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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



Figure S1 (related to Figure 1). IFI16 inhibits HIV-1 independently of IFN^β 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 (\pm 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. $n = 5 \pm SEM$. (E) HEK293T cells were cotransfected with a firefly luciferase reporter construct under the control of the IFN β 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 \pm 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 monocytederived 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 lines. (E) PMA-differentiated parental, IFI16 or STING KO THP-1 cells lines. Shown is 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; \pm 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 IF116. (A) HEK293T cells were cotransfected with expression constructs for the indicated IF116 mutant coexpressing BFP via an IRES (1 μ g) and proviral constructs for NL4-3 coexpressing eGFP via an IRES (2.5 μ g). 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 IF116 mutant (1 μ g). (**D**) 40 h post-transfection with the indicated IF116 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. $n = 3 \pm SEM$. (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. $n = 3 \pm SEM$. * 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 μ g). (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 μ g). 40 h post-transfection, infectious virus yield was determined by infection of TZM-bl cells. Shown are mean values of three experiments \pm 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-KB 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 (\pm 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 TNF α 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

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$\begin{split} & \text{IF116 0404} & \text{IF116 6464A rev} & \text{GGAAAATGACTCGCTTCCTTCAG} \\ & \text{IF116 H466A} & \text{IF116 H466A fw} & \text{CTGAAGGAAGGAAGTGCTTTCC} \\ & \text{IF116 H466A} & \text{IF116 H466A rev} & \text{GGAAAAGCACTCCCTTCTTCAG} \\ & \text{IF116 G469A} & \text{IF116 G469A fw} & \text{GTCATTTTCCAGCACCGTTCATG} \\ & \text{IF116 M472A} & \text{IF116 G469A rev} & \text{CATGAACGGTGCTGGAAAATGAC} \\ & \text{IF116 M472A} & \text{IF116 M472A rev} & \text{CCTATGCTGGTCGCGAACG} \\ & \text{IF116 V615A} & \text{IF116 V615A fw} & \text{GAAGTGCCAGCGCAACTCC} \\ & \text{IF116 V615A} & \text{IF116 S409A fw} & \text{CTACCCCAGGAACAGAGACAGCTTC} \\ & \text{IF116 S409A} & \text{IF116 S409A fw} & \text{CTACCCCAGGAACAGAGACAGCTTC} \\ & \text{IF116 N413Y} & \text{IF116 S409A fw} & \text{CTACCCATACCTTCAGAGGCC} \\ & \text{IF116 N413Y} & \text{IF116 N413Y fw} & \text{GCTCCATACCTTCAGAGGCC} \\ & \text{IF116 A413Y} & \text{IF116 A413Y fw} & \text{GCCCTATCAGAAGTGGAAGC} \\ & \text{IF116 AALS1 fw} & \text{GCCCTATCAGAAGTGGATGCTACTTCACCTG} \\ & \text{IF116 AALS1 fw} & \text{GCCCTATCAGAAGTGGATGCTACTTCACCTG} \\ & \text{IF116 AALS1 fw} & \text{GCCCTATCAGAAGTGGATGCTACTTCACCTG} \\ & \text{IF116 AALS1 rev} & \text{CATCCACTTCAGAAGGCGGAGGTGGA} \\ & \text{IF116 AALS2} & \text{IF116 AALS2 fw} & \text{GGAGCTCAGTCAACAAAAAGAAAAGGACCAGCCTA} \\ & \text{IF116 AALS2 fw} & \text{GGAGCTCAGTCAACAAAAGAAAAGGACCAGCCTA} \\ & \text{IF116 AALS2 fw} & \text{GGAGCCAATGTGAAACAAAAGGACCAGCCTA} \\ & \text{IF116 AALS2 fw} & \text{GGAGCCAATGCAAGGAAAAAGGACCAGCCCTA} \\ & \text{IF116 AALS2 fw} & \text{GGAGCCAATGCAAAAAAGGACCAGCCCTA} \\ & \text{IF116 AALS2 fw} & \text{GGCCAATGCAAGGAAAAGGACCAGCCCTA} \\ & \text{IF116 AALS2 fw} & \text{GGCCAAATGTCAAGAAAAGGACCAGCCCTA} \\ & \text{IF116 AALS2 fw} & \text{GGCCAAATGTCAAGAAAAGGACCAGCCCTA} \\ & \text{IF116 AAHiAA} fw & \text{ATGTTC} \\ \\ & \text{IF116 AHinA} fw & \text{ATGTTC} \\ \\ & \text{IF116 AHinA} fw & \text{GTGGCCAATGCAAGAAAGGAAAGGAAAGGG} \\ \\ & \text{IF116 AHinA} fw & \text{GGCCAAATGTCAAGATACTGAAGAAAGGAAGGG} \\ \\ & \text{IF116 AHinA} fw & \text{GCCCAATGTCAAGATACTGAAGAAAGGAAGGG} \\ \\ & \text{IF116 AHinA} fw & \text{GCCCAATGTAAGACATTTGGCCACTGTTTTCG} \\ \\ & \text{IF116 AHinA} fw & \text{IF116 AHinA} fw \\ \\ & \text{IF116 AHinA} fw & \text{GCCCAATGTAAGTACTAGAAGACAGT} \\ \\ & \text{IF116 AHinA} fw & \text{IF116 AHinA} fw \\ \\ & \text{IF116 AHinA} fw \\ \\ & \text{IF116 AHinA} fw \\ \\ & $	IFI16 G464A	IFI16 G464A fw	CTGAAGGAAGCGAGTCATTTTCC
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	11110 040471	IFI16 G464A rev	GGAAAATGACTCGCTTCCTTCAG
$\begin{split} \begin{tabular}{ c c c c c c c } & \mbox{IF116 H466A rev} & \mbox{GGAAAAGCACTCCCTTCCTTCAG} \\ \hline \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	IFI16 H/66A	IFI16 H466A fw	CTGAAGGAAGGGAGTGCTTTTCC
$\begin{split} \mbox{IF116}\ G469A & \begin{tabular}{ c $	11110 11 4 00A	IFI16 H466A rev	GGAAAAGCACTCCCTTCCTTCAG
$\begin{split} \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	IE116 C460A	IFI16 G469A fw	GTCATTTTCCAGCACCGTTCATG
IF116 M472AIF116 M472A fwCGTTCGCGACCAGCATAGGIF116 M472A revCCTATGCTGGTCGCGAACGIF116 V615AIF116 V615A fwGAAGTGCCAGCGCAACTCCIF116 V615AIF116 V615A revGGAGTTGCGCTGGCACTTCIF116 S409AIF116 V615A revGAAGCTGTCTCTGTCCTGGGGTAGIF116 S409AIF116 S409A fwCTACCCCAGGAACAGAGACAGGCCIF116 N413YIF116 N413Y fwGCTTCCATACCCTTCAGAGGCCIF116 N413YIF116 N413Y revGGCCTCTGAAGGGTATGGAAGCIF116 ANLS1IF116 ANLS1 fwGCCCTATCAGAAGTGGATGCTACTTCACCTG CACIF116 ANLS1IF116 ANLS1 revCATCCACTTCTGATAGGGCTGGTCCTTTACIF116 ANLS2IF116 ANLS2 revCTTTGGTTGACTGAGCTCCAGGAGATGCCCAGGAGTGCCCTCIF116 ANLS2IF116 ANLS2 revCTTTGGTTGACTGAGGCTAGGAAACGCCGAAGAIF116 APyrinIF116 APyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCTAAAAGGACCAGCCTAAAAGGACCAGGCCTAAAAGGACCAGGCAAATGTCIF116 APyrinIF116 APyrin-linkerCGTCTAGACCATGCAGGTAACTCCAGAAAGGAAAGGGAAAGGGAAGGGAAAGGCAAATGTCAGAATACTGAAAGGAAAGGGAAAGGGAAGGGAAGTTCCIF116 AHinAIF116 AHinA fwGTGGCCAAATGTCAGAATACTGAAAGGAAAGGCAAGGGAAGGGAAGGGAAGGCAAATGTCAGAATATTGGCCACTGTTTCCGGAAATGTAAAGAAAG	IF110 0409A	IFI16 G469A rev	CATGAACGGTGCTGGAAAATGAC
IFI16 MI472AIFI16 M472A revCCTATGCTGGTCGCGAACGIF116 V615AIFI16 V615A fwGAAGTGCCAGCGCAACTCCIF116 V615AIFI16 V615A revGGAGTTGCGCTGGCACTTCIF116 S409AIFI16 S409A fwCTACCCCAGGAACAGAGACAGCTTCIF116 N413YIFI16 N413Y fwGCTTCCATACCCTTCAGAGGCCIF116 N413YIF116 N413Y fwGCCTCTGAAGGGTATGGAAGCIF116 N413YIF116 ANLS1 fwGCCCTATCAGAAGTGGATGCTACTTCACCTG CACIF116 ΔNLS1IF116 ΔNLS1 fwGCCCTATCAGAAGTGGATGCTACTTCACCTG CACIF116 ΔNLS2IF116 ΔNLS2 fwGGAGCTCAGTCAGTCAACCAAAGAAAAGGCTGGA CIF116 ΔNLS2IF116 ΔNLS2 revCTTTGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIF116 ΔPyrin fwCGTCTAGACCATGCAGGTAACTCCAGAAGA AATGTTCIF116 ΔHinAIF116 ΔHinA fwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinA-IF116 ΔHinA- Iniker fwGGCCAAATGTGAAGTTTCCATAGAAGACAGCIF116 ΔHinA-IF116 ΔHinA- Iniker fwGGCCAAATGTGAAGTTTCCATAGAAGACAGC	IEI16 M472 A	IFI16 M472A fw	CGTTCGCGACCAGCATAGG
$\begin{split} & \mbox{IF116 V615A} & \begin{tabular}{ c c c c c c c } & \mbox{IF116 V615A fw} & \end{tabular} & \end{tabuar} & \end{tabular} & \end{tabular} & \end{tabular} & tabu$	IF110 W14/2A	IFI16 M472A rev	CCTATGCTGGTCGCGAACG
IFI16 V613AIFI16 V615A revGGAGTTGCGCTGGCACTTCIF116 S409AIFI16 S409A fwCTACCCCAGGAACAGAGACAGCAGCTTCIF116 S409AIFI16 S409A revGAAGCTGTCTCTGTTCCTGGGGTAGIF116 N413YIFI16 N413Y fwGCTTCCATACCCTTCAGAGGCCIF116 N413YIFI16 N413Y revGGCCTCTGAAGGGTATGGAAGCIF116 ΔNLS1IFI16 ΔNLS1 fwGCCCTATCAGAAGTGGATGCTACTTCACCTGIF116 ΔNLS1IFI16 ΔNLS1 revCATCCACTTCTGATAGGGCTGGTCCTTTACIF116 ΔNLS2IFI16 ΔNLS2 fwGGAGCTCAGTCAACCAAAGAAAAGGCTGGAIF116 ΔNLS2IFI16 ΔNLS2 revCTTTGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIFI16 ΔPyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCCTA TCIF116 ΔPyrinIFI16 ΔPyrin-linkerCGTCTAGACCATGCAGGTAACTCCCAGAAGAAIF116 ΔHinAIFI16 ΔHinA fwGTGGCCAAATGTCAGATACTGAAGAAAGGG AGTCATTTCIF116 ΔHinAIFI16 ΔHinA revCTTCAGTATCTGACATTGGCCACTGTTTCG GIF116 ΔHinA-IfFI16 ΔHinA- 		IFI16 V615A fw	GAAGTGCCAGCGCAACTCC
$\begin{split} \mbox{IF116 S409A} & \begin{tabular}{ c c c c c } IF116 S409A fw & CTACCCCAGGAACAGAGACAGCTTC \\ \hline IF116 S409A rev & GAAGCTGTCTCTGTTCTGGGGTAG \\ \hline IF116 N413Y & \end{tabular} & GCTTCCATACCCTTCAGAGGCC \\ \hline IF116 A13Y rev & GGCCTCTGAAGGGTATGGAAGC \\ \hline IF116 ANLS1 & \end{tabular} & GCCTATCAGAAGTGGATGCTACTTCACCTG \\ \hline IF116 \DeltaNLS1 & \end{tabular} & GCCTATCAGAAGTGGATGCTACTTCACCTG \\ \hline IF116 \DeltaNLS2 & \end{tabular} & GGAGCTCAGTCAACAAAGAAAAGGCTGGA \\ \hline IF116 \DeltaNLS2 & \end{tabular} & GGAGCTCAGTCAACAAAGAAAAGGCTGGA \\ \hline IF116 \DeltaNLS2 & \end{tabular} & CTTTGGTTGACTGAGCTCCAGGAGTTGCCTC \\ \hline IF116 \DeltaPyrin & IF116 \DeltaPyrin fw & CGTCTAGACCATGGTAAAAGGACCAGCCTA \\ \hline IF116 \DeltaPyrin & \end{tabular} & \end{tabular} & CGTCTAGACCATGCAGGTAACTCCAGAAAGA \\ \hline IF116 \DeltaPyrin & IF116 \DeltaPyrin-linker & CGTCTAGACCATGCAGGTAACTCCAGAAAGA \\ \hline IF116 \DeltaHinA & \end{tabular} & \en$	IF110 V013A	IFI16 V615A rev	GGAGTTGCGCTGGCACTTC
IFI16 S409AIFI16 S409A revGAAGCTGTCTCTGTTCCTGGGGTAGIFI16 N413YIFI16 N413Y fwGCTTCCATACCCTTCAGAGGCCIFI16 N413YIFI16 N413Y revGGCCTATCAGAAGTGGAAGCIFI16 ΔNLS1IFI16 ΔNLS1 fwGCCCTATCAGAAGTGGATGCTACTTCACCTG CACIFI16 ΔNLS1FWCATCCACTTCTGATAGGGCTGGTCCTTTTACIF116 ΔNLS2IFI16 ΔNLS2 fwGGAGCTCAGTCAACCAAAGAAAAGGCTGGA CIF116 ΔNLS2IFI16 ΔNLS2 revCTTTGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIFI16 ΔNLS2 revCTTTGGTTGACCATGGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIFI16 ΔPyrin fwCGTCTAGACCATGCAGGTAACTCCCAGAAGAIF116 ΔPyrinIFI16 ΔPyrin-linkerCGTCTAGACCATGCAGGTAACTCCCAGAAGAIF116 ΔHinAIFI16 ΔHinA fwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinAIFI16 ΔHinA revCTTCAGTATCTGACATTTGGCCACTGTTTTCGIF116 ΔHinA-IFI16 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGACAGTIFI16 ΔHinA-IFI16 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIFI16 ΔHinA-IFI16 ΔHinA-GCCCAATGTGAAGTTTCCATAGAAGACAGTIFI16 ΔHinA-IFI16 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIFI16 ΔHinA-IFI16 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIFI16 ΔHinA-IFI16 ΔHinA-GCCAAATGTGAAGTTTCCATAGAAGACAGTIFI16 ΔHinA-IFI16 ΔHinA-GCCAAATGTGAAGTTTCCATAGAAGACAGTIFI16 ΔHinA-IFI16 ΔHinA-GCCAAATGTGAAGTTTCCATAGAAGACAGT		IFI16 S409A fw	CTACCCCAGGAACAGAGACAGCTTC
IF116 N413YIF116 N413Y fwGCTTCCATACCCTTCAGAGGCCIF116 N413Y revGGCCTCTGAAGGGTATGGAAGCIF116 ΔNLS1IF116 ΔNLS1 fwGCCTATCAGAAGTGGATGCTACTTCACCTG CACIF116 ΔNLS1IF116 ΔNLS1 revCATCCACTTCTGATAGGGCTGGTCCTTTACIF116 ΔNLS2IF116 ΔNLS2 fwGGAGCTCAGTCAACCAAAGAAAAGGCTGGA CIF116 ΔNLS2IF116 ΔNLS2 revCTTTGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIF116 ΔPyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCCTA TCIF116 ΔPyrinIF116 ΔPyrin-linkerCGTCTAGACCATGCAGGTAACTCCAGAAGA AATGTTCIF116 ΔHinAIF116 ΔHinA fwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinAIF116 ΔHinA revCTTCAGTATCTGACATTTGGCCACTGTTTCG GIF116 ΔHinA-IF116 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA FwGTCATTTGGAAACTTCCATAGAAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GCCCAAATGTGAAGTTTCCATAGAAGACAGT	IF116 S409A	IFI16 S409A rev	GAAGCTGTCTCTGTTCCTGGGGTAG
IF116 N413 YIFI16 N413 Y revGGCCTCTGAAGGGTATGGAAGCIF116 ΔNLS1IF116 ΔNLS1 fwGCCCTATCAGAAGTGGATGCTACTTCACCTG CACIF116 ΔNLS1IF116 ΔNLS1 revCATCCACTTCTGATAGGGCTGGTCCTTTACIF116 ΔNLS2IF116 ΔNLS2 fwGGAGCTCAGTCAACCAAAGAAAAGGCTGGA CIF116 ΔNLS2IF116 ΔNLS2 revCTTTGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIF116 ΔPyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCCTA TCIF116 ΔPyrinIF116 ΔPyrin-linkerCGTCTAGACCATGCAGGTAACTCCCAGAAGA AATGTTCIF116 ΔHinAfwATGTTCIF116 ΔHinA fwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinA fwIF116 ΔHinA revIF116 ΔHinAGGCCAAATGTGAAGTTTCCATAGAAGAAGGG GIF116 ΔHinAIF116 ΔHinA-IF116 ΔHi		IFI16 N413Y fw	GCTTCCATACCCTTCAGAGGCC
IF116 ΔNLS1IF116 ΔNLS1 fwGCCCTATCAGAAGTGGATGCTACTTCACCTG CACIF116 ΔNLS1 revCATCCACTTCTGATAGGGCTGGTCCTTTACIF116 ΔNLS2IF116 ΔNLS2 fwGGAGCTCAGTCAACCAAAGAAAAGGCTGGA CIF116 ΔNLS2IF116 ΔNLS2 revCTTTGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIF116 ΔPyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCCTA TCIF116 ΔPyrin-IF116 ΔPyrin-linkerCGTCTAGACCATGCAGGTAACTCCCAGAAGA AATGTTCIF116 ΔHinAIF116 ΔHinA fwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinAIF116 ΔHinA revCTTCAGTATCTGACATTTGGCCACTGTTTCG GIF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-IF116 ΔHinA-IF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGACAGT	IF110 N413Y	IFI16 N413Y rev	GGCCTCTGAAGGGTATGGAAGC
IFI16 ΔNLS1CACIF16 ΔNLS1 revCATCCACTTCTGATAGGGCTGGTCCTTTACIF16 ΔNLS2GGAGCTCAGTCAGTCAACAAAGAAAGGCTGGAIF116 ΔNLS2 revCTTTGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIF116 ΔPyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCCTA TCIF116 ΔPyrin-IF116 ΔPyrin-linkerCGTCTAGACCATGCAGGTAACTCCCAGAAGA AATGTTCIF116 ΔHinAfwATGTCCIF116 ΔHinAFI16 ΔHinA fwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGAACAGT Inker fwIF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-IF116 ΔHinA-Iinker fwGG		IFI16 ΔNLS1 fw	GCCCTATCAGAAGTGGATGCTACTTCACCTG
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	IFI16 ANLS1		CAC
IF116 ΔNLS2IF116 ΔNLS2 fwGGAGCTCAGTCAACCAAAGAAAAGGCTGGA CIF116 ΔNLS2 revCTTTGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIF116 ΔPyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCCTA TCIF116 ΔPyrin-IF116 ΔPyrin-linkerCGTCTAGACCATGCAGGTAACTCCCAGAAGA AATGTTCIF116 ΔHinAfwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinAFI16 ΔHinA fwGTGGCCAAATGTGACATTTGGCCACTGTTTCG GIF116 ΔHinAIF116 ΔHinA revCTTCAGTATCTGACATTTGGCCACTGTTTCG GIF116 ΔHinA-IF116 ΔHinA-GGCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA-		IFI16 ΔNLS1 rev	CATCCACTTCTGATAGGGCTGGTCCTTTTAC
IFI16 ΔNLS2 C IFI16 ΔNLS2 rev CTTTGGTTGACTGAGCTCCAGGAGTTGCCTC IFI16 ΔPyrin IFI16 ΔPyrin fw CGTCTAGACCATGGTAAAAGGACCAGCCCTA TC IFI16 ΔPyrin IFI16 ΔPyrin-linker CGTCTAGACCATGCAGGTAACTCCCAGAAGA ACTGTTC IFI16 ΔHinA fw AGTCATTTC IFI16 ΔHinA IFI16 ΔHinA rev CTTCAGTATCTGACATTTGGCCACTGTTTCG G IFI16 ΔHinA- IFI16 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT IFI16 ΔHinA- IFI16 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT IFI16 ΔHinA- IFI16 ΔHinA- IFI16 ΔHinA- IFI16 ΔHinA- IFI16 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT IFI16 ΔHinA- IFI16 ΔHinA- IFI16 ΔHinA- Iinker fw G G		IFI16 ΔNLS2 fw	GGAGCTCAGTCAACCAAAGAAAAGGCTGGA
IFI16 ΔNLS2 revCTITGGTTGACTGAGCTCCAGGAGTTGCCTCIF116 ΔPyrinIFI16 ΔPyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCCTA TCIF116 ΔPyrin-IFI16 ΔPyrin-linker fwCGTCTAGACCATGCAGGTAACTCCCAGAAGA AATGTTCIF116 ΔHinAfwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinAIFI16 ΔHinA revCTTCAGTATCTGACATTTGGCCACTGTTTCG GIF116 ΔHinA- linker fwIFI16 ΔHinA- GGGCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA- linker fwCTATGGAAACTTCACATTTGGCCACTGTTTCC	IFI16 ΔNLS2		
IFI16 ΔPyrinIFI16 ΔPyrin fwCGTCTAGACCATGGTAAAAGGACCAGCCCTA TCIF116 ΔPyrin-IFI16 ΔPyrin-linkerCGTCTAGACCATGCAGGTAACTCCCAGAAGA AATGTTCInkerfwCGTCTAGACCATGCAGGTAACTCCCAGAAGA AATGTTCIF116 ΔHinAfwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinAIF116 ΔHinA revCTTCAGTATCTGACATTTGGCCACTGTTTCG GIF116 ΔHinA-IF116 ΔHinA- linker fwGGCCAAATGTGAAGTTCCATAGAAGACAGTIF116 ΔHinA-IF116 ΔHinA- linker fwGCCCAATGTGAAGTTTCCATAGAAGACAGT		IFI16 ANLS2 rev	
IF116 InkerΔPyrin- IF116 ΔPyrin-linkerICIF116 MinArIF116 ΔPyrin-linker fwCGTCTAGACCATGCAGGTAACTCCAGAAGA AATGTTCIF116 ΔHinAIF116 ΔHinA fwGTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTCIF116 ΔHinArIF116 ΔHinA revCTTCAGTATCTGACATTTGGCCACTGTTTCG GIF116 ΔHinA- linker fwIF116 ΔHinA- GGGCCAAATGTGAAGTTTCCATAGAAGACAGTIF116 ΔHinA- linker fwCTATGGAAACTTCACATTTGGCCACTGTTTCC	IFI16 ∆Pyrin	IFII6 ΔPyrin fw	
IFI10 ΔFynin- IFI10 ΔFynin-linker COTCTAGACCATOCAGOTAACTCCCAGAAAGA linker fw AATGTTC IFI16 ΔHinA fw GTGGCCAAATGTCAGATACTGAAGGAAGGG IFI16 ΔHinA fw CTTCAGTATCTGACATTTGGCCACTGTTTCG IFI16 ΔHinA rev CTTCAGTATCTGACATTTGGCCACTGTTTCG IFI16 ΔHinA- IFI16 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT IFI16 ΔHinA- Inker fw G Iinker IFI16 ΔHinA- CTATGGAAACTTCACATTTGGCCACTGTTTCC	IEI16 ADurin	IEI16 ADurin linkor	
Initial Initial file IFI16 ΔHinA IFI16 ΔHinA fw GTGGCCAAATGTCAGATACTGAAGGAAGGG AGTCATTTC IFI16 ΔHinA IFI16 ΔHinA rev CTTCAGTATCTGACATTTGGCCACTGTTTCG G IFI16 ΔHinA- IFI16 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT IFI16 ΔHinA- Inter fw G IFI16 ΔHinA- Inter fw G	$\lim_{n\to\infty} \Delta r y \lim_{n\to\infty} \Delta r y $	fw	AATGTTC
IF116 ΔHinA IF116 ΔHinA rev CTTCAGTATCTGACATTGGCCACTGTTTCG IF116 ΔHinA- IF116 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT IF116 ΔHinA- IF116 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT Iinker fw G G		IFI16 AHinA fiv	GTGGCCAAATGTCAGATACTGAAGGAAGGG
IFI16 ΔHinA IFI16 ΔHinA rev CTTCAGTATCTGACATTTGGCCACTGTTTTCG IFI16 ΔHinA- IFI16 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT IFI16 ΔHinA- Inker fw G Iinker IFI16 ΔHinA- CTATGGAAACTTCACATTTGGCCACTGTTTTC	IFI16 ∆HinA		AGTCATTTTC
IFI16 ΔHinA- G IFI16 ΔHinA- IFI16 ΔHinA- Iinker fw G Iinker IFI16 ΔHinA-		IFI16 AHinA rev	CTTCAGTATCTGACATTTGGCCACTGTTTTCG
IFI16 ΔHinA- IFI16 ΔHinA- GGCCAAATGTGAAGTTTCCATAGAAGACAGT IFI16 ΔHinA- G G Linker IFI16 ΔHinA- CTATGGAAACTTCACATTTGGCCACTGTTTTC			G
IFI16 ΔHinA- linker fw G		IFI16 ΔHinA -	GGCCAAATGTGAAGTTTCCATAGAAGACAGT
linker IFU6 AHinA- CTATGGAAACTTCACATTTGGCCACTGTTTTC	IFI16 ∆HinA-	linker fw	G
	linker	IFI16 ∆HinA-	CTATGGAAACTTCACATTTGGCCACTGTTTTC
linker rev		linker rev	
IFI16 Δ HinB IFI16 Δ HinB rev CTACGCGTTAAGCGTAATCTGGAACATCGTA	IFI16 ∆HinB	IFI16 ΔHinB rev	CTACGCGTTAAGCGTAATCTGGAACATCGTA
TGGGTAGTTTGTTTTTTTTTTGG			TGGGTAGTTTGTTTTTTTTTTTTTTTGG
IFI16 Pyrin-linker IFI16 Pyrin-linker CTACGCGTTAAGCGTAATCTGGAACATCGTA	IFI16 Pyrin-linker	IFI16 Pyrin-linker	CTACGCGTTAAGCGTAATCTGGAACATCGTA
rev TGGGTAACATTTGGCCACTGTTTTC		rev	IGGGTAACATTIGGCCACIGTTTTC
IFI16 Pyrin-only IFI16 Pyrin-only CTACGCGTTAAGCGTAATCTGGAACATCGTA	IFI16 Pyrin-only	IFI16 Pyrin-only	CTACGCGTTAAGCGTAATCTGGAACATCGTA
		rev	

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

	1	
IFI16 + SV40	IFI16 + SV40 NLS	ATCACGCGTTTACACCTTCCGCTTCTTCTCG
NLS	rev	GGAAGAAAAAGTCTGGTGAAGTTTCC
IFI16 + Rev NES	IFI16 + Rev NES	ATCACGCGTTTAAAGAGTAAGTCTCTCAAGC
	rev	GGTGGTAGGAAGAAAAAGTCTGGTGAAGTTT
		CC
	IFI16 NLS1 mut	ATCAGCCGCTGCCGCCGCTGAAGTGGATGCT
IEI16 NI S1 mut	fw	ACTTCACCTG
IFIIO NLSI mut	IFI16 NLS1 mut	CTTCAGCGGCGGCAGCGGCTGATAGGGCTGG
	rev	TCCTTTTAC
	IFI16 NLS2/3/4	ACCGCCGAAGCTGCTGGACCCGCCGGGAGTG
IFI16 NLS2/3/4	mut fw	CCGTGTCCGAGGAACAGACTCAG
mut	IFI16 NLS2/3/4	GGGTCCAGCAGCTTCGGCGGTTGATGCAGCG
	mut rev	GCTGCCTGAGCTCCAGGAGTTGCC
IEI16 K 00 A	IFI16 K99A fw	CAAGAAAGAGGGCGAAGGAAGTG
II'II0 K99A	IFI16 K99A rev	CACTTCCTTCGCCCTCTTTCTTG
IEI16 V 199A	IFI16 K128A fw	GAGCTCAGGCAAGAAAAAAATCAACCAAAG
IF110 K120A	IFI16 K128A rev	CTTTGGTTGATTTTTTTTTTCTTGCCTGAGCTC
	cpz IFI16 XbaI fw	CTTCTAGACCATGGAAAAAAAAAAAAAAAAAAAAAAAAA
	1	ATTGTTCTACTG
cpz IFI16 C-HA	cpz IFI16 C-HA	CTACGCGTTAAGCGTAATCTGGAACATCGTA
	MluI rev	TGGGTAGAAGAAAAAGTCTGGTGAAGTTTCC
		ATAC
	agm IFI16 XbaI fw	CTTCTAGACCATGGAAAAAAAATACAAGAAC
		ATTGTTCTACTG
agm IFI16 C-HA	agm IFI16 C-HA	CTACGCGTTAAGCGTAATCTGGAACATCGTA
	MluI rev	TGGGTAGAAAAAAAAGTCTGGTGAAGTTTCC
		ATAC
mac IFI16 C-HA	mac IFI16 XbaI fw	CTTCTAGACCATGGAAAAAAAAAAAAAAAAAAAAAAAAA
		ATIGITCTACIG
	mac IFI16 C-HA	
	MluI rev	
	204 3/1 1 0	
р204 С-НА	p204 Xbal fw	CGICIAGACCATGGIGAATGAATACAAGAG
	p204 C-HA Mlul	CTACGCGTTAAGCGTAATCTGGAACATCGTA
	rev	TGGGTACTITCTAGCATIGATGACC
AIM2 C-HA	AIM2 XbaI fw	CGTCTAGACCATGGAGAGTAAATACAAGG
	AIM2 C-HA MluI	CTACGCGTTAAGCGTAATCTGGAACATCGTA
	rev	TGGGTATGTTTTTTTTTTGGCCTTAATAAC
STING	STING XbaI fw	CGTCTAGACCATGCCCCACTCCAGC
	STING MluI rev	CTACGCGTTCAAGAGAAATCCGTGCG
Sp1 C-HA	Sp1 XbaI fw	CATCTAGACCATGAGCGACCAAGATCACTCC
		ATG
	Sp1 C-HA MluI	CGAACGCGTTCAAGCGTAATCTGGAACATCG
	rev	TATGGGTAGAAGCCATTGCCACTGATATTAA
		TGGAC

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	CGTCTAGACCATGGGAAAAAAATACAAGA
	HIV-1 LTR XhoI rev	CTACGCGTTTAGAAGAAAAAGTCTGGTGA AGTTTCCATAC

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

Construct name	Primer name	Primer sequence
	THRO_NotI_5'LTR_for	GCATGCTCGAGCGGCCGCCAGTGTGATG
	THRO_5'LTR in	
	CH058_OE_rev	CTCTAGCAGTGGCGCCCGAACAGGGACCTG
CH058	THRO 5'LTR in	GTCAGTGTGGAAAATCTCTAGCAGTGGCGC
with LTRs	CH058_OE_fw	CCG
of THRO	CH058_BstZ17I_5'LTR_rev	GATGAAGACTTCAGGAAGTATACGCCGAAT TGGGCCCTCTAGATGCATGCTCGA
	CH058_StuI_3'LTR_fw	CGAGGCCTGTCCAAAGGTATCTTTTCAGC
	THRO 3'LTR in	GGACTGGAAGGGCTAACTTACTCCCAAAAG
	CH058 OE rev	AG
	THRO_3'LTR in	GATCTTAGCCACTTTTTAAAAGAAAAGGGG
	CH058_OE_fw	GGACTGGAAGG
	THRO_MluI_3'LTR_rev	GCTTGATGCATAGCTTGAGTATTCTAACGCG TCAC
	THRO Notl 5'LTR fw	GCATGCTCGAGCGGCCGCCAGTGTGATG
	THRO 5'LTR in	
	CH040_OE_rev	CTCTAGCAGTGGCGCCCGAACAGGGACTTG
	THRO 5'LTR in	GTCAGTGTGGAAAATCTCTAGCAGTGGCGC
CH040	CH040_OE_fw	CCG
with LTRs	CH040_AgeI_5'LTR_rev	GAGACTATGTAGACCGGTCG
of THRO	CH040_BlpI_3'LTR_fw	CGGCTGAGCCAGCAGCAGAG
	THRO_3'LTR in	GGACTGGAAGGGCTAACTTACTCCCAAAAG
	CH040_OE_rev	AG
	THRO_3'LTR in	GATCTTAGCCACTTTTTAAAAGAAAAGGGG
	CH040_OE_fw	GGACTGGAAGG
	THRO_MluI_3'LTR_rev	GCTTGATGCATAGCTTGAGTATTCTAACGCG TCAC
THRO with LTRs of CH058	CH058_NotI_5'LTR_fw	CTAGATGCATGCTCGAGCGGCCGCTGGAAG
	CH058_5'LTR in	
	THRO_OE_rev	CTCTAGCAGTGGCGCCCGAACAGGGACC
	CH058_5'LTR in	CAGTGTGGAAAATCTCTAGCAGTGGCGCCC
	THRO_OE_fw	GAAC
	CH042_HpaI_5'LTR_rev	GCCCACACTAATGATGTAAAACAGTTAACA
		GAGGCAGTGC
	THRO_BbvCI_3'LTR_fw	GCTATAGCAGTAGCTGAGGGGACAGATAG
	CH058_3'LTR in	GGGGACTGGAAGGGCTAATTCACTCCCAGA
	THRO_OE_rev	AAAGAC
	CH058_3'LTR in	GCCACTTTTTAAAAGAAAAGGGGGGGGACTGG
	THRO_OE_fw	AAGGGC
	CH058_MluI_3'LTR_rev	GATGCATAGCTTGAGTATTCTAACGCGTCAC

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	<i>nef</i> for	GGTGGGAGCAGYATCTCGAGA
	<i>nef</i> rev	TGTAAGTCATTGGTCTTAAAGGTACCTGAGG
	<i>nef</i> probe FAM/BBQ	6FAM-
		GCTTCYAGCCAGGCACAAKCAGCATT-BBQ
U3-polyA	U3-polyA for	GCCCTCAGATGCTRCATATAA
	U3-polyA rev	TTTTTTTTTTTTTTTTTTTTTTTTTGAAG
	U3-polyA FAM/BBQ	6FAM-TGCCTGTACTGGGTCTCTCTGGTTAG-
		BBQ

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