** Genomic organization of the NL4.3-deltaEnv-nLuc-2ANef construct and outline of the experimental approach to analyze the effect of the Sp1 inhibitor Mithramycin on HIV gene expression and reactivation in primary CD4+ T cells. (**D**) Frequency of p24+ T cells and (**E**) cell viability measured by flow cytometry at day 21. (**F**) In myeloid cells, IFI16 acts as a cytosolic immune sensor of HIV-1 DNA species (Jakobsen et al., 2013) that promotes IFN induction via the cGAS-STING pathway (Jønsson et al., 2017). In lymphoid CD4+ T cells abortively infected with HIV-1, IFI16 might sense cytosolic HIV-1 RT intermediates to mediate pyroptotic death in a cGAS-STING-independent manner (Monroe et al., 2014). The present study shows that nuclear IFI16 inhibits HIV-1 gene expression in an Sp1-dependent manner.

SUPPLEMENTAL TABLES

Table S1: Primers used to generate pCG IRES BFP expression constructs

Construct name	Primer name	Primer sequence
IFI16	IFI16 XbaI fw	CGTCTAGACCATGGGAAAAAAATACAAGAACATTGTTC
	IFI16 MluI rev	CTACGCGTTTAGAAGAAAAAGTCTGGTGAAGTTTCCATAC
IFI16 C-HA	IFI16 C-HA MluI rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTAGAAGAAAAAGTCTGGTGAAGTTTCC
IFI16 R427A	IFI16 R427A fw	CCATCTTGCGACTCCTCAGATG
	IFI16 R427A rev	CATCTGAGGAGTCGCAAGATGG
IFI16 E463A	IFI16 E463A fw	CTGAAGGCAGGGAGTCATTTTCC
	IFI16 E463A rev	GGAAAATGACTCCCTGCCTTCAG
IFI16 G464A	IFI16 G464A fw	CTGAAGGAAGCGAGTCATTTTCC
	IFI16 G464A rev	GGAAAATGACTCGCTTCCTTCAG
IFI16 H466A	IFI16 H466A fw	CTGAAGGAAGGGAGTGCTTTTCC
	IFI16 H466A rev	GGAAAAGCACTCCCTTCCTTCAG
IFI16 G469A	IFI16 G469A fw	GTCATTTTCCAGCACCGTTCATG
	IFI16 G469A rev	CATGAACGGTGCTGGAAAATGAC
IFI16 M472A	IFI16 M472A fw	CGTTCGCGACCAGCATAGG
	IFI16 M472A rev	CCTATGCTGGTCGCGAACG
IFI16 V615A	IFI16 V615A fw	GAAGTGCCAGCGCAACTCC
	IFI16 V615A rev	GGAGTTGCGCTGGCACTTC
IFI16 S409A	IFI16 S409A fw	CTACCCCAGGAACAGAGACAGCTTC
	IFI16 S409A rev	GAAGCTGTCTCTGTTCCTGGGGTAG
IFI16 N413Y	IFI16 N413Y fw	GCTTCCATACCCTTCAGAGGCC
	IFI16 N413Y rev	GGCCTCTGAAGGGTATGGAAGC
IFI16 ΔNLS1	IFI16 ΔNLS1 fw	GCCCTATCAGAAGTGGATGCTACTTCACCTGCAC
	IFI16 ΔNLS1 rev	CATCCACTTCTGATAGGGCTGGTCCTTTTAC
IFI16 ΔNLS2	IFI16 ΔNLS2 fw	GGAGCTCAGTCAACCAAAGAAAAGGCTGGAC
	IFI16 ΔNLS2 rev	CTTTGGTTGACTGAGCTCCAGGAGTTGCCTC
IFI16 ΔPyrin	IFI16 ΔPyrin fw	CGTCTAGACCATGGTAAAAGGACCAGCCCTATC
IFI16 ΔPyrin-linker	IFI16 ΔPyrin-linker fw	CGTCTAGACCATGCAGGTAACTCCCAGAAGAAATGTTC
IFI16 ΔHinA	IFI16 ΔHinA fw	GTGGCCAAATGTCAGATACTGAAGGAAGGGAGTCATTTTC
	IFI16 ΔHinA rev	CTTCAGTATCTGACATTTGGCCACTGTTTTCGG
IFI16 ΔHinA-linker	IFI16 ΔHinA-linker fw	GGCCAAATGTGAAGTTTCCATAGAAGACAGTG
	IFI16 ΔHinA-linker rev	CTATGGAAACTTCACATTTGGCCACTGTTTTC
IFI16 ΔHinB	IFI16 ΔHinB rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTAGTTTGTTTTTTCTTTATCTGG
IFI16 Pyrin-linker	IFI16 Pyrin-linker rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTAACATTTGGCCACTGTTTTC
IFI16 Pyrin-only	IFI16 Pyrin-only rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTATTTTAACTTTTCTTTTTAAGAGTTTCAGC

IFI16 + SV40 NLS	IFI16 + SV40 NLS rev	ATCACGCGTTTACACCTTCCGCTTCTTCTTCGGGAAGAAAAAGTCTGGTGAAGTTTCC
IFI16 + Rev NES	IFI16 + Rev NES rev	ATCACGCGTTTAAAGAGTAAGTCTCTCAAGCGGTGGTAGGAAGAAAAAGTCTGGTGAAGTTTCC
IFI16 NLS1 mut	IFI16 NLS1 mut fw	ATCAGCCGCTGCCGCCGCTGAAGTGGATGCTACTTCACCTG
	IFI16 NLS1 mut rev	CTTCAGCGGCGGCAGCGGCTGATAGGGCTGGTCCTTTTAC
IFI16 NLS2/3/4 mut	IFI16 NLS2/3/4 mut fw	ACCGCCGAAGCTGCTGGACCCGCCGGGAGTGCCGTGTCCGAGGAACAGACTCAG
	IFI16 NLS2/3/4 mut rev	GGGTCCAGCAGCTTCGGCGGTTGATGCAGCGGCTGCCTGAGCTCCAGGAGTTGCC
IFI16 K99A	IFI16 K99A fw	CAAGAAAGAGGGCGAAGGAAGTG
	IFI16 K99A rev	CACTTCCTTCGCCCTCTTTCTTG
IFI16 K128A	IFI16 K128A fw	GAGCTCAGGCAAGAAAAAAATCAACCAAAG
	IFI16 K128A rev	CTTTGGTTGATTTTTTTCTTGCCTGAGCTC
cpz IFI16 C-HA	cpz IFI16 XbaI fw	CTTCTAGACCATGGAAAAAAAATACAAGAACATTGTTCTACTG
	cpz IFI16 C-HA MluI rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTAGAAGAAAAAGTCTGGTGAAGTTTCCATAC
agm IFI16 C-HA	agm IFI16 XbaI fw	CTTCTAGACCATGGAAAAAAAATACAAGAACATTGTTCTACTG
	agm IFI16 C-HA MluI rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTAGAAAAAAAAGTCTGGTGAAGTTTCCATAC
mac IFI16 C-HA	mac IFI16 XbaI fw	CTTCTAGACCATGGAAAAAAAATACAAGAACATTGTTCTACTG
	mac IFI16 C-HA MluI rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTAGAAAAAAAAGCCTGGTGAAGTTTCCATAC
p204 C-HA	p204 XbaI fw	CGTCTAGACCATGGTGAATGAATACAAGAG
	p204 C-HA MluI rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTACTTTCTAGCATTGATGACC
AIM2 C-HA	AIM2 XbaI fw	CGTCTAGACCATGGAGAGTAAATACAAGG
	AIM2 C-HA MluI rev	CTACGCGTTAAGCGTAATCTGGAACATCGTATGGGTATGTTTTTTTTTGGCCTTAATAAC
STING	STING XbaI fw	CGTCTAGACCATGCCCCACTCCAGC
	STING MluI rev	CTACGCGTTCAAGAGAAATCCGTGCG
Sp1 C-HA	Sp1 XbaI fw	CATCTAGACCATGAGCGACCAAGATCACTCCATG
	Sp1 C-HA MluI rev	CGAACGCGTTCAAGCGTAATCTGGAACATCGTATGGGTAGAAGCCATTGCCACTGATATTAATGGAC

Table S2: Primers used to generate HIV-1 LTR F-Luc reporter constructs

Construct name	Primer name	Primer sequence
HIV-1 LTR F-luc	HIV-1 LTR MluI fw	CGTCTAGACCATGGAAAAAAATACAAGAACATTGTTC
	HIV-1 LTR XhoI rev	CTACGCGTTTAGAAGAAAAAGTCTGGTGAAGTTTCCATAC

Table S3: Primers used to generate chimeric HIV-1 proviral constructs

Construct name	Primer name	Primer sequence
CH058 with LTRs of THRO	THRO_NotI_5'LTR_for	GCATGCTCGAGCGGCCGCCAGTGTGATG
	THRO_5'LTR in CH058_OE_rev	CTCTAGCAGTGGCGCCCGAACAGGGACCTG
	THRO_5'LTR in CH058_OE_fw	GTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCG
	CH058_BstZ17I_5'LTR_rev	GATGAAGACTTCAGGAAGTATACGCCGAATTGGGCCCTCTAGATGCATGCTCGA
	CH058_StuI_3'LTR_fw	CGAGGCCTGTCCAAAGGTATCTTTTCAGC
	THRO_3'LTR in CH058_OE_rev	GGACTGGAAGGGCTAACTTACTCCCAAAAGAG
	THRO_3'LTR in CH058_OE_fw	GATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGG
	THRO_MluI_3'LTR_rev	GCTTGATGCATAGCTTGAGTATTCTAACGCGTCAC
CH040 with LTRs of THRO	THRO_NotI_5'LTR_fw	GCATGCTCGAGCGGCCGCCAGTGTGATG
	THRO_5'LTR in CH040_OE_rev	CTCTAGCAGTGGCGCCCGAACAGGGACTTG
	THRO_5'LTR in CH040_OE_fw	GTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCG
	CH040_AgeI_5'LTR_rev	GAGACTATGTAGACCGGTCG
	CH040_BlpI_3'LTR_fw	CGGCTGAGCCAGCAGCAGAG
	THRO_3'LTR in CH040_OE_rev	GGACTGGAAGGGCTAACTTACTCCCAAAAGAG
	THRO_3'LTR in CH040_OE_fw	GATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGG
	THRO_MluI_3'LTR_rev	GCTTGATGCATAGCTTGAGTATTCTAACGCGTCAC
THRO with LTRs of CH058	CH058_NotI_5'LTR_fw	CTAGATGCATGCTCGAGCGGCCGCTGGAAG
	CH058_5'LTR in THRO_OE_rev	CTCTAGCAGTGGCGCCCGAACAGGGACC
	CH058_5'LTR in THRO_OE_fw	CAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAAC
	CH042_HpaI_5'LTR_rev	GCCCACACTAATGATGTAAAACAGTTAACAGAGGCAGTGC
	THRO_BbvCI_3'LTR_fw	GCTATAGCAGTAGCTGAGGGGACAGATAG
	CH058_3'LTR in THRO_OE_rev	GGGGACTGGAAGGGCTAATTCACTCCCAGAAAAGAC
	CH058_3'LTR in THRO_OE_fw	GCCACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGC
	CH058_MluI_3'LTR_rev	GATGCATAGCTTGAGTATTCTAACGCGTCAC

Table S4: Primers and fluorescent probes used for qRT-PCR

Viral transcript	**Primer/probe name**	**Primer/probe sequence**
R-U5/*gag*	R-U5/gag for	GCCTCAATAAAGCTTGCCTTGA
	R-U5/gag rev	GGGCGCCACTGCTAGAGA
	R-U5/gag probe FAM/BBQ	6FAM-CAGAGTCACACAACAGACGGGCACA-BBQ
nef	*nef* for	GGTGGGAGCAGYATCTCGAGA
	nef rev	TGTAAGTCATTGGTCTTAAAGGTACCTGAGG
	nef probe FAM/BBQ	6FAM-GCTTCYAGCCAGGCACAAKCAGCATT-BBQ
U3-polyA	U3-polyA for	GCCCTCAGATGCTRCATATAA
	U3-polyA rev	TTTTTTTTTTTTTTTTTTTTTTTTTGAAG
	U3-polyA FAM/BBQ	6FAM-TGCCTGTACTGGGTCTCTCTGGTTAG-BBQ