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The role of MxB in interferon-α mediated inhibition of HIV-1 infection

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Abstract

Type I interferon inhibits viruses through inducing the expression of antiviral proteins, including the myxovirus resistance (Mx) proteins. Compared to human MxA protein that inhibits a wide range of viruses, the MxB protein has been reported to specifically inhibit primate lentiviruses including HIV-1, and herpesviruses. Further, the role of endogenous MxB in interferon-α mediated inhibition of HIV-1 infection was questioned by a recent study showing that MxB knockout did not increase the infection of HIV-1 which carried the G protein of vesicular stomatitis virus (VSV), and thus was able to infect CD4-negative HT1080 cells. In order to further examine the anti-HIV-1 activity of endogenous MxB, we have used CRISPR/Cas9 to deplete MxB in different cell lines, and observed substantial restoration of HIV-1 infection in the presence of interferon-α treatment. However, this rescue effect of MxB knockout became much less pronounced when infection was performed with HIV-1 carrying VSV G protein. Interestingly, a CRISPR/Cas9 knockout screen of interferon-α stimulated genes in U87-MG cells revealed that the interferon induced transmembrane proteins 2 and 3 (IFITM2 and IFITM3) inhibited VSV G pseudotyped HIV-1 much more strongly than the rest of genes tested, including MxB. Therefore, our results demonstrate the importance of MxB in interferon-α mediated inhibition of HIV-1 infection, which however can be underestimated if infection is performed with VSV G protein pseudotyped HIV-1, due to the high sensitivity of VSV G mediated infection to the inhibition by IFITM proteins.

Importance. Results of this study has reconciled the controversial reports regarding the anti-HIV-1 function of interferon-α induced MxB protein. In addition to the different cell types that may have contributed to the different observations, our data also suggest that VSV G protein pseudotyped HIV-1 is much less inhibited by interferon-α induced MxB than HIV-1 itself. Our

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35 results clearly demonstrate an important contribution of MxB to interferon-α mediated inhibition
36 of HIV-1 in CD4+ T cells, which calls for using HIV-1 target cells and wild type virus to test the
37 relevance of the anti-HIV-1 activity of endogenous MxB and other restriction factors.

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Introduction

HIV-1 infection is inhibited by type I interferon (IFN) (reviewed in (1)). Several genes that are stimulated by type I IFN, called ISGs, have been reported to bear anti-HIV-1 activity (1-5), including the myxovirus resistance gene B (*MxB*) (6-8). Humans carry two *Mx* genes, *MxA* and *MxB* (also called *Mx1* and *Mx2*), both of which respond to IFN stimulation (reviewed in (9)). The antiviral function of MxA has been well received for its inhibition of a range of different viruses, including influenza viruses, human parainfluenza virus type 3, measles virus, hepatitis B virus, and others (10). Discovery of the antiviral activity of MxB came relatively late, which was made in 2013 when HIV-1 was reported subject to inhibition by MxB (6-8). Recently, human herpesviruses were also shown to be restricted by MxB (11).

MxA and MxB are large dynamin-like GTPases (reviewed in (9)). Both proteins fold into similar structures, comprising a stalk that mediates oligomerization of Mx protein, and a globular domain that bears the GTPase activity (12-15). Forming oligomers is indispensable for both MxA and MxB to restrict their target viruses. While MxA depends on its GTPase activity for its antiviral function, the GTPase activity for MxB is required to inhibit human herpesviruses but not HIV-1 (6, 8, 11-19). MxA is cytoplasmic, whereas MxB is more seen at the cytoplasmic face of the nuclear envelope. In contrast to MxA which exploits its unstructured loop 4 to recognize viral nucleoproteins (20, 21), MxB uses its N-terminal region to achieve targeting of HIV-1 capsid core (22-26).

Results of mutagenesis studies and viral escape experiments suggest that HIV-1 capsid protein is the viral target of MxB (6-8, 22, 24), which is further supported by the association of MxB with

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HIV-1 capsid assembly *in vitro* (13, 27). MxB does not affect HIV-1 reverse transcription, but rather reduces the levels of HIV-1 2-LTR circles and the integrated viral DNA (6-8), as well as alters the distribution of HIV-1 DNA integration sites in cellular DNA (24).

Several studies indicate a role of MxB in suppressing HIV-1 acquisition and replication in patients. First, significantly higher levels of MxB mRNA were reported in the highly exposed HIV seronegative Kenyan sex workers, suggesting an association of MxB with reduced HIV-1 susceptibility in this cohort (28). Second, a MxB haplotype, rs2074560, showed higher association with HIV-1 exposed seronegative individuals, likely as a result of increased MxB expression associated with rs2074560 and lower HIV-1 replication (29). Equally important, MxB-resistance mutations in viral capsid protein, such as the H87Q and G116A mutations, have high prevalence in the circulating HIV-1 strains (30-32). Moreover, the CRF08 BC strain, a result of recombination of the A116 subtype C strain and the 116G subtype B strain, carries the MxB-sensitive G116 capsid, but regains the MxB-resistant A116 mutation in more than half of its descendants after 13 years of circulating in China (31). All these findings support an inhibitory pressure of MxB on HIV-1 in patients.

However, it remains unsettled regarding the role of MxB in type I IFN-mediated inhibition of HIV-1 infection. When the anti-HIV-1 activity of MxB was reported in 2013, three labs used shRNA to knock down MxB in different cell lines, including SupT1, THP-1, U87/CD4/CXCR4 and HOS cells, and all observed substantial restoration of HIV-1 infection in the presence of IFNα (6-8). A subsequent study by Opp et al utilized Cas9/gRNA to knock out MxB in THP-1 cells and HT-1080 cells, and reported no role of MxB in IFNα-mediated inhibition of HIV-1

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infection (33). Subsequent to this report, the MxB knockout experiment in THP-1 cells was repeated by Bulli et al, and the data showed significant alleviation of HIV-1 inhibition by IFNα (34). With the aim to reconcile these inconsistent reports and further elucidate the importance of IFNα-induced MxB in controlling HIV-1 infection, we have used Cas9/gRNA to knock out MxB in different cell lines, including HT-1080, U87, and SupT1 cells. The results suggest that the anti-HIV-1 potency of IFNα-induced endogenous MxB can be cell-type dependent, with the weakest anti-HIV-1 effect in HT-1080 cells. The data also suggest that VSV G-pseudotyped HIV-1 is less sensitive to the inhibition by IFNα-induced endogenous MxB. Overall, our results further support an important role of MxB in IFNα-mediated anti-HIV-1 response in HIV-1 target cells.

Results

Knockout of MxB in HT1080 cells marginally alleviates the inhibition of HIV-1 infection by IFNα2b. We first knocked out MxB in HT1080 cells and measured the effect on HIV-1 infection. Two gRNAs were accordingly designed (Figure 1A). The gRNA1 targets exon II, downstream of the ATG start codon, which overlaps with one of the gRNAs used by Opp et al in their study (33). The gRNA2 targets exon IV. Both gRNAs were cloned into the lentiCRISPRv2 vector which carries the U6 promoter cassette to express gRNA and the EF-1α promoter to express Cas9 (35). HT1080 cells were transduced with lentiviruses that packaged the Cas9/gRNA sequence, and then subjected to selection with puromycin, to generate stably transduced cell lines. The lentiCRISPRv2 vector that does not have a gRNA inserted was used to create the control cell line. We also designed one gRNA to target *STAT1* gene. STAT1, together with STAT2, is phosphorylated by the IFNα-activated JAK1/TYK2, and activates the expression

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of ISGs (36). Therefore, depletion of STAT1 is expected to block IFNα signaling transduction and prevent the expression of ISGs. Results of western blotting of the generated cell lines showed that both MxB gRNAs efficiently depleted the expression of MxB upon IFNα2b stimulation, but did not affect the expression of STAT1, suggesting that MxB knockout does not exert a general effect on cellular response to IFNα stimulation (Figure 1B). The STAT1 gRNA depleted STAT1 expression that was enhanced by IFNα2b, and dramatically reduced MxB expression, which confirmed the impaired IFNα response due to STAT1 depletion (Figure 1B). As HT1080 cells do not express HIV-1 receptors CD4 and CXCR4/CCR5, we challenged these cells with VSV G pseudotyped HIV-1. Two doses of IFNα2b, 500 U/ml and 1000 U/ml, were used to treat HT1080 cells prior to exposure to HIV-1/VSV G. HIV-1 infectivity was determined by infecting the TZM-bl indicator cells (37). IFNα2b treatment decreased HIV-1 infection in control cells to 15% (500 U/ml IFNα2b) and 6% (1000 U/ml IFNα2b) compared to infection without IFNα2b treatment (Figure 1C). When STAT1 was depleted, HIV-1 infection, in the presence of 1000 U/ml IFNα2b, rose from 6% to 40%, indicating a significant loss of IFNα2b inhibition (Figure 1C). In contrast, IFNα2b diminished HIV-1 infection in MxB knockout cells as strongly as in the control cells (Figure 1C), which suggests that in HT1080 cells, IFNα2b-induced MxB does not contribute to the maximal inhibition of VSV G pseudotyped HIV-1 by IFNα2b, in agreement with the results by Opp et al (33).

Knockout of MxB in SupT1 cells and U87/CD4 cells significantly restores HIV-1 infection in the presence of IFNα2b. HT1080 cells are epithelial cells in nature and are not target cells for HIV-1. We therefore asked whether IFNα-induced MxB can suppress HIV-1 infection in

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CD4+ T cells, and whether HIV-1, without the VSV G protein, is more susceptible to inhibition by IFNα2b-induced MxB. We chose to knockout MxB in a CD4+ T cell line SupT1(Figure 2A) and then, compared the response of HIV-1 to IFNα2b treatment in the control and the MxB knockout SupT1 cell lines. We also depleted STAT1 using Cas9/gRNA, and observed that depletion of STAT1, which impairs cellular response to IFNα, restored HIV-1 infection under IFNα2b treatment from 10% in the control cells to 60% in STAT1 knockout cells (Figure 2B). Depletion of MxB by either gRNA1 or gRNA2 elevated HIV-1 infection in the presence of IFNα2b from 10% to 40% (Figure 2B), which indicates a marked attenuation of IFNα2b-mediated HIV-1 inhibition as a result of MxB knockout. Interestingly, when the infection was performed with HIV-1 carrying the VSV G protein, although depletion of STAT1 still effectively rescued HIV-1 infection, knockout of MxB increased HIV-1 infection from 10% to only 20%, which is significantly lower than the 40% restoration when infection was performed with HIV-1 itself (Figure 2B). These results suggest that MxB plays a major role in IFNα-mediated inhibition of HIV-1 in T cells. This inhibitory role of MxB becomes less pronounced against HIV-1 that is pseudotyped with VSV G protein.

We further investigated the anti-HIV-1 activity of MxB in U87 cells, a human glioblastoma cell line. When MxB was knocked out in U87/CD4/CXCR4 cells (Figure 2C), HIV-1 infection in the presence of IFNα2b rose from 10% (control cells) to 40% (MxB gRNA1) and 30% (MxB gRNA2), an increase similar to that when STAT1 was knocked out (Figure 2D). However, marginal increases were observed in MxB knockout U87/CD4/CXCR4 cells when infection was performed with HIV-1 carrying VSV G, in contrast to an increase from 5% (control cells) to 40% as a result of STAT1 knockout. Similar results were obtained when MxB was knocked out in

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U87/CD4/CCR5 cells, and infections were performed with either the R5-tropic HIV-1 strain AD8-1 or AD8-1 carrying VSV G protein (Figure 2E and 2F). These data further demonstrate a strong inhibition of HIV-1 by IFNα-induced MxB, but this inhibition is weakened against VSV G-pseudotyped HIV-1.

The MxB-resistant capsid mutation H87Q is less inhibited by IFNα2b. Mutations in HIV-1 capsid mediate resistance to MxB (6-8). We therefore chose to test two such capsid mutations, H87Q and G208A (22, 30), for their ability to resist IFNα2b inhibition. The results showed that the G208A mutant was inhibited by IFNα2b in SupT1 cells to similar degrees as the wild type HIV-1 (Figure 2G). In contrast, IFNα2b decreased the infection of the H87Q mutant to only 40% of that without exposure to IFNα2b, which is significantly higher than the 15% observed for wild type HIV-1 (Figure 2G). Again, knockout of MxB significantly increased the infection of wild type HIV-1 and the G208A mutant in the presence of IFNα2b treatment (Figure 2G). These results suggest that some MxB-resistance capsid mutations render HIV-1 resistant to IFNα inhibition.

Knockout of MxB alleviates the inhibition of HIV-1 reporter viruses by both IFNα and IFNβ. Given that MxB impairs the nuclear import of HIV-1 DNA (6, 8), we further investigated the effect of MxB knockout on IFNα2b-mediated inhibition of HIV-1 using reporter viruses NLENY1 and NLENY1-ES/VSV G which express YFP and allow quantification of the infected cells by flow cytometry (38). Virus NLENY1 carries HIV-1 Env protein, whereas NLENY1-ES/VSV-G is pseudotyped with VSV G protein. Results of Figure 3 show that IFNα2b led to a 2-fold reduction of SupT1 cells that were infected either by NLENY1 or NLENY1-ES/VSV G.

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Knockout of STAT1 ablated IFNα2b-mediated inhibition of both viruses. As opposed to a nearly complete rescue of NLENY1 virus infection from IFNα2b inhibition by MxB knockout, the rescue effect on NLENY1-ES/VSV G was much less pronounced (Figure 3). We further tested IFNβ on these two HIV-1 reporter viruses. As expected, MxB protein was induced in the control SupT1 cells by IFNβ, but not expressed in the MxB knockout or STAT1 knockout SupT1 cell lines (Figure 4A). Infection with both NLENY1 and NLENY1-ES/VSV G decreased 2-fold with IFNβ treatment (Figure 4B and 4C). This inhibition was lost in MxB knockout cells for the NLENY1 virus, but persisted for NLENY1-ES/VSV G. These data further demonstrate that MxB has an important role in type I interferon mediated control of HIV-1 infection, including both IFNα and IFNβ, albeit the role of MxB is less prominent when HIV-1 is pseudotyped with VSV G protein.

Ectopically expressed MxB inhibits HIV-1 carrying VSV G protein. We next asked whether MxB inhibits HIV-1 to a lesser degree when the virus carries VSV G protein. This may explain why VSV G pseudotyped HIV-1 responded poorly to the rescue by MxB depletion in the presence of IFNα treatment. To this end, we used a SupT1 cell line expressing exogenous MxB protein upon induction by doxycycline (7). We treated the MxB SupT1 cell lines with a range of doxycycline concentrations, and induced MxB expression from low (induced by 5 ng/ml doxycycline) to high (induced by 100 ng/ml) levels of MxB (Figure 5A). When the treated cells were infected with either NL4-3 or NL4-3 carrying VSV G protein, expression of viral Gag and p24/CA proteins diminished gradually along with increasing MxB expression, indicating suppression of viral infection (Figure 5A and 5B). We also performed western blotting to measure the level of endogenous MxB in SupT1 cells upon treatment with IFNα2b (500 U/ml),

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198 and found that its level was equivalent to the MxB amount that was induced by doxycycline of 5
199 ng/ml to 10 ng/ml in the MxB SupT1 cell line (Figure 5C). We then measured the levels of
200 infectious HIV-1 particles that were produced by the infected SupT1 cells. Doxycycline did not
201 markedly affect HIV-1 production from control SupT1 cells that did not express exogenous MxB
202 (Figure 5D). The doxycycline-induced MxB inhibited both HIV-1 NL4-3 and HIV-1 NL4-3
203 carrying VSV G to similar degrees, across all doxycycline concentrations tested (Figure 5D).
204 The inhibition of HIV-1 by exogenous MxB expression was slightly greater than inhibition of
205 HIV-1 carrying VSV G, but the differences were not significant. These data suggest that MxB,
206 when ectopically expressed in the absence of other IFNα induced ISGs, inhibits HIV-1 and VSV
207 G pseudotyped HIV-1 equally well.
208
209 **VSV G mediated infection is strongly suppressed by IFNα-induced IFITM proteins.** Since
210 VSV G pseudotyped HIV-1 is strongly suppressed by IFNα and knockout of MxB only slightly
211 reduces this inhibition, we were interested in knowing which IFNα induced protein(s) play the
212 major inhibitory role. To this end, we designed gRNAs to target 53 ISGs which were reported to
213 be upregulated by IFNα in U87-MG cells by more than 3-fold (8). In order to achieve efficient
214 knockout, three gRNAs were designed for each ISG (Table S1). The three gRNAs of each ISG
215 were pooled together to transduce the U87-MG cells. The stably transduced cell lines, selected
216 with puromycin, were treated with IFNα2b to induce the expression of ISGs, prior to the
217 challenge with VSV G-pseudotyped HIV-1. HIV-1 infection was monitored by measuring levels
218 of infectious HIV-1 in the culture supernatants through infecting the TZM-bl indicator cells. We
219 calculated IFNα2b inhibition of HIV-1 in each ISG knockout cell line by dividing HIV-1
220 production in the presence of IFNα2b treatment with HIV-1 production without IFNα2b

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treatment. We set this value from the control cells as 1 and presented the data for all ISG knockout cell lines in Figure 6A. As the positive control of this screen study, STAT1 was knocked out, which led to 25 to 30-fold restoration of HIV-1 infection in the presence of IFNα2b (Figure 6A). In agreement with the data in Figure 2, MxB knockout only moderately relieved IFNα2b inhibition of HIV-1/VSV G (Figure 6A). Remarkably, knockout of IFITM3 increased HIV-1/VSV G infection in the presence of IFNα by 10-fold, knockout of IFITM2 led to a 5-fold increase (Figure 6A), an enhancement of HIV-1/VSV G infection that is markedly higher than knocking out any of the other ISGs. Knockout of the majority of the ISGs did not alleviate inhibition of HIV-1 infection by IFNα2b. In addition, knockout of some ISGs, including ISG15, USP18, DHX58 and IFI44L, moderately enhanced IFNα-mediated inhibition, which suggests a possible negative regulation of interferon response by these ISGs.

To confirm the dominant contribution of IFITM2 and IFITM3 to IFNα2b-mediated inhibition of HIV-1/VSV G infection, we generated cell lines which were stably transduced with one gRNA for IFITM2 or IFITM3. The IFITM2 gRNA1 and gRNA3 efficiently depleted IFITM2 (Figure 6B) and increased HIV-1/VSV G infection in the presence of IFNα2b by 5-fold (Figure 6C). For IFITM3, gRNA2 diminished IFITM3 greater compared to the other two gRNAs (Figure 6B), and elevated HIV-1/VSV G infection by 8-fold (Figure 6C). When all three gRNAs for IFITM2 or IFITM3 were used, maximal depletion of each gene was observed (Figure 6B), which consequently generated the highest rescue effect on HIV-1/VSV G infection in the presence of IFNα2b, compared to when an individual gRNA was used for knockout (Figure 6C). Together, these data support the dominant role of IFITM3, and IFITM2, in IFNα2b-mediated inhibition of VSV G-pseudotyped HIV-1.

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Discussion

Results of this study demonstrate that depletion of MxB with Cas9/gRNA markedly alleviates IFNα-mediated inhibition of HIV-1 infection. This significant role of MxB in the anti-HIV-1 response by IFNα is also supported by several studies that used shRNA or Cas9/gRNA to deplete endogenous MxB (6-8). Particularly, Bulli et al. recently used two gRNAs to knock out MxB in THP-1 cells, and observed a significant increase of HIV-1 infection in the presence IFNα treatment (34), which is in agreement with the observation of using shRNA to deplete MxB in THP-1 cells (6, 8). Further, we observed that this role of MxB can be underestimated when the VSV G protein is used to pseudotype HIV-1, with the possibility of no effect of MxB depletion on IFNα inhibition of HIV-1 infection in some cell lines including HT1080 cells. The inhibitory pressure of endogenous MxB in the context of IFNα stimulation, as observed in tissue culture studies by multiple groups, is further supported by the association of high MxB levels with low HIV-1 acquisition *in vivo* (28, 29) and the prevalence of MxB-resistance capsid mutations in the circulating HIV-1 strains (30-32). The role of MxB in type I interferon mediated antiviral defense is also supported by the recent report showing that knockdown of MxB markedly rescues the infection of herpes simplex virus type 1 (HSV-1) in the presence of IFNα treatment (11). Further study is warranted to investigate the contribution of MxB to interferon mediated inhibition of viruses in primary cells, including knockout of MxB in human PBMCs (peripheral blood mononuclear cells) and primary CD4+ T cells. Technical challenges include the relatively low transfection and transduction efficiency of primary cells, and the difficulty to select for and enrich knockout primary cells as often practiced with transformed cell lines.

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MxB strongly suppresses VSV G pseudotyped HIV-1 when ectopically expressed in the absence of other ISGs (33). In agreement with this, when we compared MxB inhibition of HIV-1 with or without VSV G pseudotyping, we only observed modestly less suppression of VSV G pseudotyped HIV-1 compared to HIV-1 itself, along a range of MxB expression levels that were induced with different doses of doxycycline (Figure 5). We suspect that in the context of IFNα stimulation, other ISGs might have influenced the anti-HIV-1 effect of the endogenous MxB. In support of this possibility, results from our knockout screen of ISGs in U87-MG cells showed a dominant inhibition of VSV G-pseudotyped HIV-1 by IFNα induced IFITM2 and IFITM3 proteins. This observation may explain the relatively moderate increase in HIV-1/VSV G infection when the other ISGs, including MxB, were knocked out (Figure 6). This is likely because IFITM2 and IFITM3 are so potent in inhibiting VSV G-mediated infection that HIV-1/VSV G infection is still substantially suppressed when other anti-HIV-1 ISGs are depleted.

Mutations across the N- and C-terminal domains of the capsid protein have been reported to confer resistance to MxB at varying degrees (6-8, 22, 24, 30). One group of mutations are located to the cyclophilin A binding loop in the N-terminal domain of capsid, including mutations at amino acid positions V86, H87, G89 and P90. A second cluster is found in a solvent accessible loop between helices 10 and 11 in the C terminal domain of capsid, including amino acid positions P207 and G208. Several more MxB-resistant capsid mutations, such as the cell-cycle dependent mutation N57S, were also identified, and are predominantly located to the surface of capsid hexamer. These MxB-resistant capsid mutations modulate HIV-1 sensitivity to IFNα at different degrees. Some mutations, such as N57S, exhibit modest resistance to IFNα (6), some such as G208A do not confer any resistance in this regard (33), while others, such as N74D

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and P90A, render HIV-1 more sensitive to IFNα inhibition (34). Results of our study showed that the MxB-resistance mutation H87Q is significantly refractory to IFNα. These different responses of MxB-resistant HIV-1 mutations to IFNα may result from the diverse effects of these mutations on the functions of capsid, ranging from capsid stability, core uncoating, routes of nuclear entry, and interaction with a plethora of host factors. Thus, it is expected that in spite of the marked inhibition of HIV-1 by MxB, resistance to MxB may not always lead to resistance to IFNα. All the same, it is encouraging to observe that some of these MxB-resistance mutations, such as N57S and H87Q, do confer resistance to IFNα inhibition of HIV-1.

In summary, our data further support the important contribution of MxB to IFNα-mediated inhibition of HIV-1 and other viruses including human herpesviruses. The anti-HIV-1 activity of MxB in the context of IFNα stimulation can be underestimated when infection is performed with VSV G pseudotyped HIV-1. This is partially due to the high sensitivity of VSV G-mediated virus entry to IFNα-induced IFITM proteins, which might have shadowed the anti-HIV-1 effect of other ISGs.

Materials and methods

Plasmid DNA. The infectious HIV-1 DNA clones NL4-3 and AD8-1 were obtained from NIH AIDS Reagent Program. Site-directed mutagenesis (Clontech) was performed to generate HIV-1 NL4-3 mutants that carry capsid mutations H87Q or G208A. The LentiCRISPRv2 plasmid was purchased from Addgene. The guide RNA primers were synthesized by Invitrogen, and inserted into the BstBI site in LentiCRISPRv2. HIV-1 reporter virus DNA NLENY1-IRES and NLENY1-IRES-ES were kindly provided by David Levy (38).

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313 **Cell culture.** HEK293T, HT1080, TZM-bl, U87-MG, U87/CD4/CXCR4 and U87/CD4/CCR5
314 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented
315 with 10% fetal bovine serum (FBS) (Invitrogen), 50 U/ml penicillin, and 50 μg/ml streptomycin
316 (Invitrogen). SupT1 cells were grown in RPMI160 medium supplemented with 10% FBS, 50
317 U/ml penicillin, and 50 μg/ml streptomycin (Invitrogen). TZM-bl is an HIV indicator cell line
318 that carries the HIV-1 LTR-luc expression cassette, which responds to the transactivation by
319 HIV-1 Tat (37). HEK293T and HT1080 were purchased from ATCC. U87 cell lines were
320 obtained from NIH AIDS Reagent Program. Transfection of HEK293T cells was conducted with
321 Lipofectamine (Invitrogen) in accordance with the manufacturer's instructions.

322

323 **Generating MxB knockout cell lines.** Lentivirus particles were produced through transfecting
324 HEK293T cells using plasmid DNA clones LentiCRISPRv2 (expressing sgRNA targeting MxB),
325 psPAX2 (expressing Gag and Gag-Pol), and pVSV-G, as described in (39). Next, HT1080,
326 SupT1 and U87 cells were infected with these lentiviruses, followed by puromycin selection to
327 generate stable cell lines. The control cell line was created using the LentiCRISPRv2 vector that
328 does not express sgRNA. MxB knockout efficiency was determined by western blotting,
329 examining levels of MxB in the created cell lines with IFNα2b (500 U/ml, Sigma) to induce
330 MxB expression for 24 hours. The anti-MxB antibodies were generated as we previously
331 described (7). The sequences of MxB sgRNAs are, sgRNA1: 5'-
332 TGTGGTGGCACTGTGCCGAA-3', sgRNA2: 5'-GTTCCGGTAGCTGATCCTTC-3'. The
333 STAT1 knockout cell lines were generated using the same method. The sequence of STAT1
334 sgRNA is 5'-GCAGCTTGACTCAAAATTCC-3'.

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HIV-1 infection. HIV-1 was produced by transfecting the HEK293T cells with proviral DNA clones NL4-3, AD8-1, NL4-3/H87Q or NL4-3/G208A, or NLENY1. The pVSV-G plasmid was co-transfected to produce HIV-1 particles that carry VSV glycoprotein. Amounts of HIV-1 produced were determined either by measuring HIV-1 capsid/p24 with ELISA or measuring HIV-1 RT activity, as previously described (40). HIV-1 equivalent to 100 ng p24 was used to infect different cell lines. For HIV-1 that was pseudotyped with VSV G protein, viruses of 10 ng p24 were used for infection because of the high entry efficiency of VSV G protein. Prior to HIV-1 infection, cells were either treated with IFNα2b (Sigma) or IFNβ (Sigma) for 24 hours or left untreated. Each infection was performed in duplicate. Each experiment was independently repeated at least three times. The inoculated HIV-1 was removed 16 hours after infection. After an additional 24-hour culture, 50 μl of the culture supernatants were used to infect the TZM-bl indicator cells in duplicate. Forty hours after, cells were collected, and luciferase activity in cell lysates was measured using the Luciferase Assay System (Promega). The results represent the levels of infectious HIV-1 that were produced. For infections by NLENY1 and NLENY1-ES/VSV-G, the infected cells were harvested and fixed in 4% paraformaldehyde. Number of YFP-positive cells was scored with flow cytometry. All HIV-1 infection experiments were performed in the certified biocontainment level 3 lab at the Lady Davis Institute.

ISG knockout screen in U87-MG cells. We selected 53 ISGs whose levels increased in U87-MG cells by more than 3-fold after IFNα2b treatment, as shown in the microarray data by Goujon C et al in (8). Three sgRNAs were designed to target each of these 53 ISGs, and were cloned into LentiCRISPRv2. Sequences of all sgRNAs are listed in supplemental Table S1. For

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each ISG, viruses that encode individual sgRNA were pooled and used to transduce U87-MG cells. After selection with puromycin for stably transduced cell lines, HIV-1/VSV G (equivalent to 15 ng capsid/p24, carrying the VSV glycoprotein) was used to infect these cell lines that had been treated with IFNα2b (100 U/ml), or left untreated. Forty hours post infection, levels of infectious HIV-1 in the culture supernatants were determined by infecting TZM-bl cells. The knockout efficiency of STAT1, IFITM2 and IFITM3 was examined by western blotting. The anti-STAT1 antibody was purchased from Santa Cruz Biotechnology (C-111), anti-IFITM2 (12769-1-AP) and IFITM3 (11714-1-AP) antibodies from Proteintech Group.

Acknowledgements

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Figure legends

Figure 1. Effect of MxB depletion on IFNα2b inhibition of HIV-1/VSV G infection in HT1080 cells. (A) Illustration of the gRNA target sites (in blue) in the *MxB* gene. The first 5 exons (I to V) of MxB are shown. The boundary of adjacent exons is specified by the nucleotide positions in MxB mRNA (NM_002463.1). The start codon ATG (at nucleotide position 105) is highlighted in red. (B) Levels of MxB and STAT1 in control and Cas9/gRNA stably transduced cells, in the presence and absence of IFNα2b (500 U/ml). Levels of tubulin were also determined as the internal control. (C) Inhibition of HIV-1/VSV G infection by IFNα2b (500 U/ml and 1000 U/ml). Levels of infectious HIV-1 were determined by infecting the TZM-bl indicator cells. Suppression of HIV-1 infection in the control and knockout cell lines is calculated by dividing the values of HIV-1 production with IFNα2b treatment by those without IFNα2b treatment. Data shown are the averaged of three independent infections of HT1080 cells. Error bars indicate standard deviations. ns, not significant.

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Figure 2. Depletion of MxB in SupT1 and U87/CD4 cells rescues HIV-1 infection under IFNα2b treatment. (A) Levels of MxB and STAT1 in the control SupT1 cells and in SupT1 cells that were stably transduced with Cas9/gRNA, in the absence and presence of IFNα2b (500 U/ml). Levels of tubulin serve as the internal control. (B) IFNα2b (500 U/ml) suppression of HIV-1 infection, without or with VSV G protein pseudotyping, in the control and knockout SupT1 cells. Relative HIV-1 infection was calculated with the values in the absence IFNα2b arbitrarily set as 1. (C) Levels of MxB and STAT1 in the control and knockout U87/CD4/CXCR4 cells in the absence and presence of IFNα2b. (D) IFNα2b (100 U/ml) suppression of HIV-1 NL4-3 or NL4-3/VSV G in the control and knockout U87/CD4/CXCR4 cells. (E) Results of western blotting to show the depletion of MxB and STAT1 in U87/CD4/CCR5 cell lines that were stably transduced with Cas9/gRNA. (F) IFNα2b (100 U/ml) suppression of HIV-1 AD8-1 or AD8-1/VSV G in the control and MxB knockout U87/CD4/CCR5 cell lines. Results shown are the average of three independent infections of the knockout cell lines. (G) IFNα2b (500 U/ml) suppression of HIV-1 mutants H87Q and G208A in the control SupT1 cells or in SupT1 cells with MxB or STAT1 knockout. The level of wild type HIV-1 NL4-3 infection in the control SupT1 cells in the absence of IFNα2b is arbitrarily set as 1. Infection levels of H87Q and G208A are accordingly calculated. Results shown are the average of three independent infection experiments. Error bars indicate the standard deviations. * denotes $p<0.05$; ** denotes $p<0.005$; ns, not significant.

Figure 3. Knockout of MxB alleviates inhibition of HIV-1 reporter virus by IFNα2b. IFNα2b (500 U/ml) was used to treat SupT1 cells, including control, MxB/gRNA1, MxB/gRNA2 or STAT1/gRNA. Sixteen hours after, cells were infected with HIV-1 reporter

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526 viruses NLENY1 or NLENY1-ES/VSV G. The number of infected cells (YFP positive) was
527 scored with flow cytometry. The results of one representative infection experiment are shown in
528 (A). The average of three independent experiments are summarized in the bar graph shown in
529 (B). The values of IFNα2b inhibition were calculated as described in the legends to Figure 1C.
530 Error bars indicate the standard deviations. * denotes $p<0.05$; ** denotes $p<0.005$. SSC-A, side
531 scatter area.
532
533 **Figure 4. IFNβ inhibition of HIV-1 is mitigated by MxB knockout.** The control, MxB
534 knockout or STAT1 knockout SupT1 cell lines were treated with IFNβ (100 U/ml) for 16 hours
535 before being infected with NLENY1 or NLENY1-ES/VSV G. (A) Expression of MxB and
536 STAT1 with or without IFNβ treatment was measured by western blotting. (B) Number of
537 infected cells (YFP positive) was scored with flow cytometry. Results of one representative
538 infection experiment are shown. (C) The values of IFNβ inhibition were determined as described
539 in the legends to Figure 1C. The average of three independent infection experiments were
540 calculated. Error bars indicate the standard deviations. * denotes $p<0.05$; ** denotes $p<0.005$.
541 SSC-A, side scatter area.
542
543 **Figure 5. Suppression of HIV-1 NL4-3 or NL4-3/VSV G by ectopically expressed MxB.** (A,
544 B) The SupT1 cells, that were stably transduced with tetracycline-inducible MxB expression
545 retroviral vector, were treated with increasing doses of doxycycline, followed by challenge with
546 NL4-3 (equivalent to 100 ng p24) or NL4-3/VSV G (equivalent to 10 ng p24) viruses. Levels of
547 MxB and viral Gag/p24 proteins were measured by western blotting, representative blots from
548 one of the three independent infection experiments are shown in (A) for NL4-3 infected cells, in

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(B) for NL4-3/VSV G infected cells. The p24 signals were quantified using Image J, and presented with the value of p24 for NL4-3 in control cells (0 ng Dox treatment) arbitrarily set as 100. (C) SupT1 cells were treated with 500 U/ml IFNα2b for 16 hours to induce the expression of endogenous MxB. The MxB SupT1 cell line was treated with Dox of different doses (ng/ml) for 16 hours. Cell lysates were examined by western blotting using anti-MxB antibody. Levels of Tubulin were also probed as the internal control. (D) Levels of viruses in the supernatants were determined by infecting the TZM-bl indicator cells. The virus amount from the infection without doxycycline treatment is arbitrarily set as 1. The data shown are the average of three independent transfections. Error bars indicate the standard deviations.

Figure 6. Cas9/gRNA knockout screen in U87-MG cells for ISGs that alleviate IFNα2b inhibition of HIV-1 NL4-3/VSV G. (A) Effects of ISG knockout on IFNα2b (100 U/ml) suppression of HIV-1 NL4-3/VSV G infection. A number of 53 ISGs were selected for the knockout study. For each ISG, a pool of three gRNAs were used to stably transduce U87-MG cells. NL4-3/VSV G infection was monitored by measuring levels of viruses produced, through infecting the TZM-bl cells. IFNα2b suppression of NL4-3/VSV G infection was calculated by dividing the level of viruses produced in the presence of IFNα2b by the amount of viruses without IFNα2b treatment. The value from control cells in each screen experiment is arbitrarily set as 1, so that all the data from multiple experiments can be summarized in the bar graph. If the value from an ISG knockout cell line is greater than 1, then knockout of this ISG leads to an increase in HIV-1/VSV G infection in the presence of IFNα2b. Results shown are the average of two independent screens of all 53 ISGs. For clarity, data are presented in two graphs. (B) Results of western blotting to show the levels of IFITM2 or IFITM3 in the U87-MG knockout cell lines.

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572 Three gRNAs were tested for IFITM2 or IFITM3. One cell line was created using all three
573 gRNAs (1/2/3) to achieve maximal knockout efficiency. (C) IFNα2b (100 U/ml) inhibition of
574 NL4-3/VSV G infection in IFITM2 or IFITM3 knockout U87-MG cell lines. Levels of viruses
575 that were produced in the absence or presence of IFNα2b were determined by infecting the
576 TZM-bl indicator cells. Results shown are the average of three independent infections. RLU,
577 relative luciferase unit. Error bars indicated the standard deviations.
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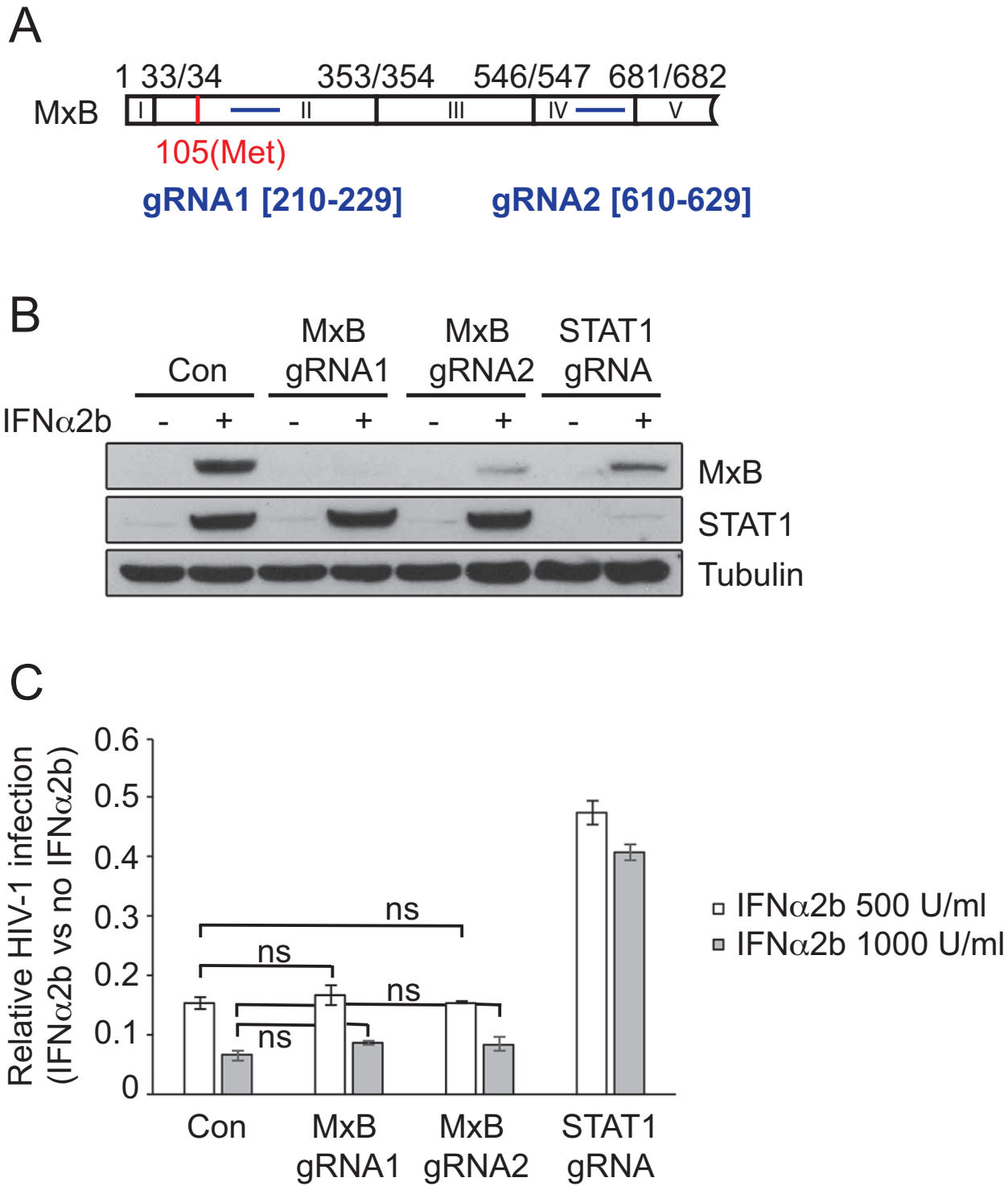

Figure 1

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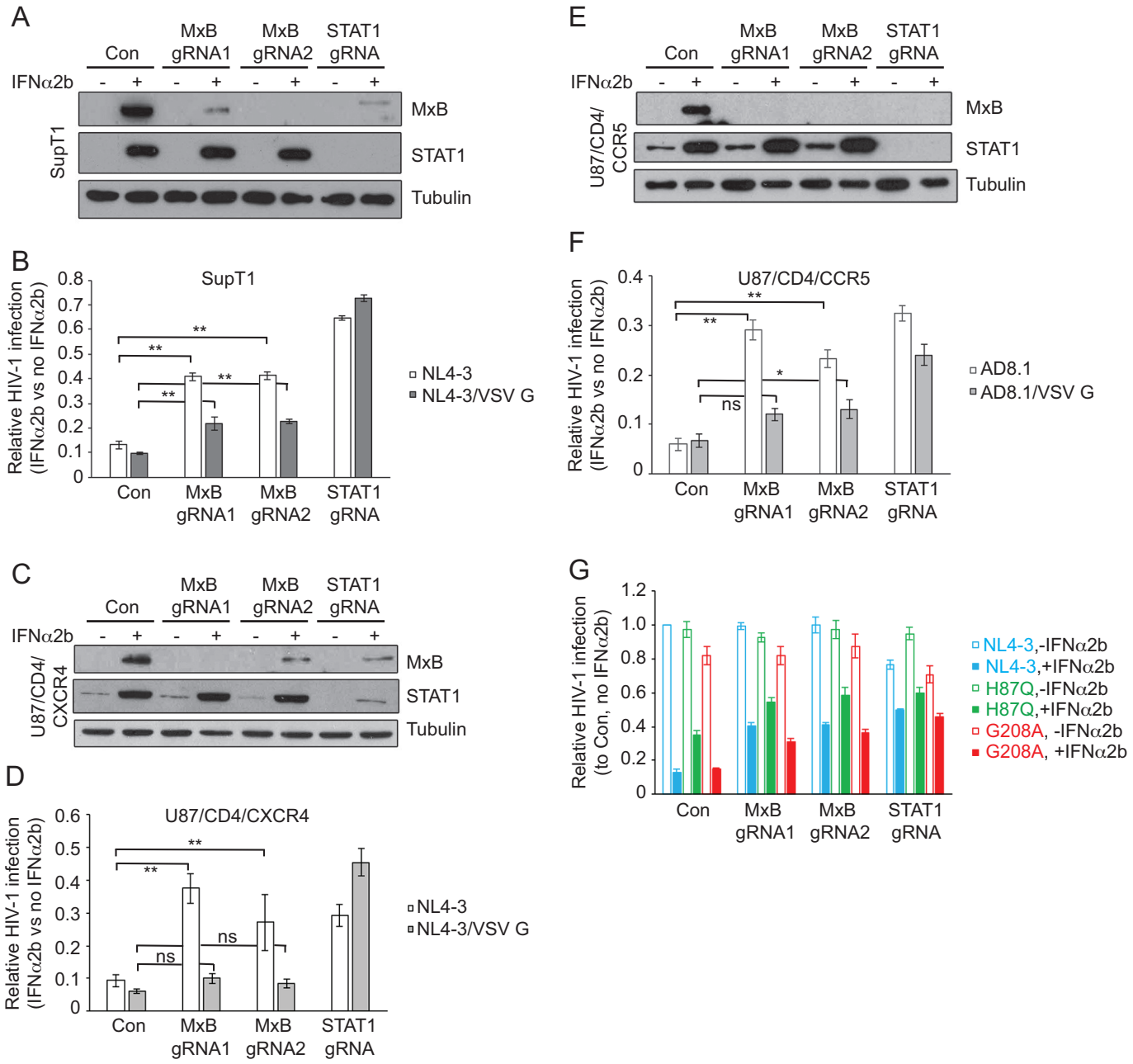

Figure 2

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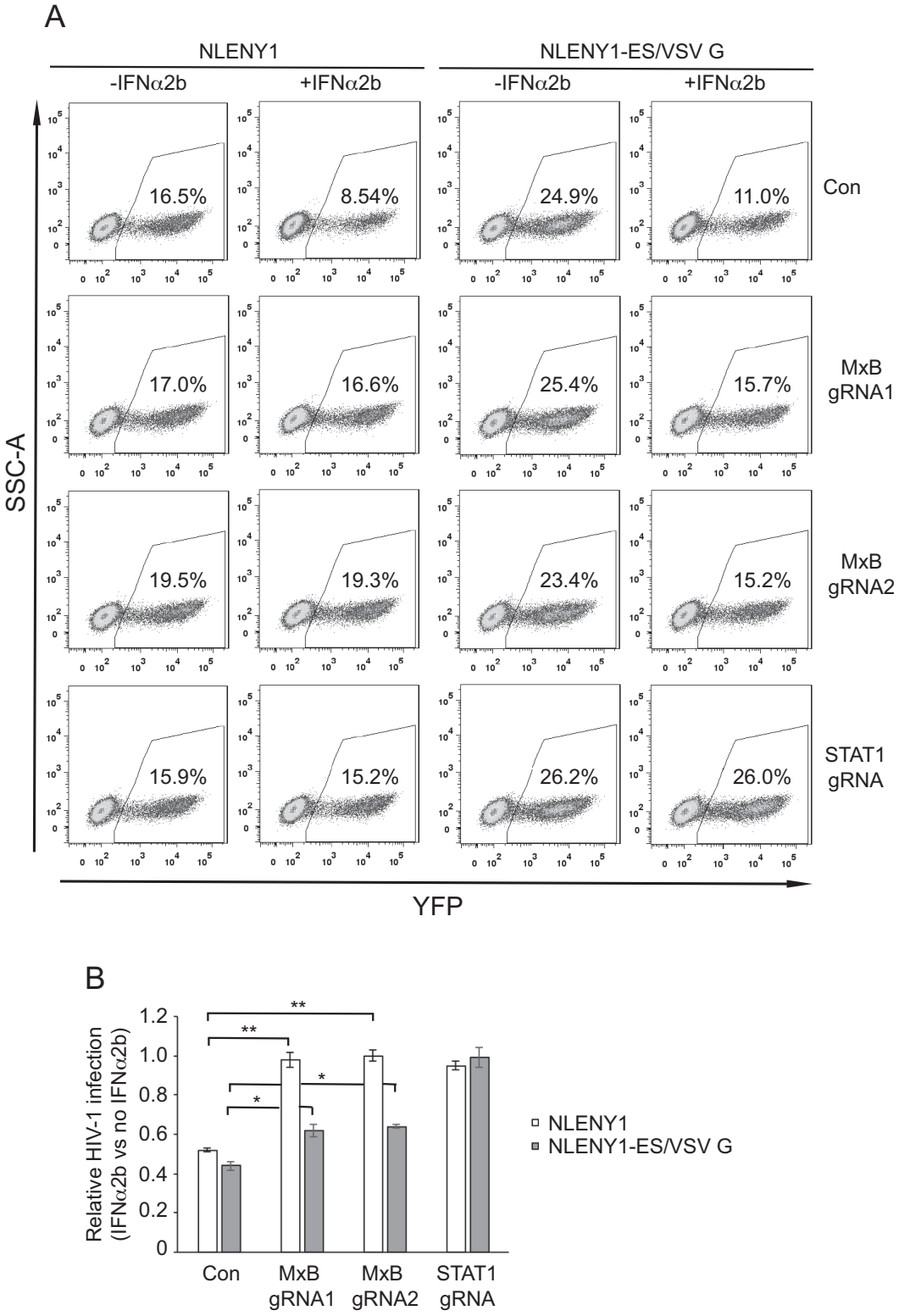

Figure 3

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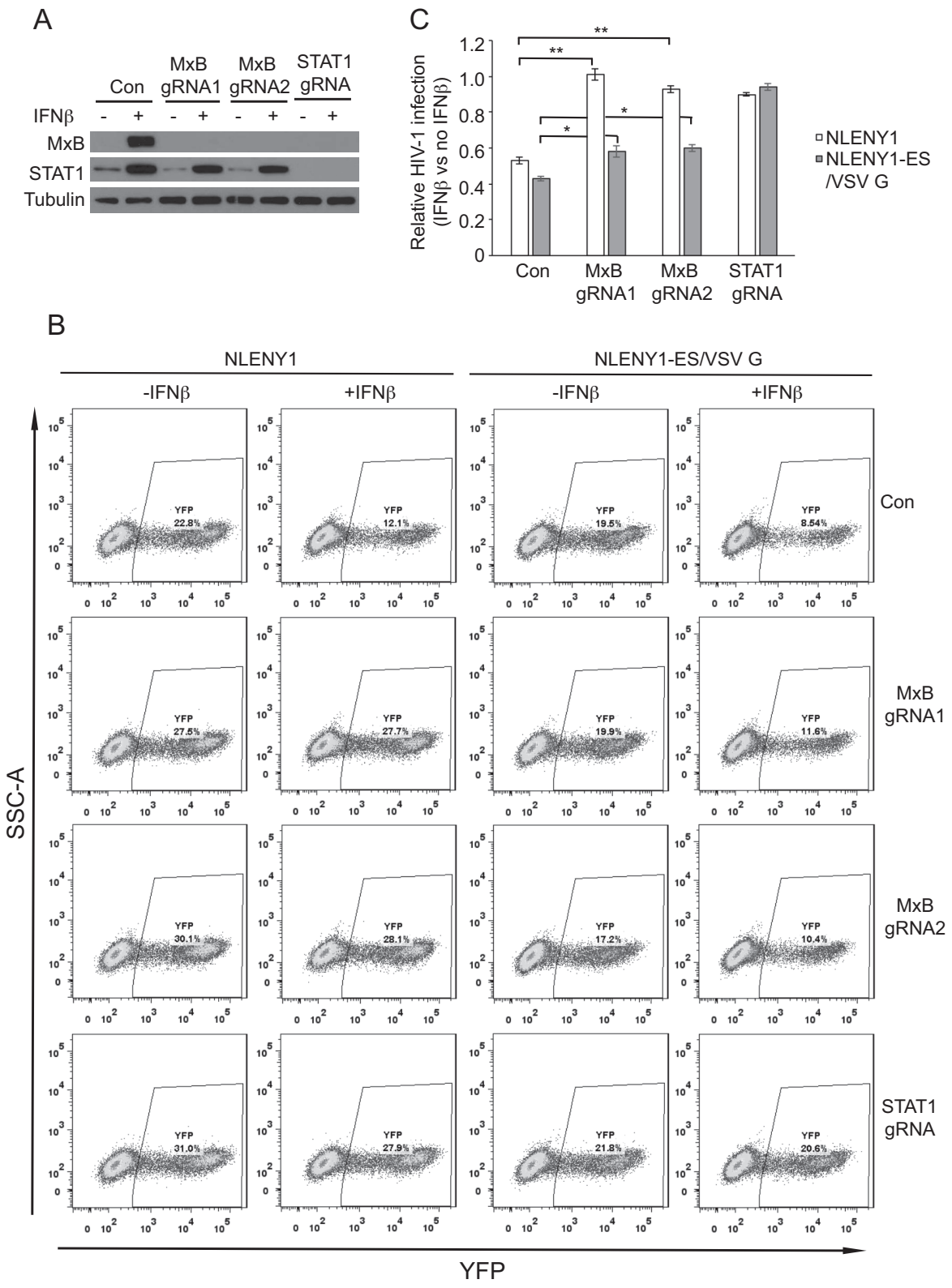

Figure 4

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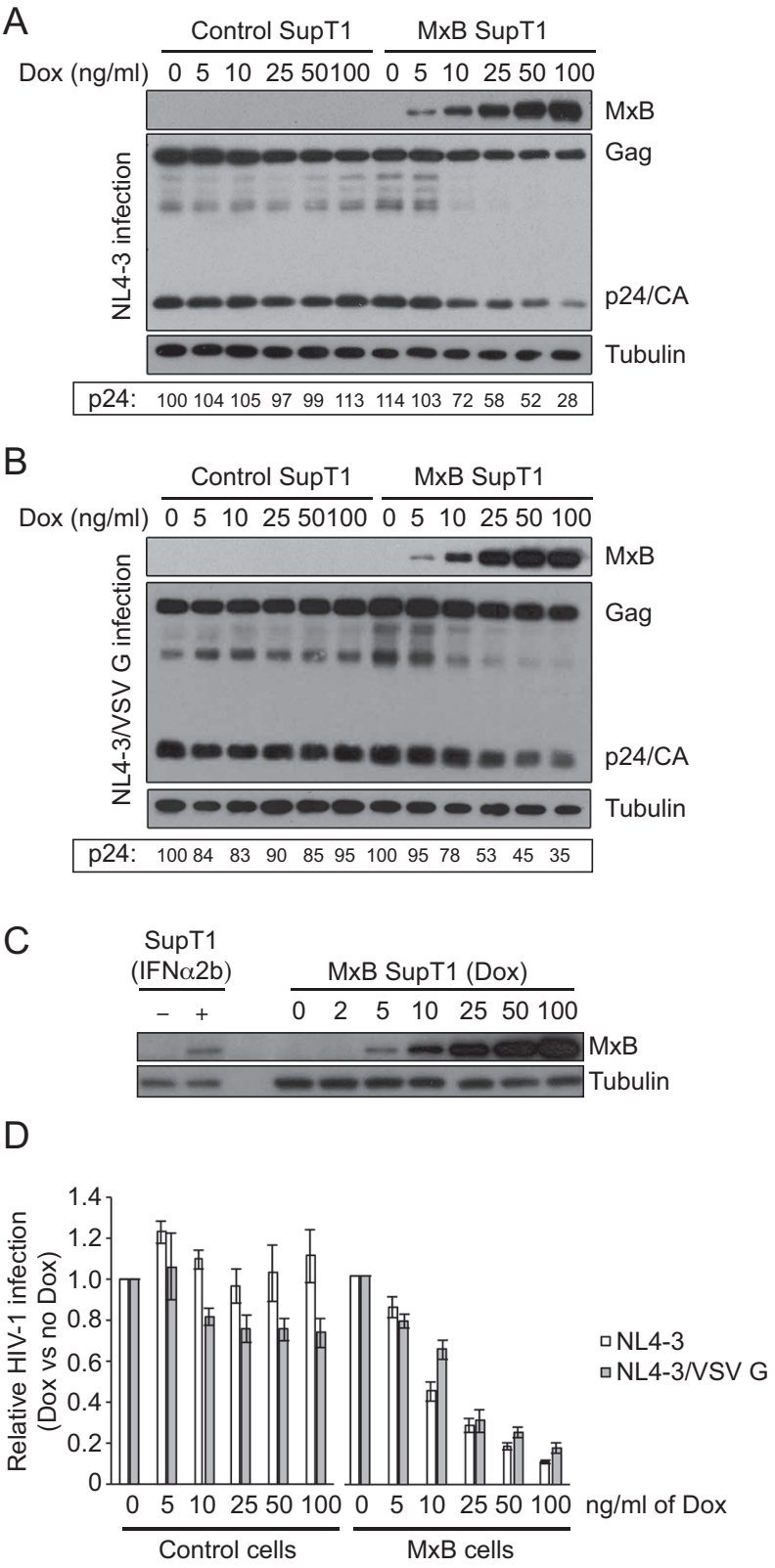

Figure 5

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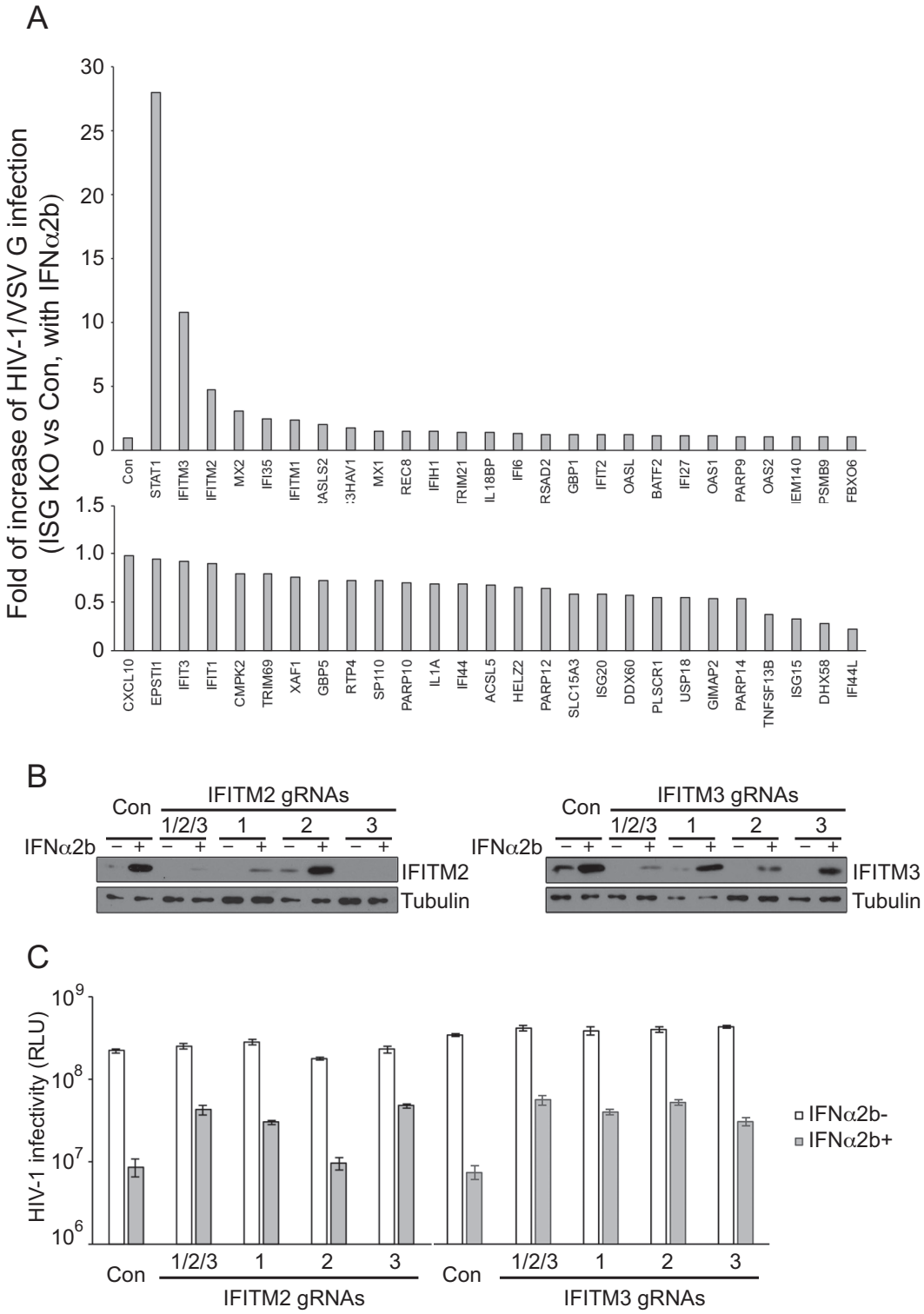

Figure 6