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Research Paper

HIV-1 Promotes the Degradation of Components of the Type 1 IFN JAK/STAT Pathway and Blocks Anti-viral ISG Induction

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ABSTRACT

Anti-retroviral therapy successfully suppresses HIV-1 infection, but fails to provide a cure. During infection Type 1 IFNs normally play an essential role in viral clearance, but in vivo IFN-α only has a modest impact on HIV-1 infection, suggesting its possible targeting by HIV. Here, we report that the HIV protein, Vif, inhibits effective IFN-α signalling via degradation of essential JAK/STAT pathway components. We found that STAT1 and STAT3 are specifically reduced in HEK293T cells expressing Vif and that full length, infectious HIV-1 IIIB strain promotes their degradation in a Vif-dependent manner. HIV-1 IIIB infection of myeloid ThP-1 cells also reduced the IFN-α-mediated induction of the anti-viral gene, ISG15, but not MxA, revealing a functional consequence of this HIV-1-mediated immune evasion strategy. Interestingly, while total STAT levels were not reduced upon in vitro IIIB infection of primary human PBMCs, IFN-α-mediated phosphorylation of STAT1 and STAT3 and ISG induction were starkly reduced, with removal of Vif (IIIBΔVif), partially restoring pSTATs, ISG15 and MxB induction. Similarly, pSTAT1 and pSTAT3 expression and IFN-α-induced ISG15 were reduced in PBMCs from HIV-infected patients, compared to healthy controls. Furthermore, IFN-α pre-treatment of a CEM T lymphoblast cells significantly inhibited HIV infection/replication (measured by cellular p24), only in the absence of Vif (IIIBΔVif), but was unable to suppress full length IIIB infection. When analysing the mechanism by which Vif might target the JAK/STAT pathway, we found Vif interacts with both STAT1 and STAT3, (but not STAT2), and its expression promotes ubiquitination and MG132-sensitive, proteosomal degradation of both proteins. Vif's Elongin-Cullin-SOCS-box binding motif enables the formation of an active E3 ligase complex, which we found to be required for Vif's degradation of STAT1 and STAT3. In fact, the E3 ligase scaffold proteins, Cul5 and Rbx2, were also found to be essential for Vif-mediated proteasomal degradation of STAT1 and STAT3. These results reveal a target for HIV-1-Vif and demonstrate how HIV-1 impairs the anti-viral activity of Type 1 IFNs, possibly explaining why both endogenous and therapeutic IFN-α fail to activate more effective control over HIV infection.

1. Introduction

Human immunodeficiency virus (HIV) remains a major global health challenge, with over 40 million people infected worldwide (Mayer and Beyrer, 2007). Anti-retroviral therapy (ART) suppresses HIV-1 replication (Potthoff and Brockmeyer, 2010), but a drug that fully clears infection remains undiscovered. Lifelong ART is costly and brings with it issues of adherence, side-effects and mutational resistance (Reust, 2011), highlighting the remaining need for new therapeutic strategies to cure HIV. Harnessing endogenous anti-viral immune responses provides a possible alternative for therapeutic discovery. Type 1 Interferons (IFNs), such as IFN-α, upregulate IFN Stimulated Genes (ISGs), that exert a broad range of anti-viral effector functions. The anti-viral mechanisms of several ISGs are well documented, in fact, by blocking the IFN-I receptor of Simian Immunodeficiency Virus (SIV)-infected rhesus macaques, Sandler et al., eloquently highlighted the importance of Type I IFNs for anti-viral gene expression, suppression of viral reservoir and control of CD4 T-cell depletion (Sandler et al., 2014). ISGs suppress HIV replication at multiple stages of the viral lifecycle (Doyle et al., 2015), but therapeutic trials of exogenous IFN-α have had surprisingly weak clinical response, with suppression of viral load poorly sustained (Lane, 1991, Bosinger and Utay, 2015, Asmuth et al., 2010). Therefore, we hypothesised that, as with other viruses (Elliott et al., 2007; Stevenson et al., 2013; Ulane et al., 2003), HIV may promote the degradation of Type 1 IFN Janus Kinase/Signal Transducers

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and Activators of Transcription (JAK/STAT) signalling pathway components, thus explaining the block in anti-viral ISG induction and HIV's in vivo resistance to IFN-α.

During infection viruses are detected by Pattern Recognition Receptors (PRRs) that induce Type I IFN production (Perry et al., 2005). Type I IFNs bind to the Type I IFN Receptor (IFNAR) and signal through the JAK/STAT pathway (Haller et al., 2006). IFN-α receptor engagement specifically results in tyrosine phosphorylation of JAK1 and Tyrosine Kinase (Tyk)2 (Colamonici et al., 1994; Shuai et al., 1993), leading to receptor phosphorylation and recruitment of STAT proteins (Platanias, 2005), which, when activated, dissociate from the receptor and form homo- or hetero-dimers that translocate to the nucleus. Type I IFNs classically signal via a trimer of STAT1, STAT2 and IFN Regulatory Factor (IRF)9, called the IFN-Stimulated Gene Factor (ISGF)3. Upon translocation to the nucleus, the ISGF3 complex binds IFN-Stimulated Response Elements (ISREs) (Levy et al., 1988) and promotes the induction of >500 ISGs (de Veer et al., 2001; Der et al., 1998a).

In this study we analysed the effects of HIV-1-Vif upon the expression of three well known, but functionally different, ISGs: ISG15, MxA and MxB. The ubiquitin like protein, ISG15, acts by targeting proteins for proteasomal degradation (Zhao et al., 2013) and has been shown to effectively inhibit the cellular release of the HIV-1 virion by inhibiting the endosomal trafficking pathway (Okumura et al., 2006). Furthermore, ISG15 can inhibit the release of HIV virions from the plasma membrane (Pincetic et al., 2010). Indeed, the expression of ISG15 is enhanced in CD4+ T cells of HIV-infected patients (Scagnolari et al., 2016) and HIV-1-Gag protein is post-translationally conjugated with ISG15, further highlighting its anti-viral importance (Woods et al., 2011). MxA shares structural homology with the dynamin family of GTPases and inhibits viral replication via physical formation of oligomeric rings around viral nucleocapsids (Haller and Kochs, 2011). It is well documented to inhibit a broad range of viruses, including influenza A virus, La Crosse encephalitis virus, bunyaviruses, phleboviruses, hantaviruses and Hepatitis C and B Viruses (HCV and HBV) (Pavlovic et al., 1990; Kochs et al., 2002; Frese et al., 1996; Gordien et al., 2001; Shi et al., 2017; Stevenson et al., 2011), but its direct anti-viral function against HIV-1 is not currently known. In contrast to MxA, MxB has been shown to inhibit HIV viral infection (Goujon et al., 2013). MxB prevents the nuclear import and integration of HIV-1 viral DNA and mutation of HIV capsid protein attenuates MxB's inhibition of HIV-1 (Kane et al., 2013; Liu et al., 2013).

Anti-viral ISGs provide Type I IFNs with an arsenal of weapons against viral infection, however, viral immune evasion strategies have evolved to block many of these immune mechanisms. We and others have discovered that several viruses, including HCV (Stevenson et al., 2013), Respiratory Syncytial Virus (RSV) (Elliott et al., 2007), Mumps (Ulane et al., 2003) Simian Virus 5 and Human Parainfluenza Virus Type 2 (Andrejeva et al., 2002), use a conserved mechanism to promote STAT proteasomal degradation, thus cutting the intracellular "lifeline" of ISG induction. Given the strong anti-viral effects of IFN-α, it is not surprising that diverse viral species have evolved this mechanism to inhibit its JAK/STAT signalling pathway. In fact, these viruses "hijack" an immunoregulatory mechanism commonly used by Suppressor Of Cytokine Signalling (SOCS) proteins to endogenously control JAK/STAT signalling (Yoshimura et al., 2007). SOCS all share a conserved "SOCS box" motif, which interacts with Elongin B, Elongin C, Cullin (Cul) and Ring box (RBX) proteins, forming a functional Elongin-Cul-SOCS box (ECS) E3 ligase complex (Kamura et al., 2004). This ECS enzymatic complex catalyses the poly-ubiquitination of target proteins, such as JAK2, thus marking them for degradation via the 26S proteasome (Kamizono et al., 2001). The RSV protein, NS1, interacts with Elongin C and Cul2, thereby forming an E3 ligase complex, which specifically targets STAT2 for proteasomal degradation (Elliott et al., 2007). Similarly, Paramyxovirus protein V complexes with DNA damage binding protein-1 (DDB1) and Cul4A, thus promoting STAT1 and STAT2 degradation (Ulane and Horvath, 2002); while Mumps protein V promotes degradation of STAT1, STAT2 and STAT3 via formation of a Cul4A-DDB1-dependent E3 complex (Ulane et al., 2003). Furthermore, we recently discovered that HCV also targets STAT1 and STAT3 for ubiquitination and proteasomal degradation, revealing yet another virus that suppresses anti-viral IFN-α signalling (Stevenson et al., 2013).

HIV-1 has evolved several mechanisms for disabling specific anti-viral ISGs. While the powerful immune evasion strategies HIV-1-Vif are clearly displayed by its ability to down-regulate APOBEC3 gene transcription (Anderson and Harris, 2015), it also contains SOCS box-like motifs that promote the formation of an ECS E3 ligase complex, which ubiquitinates and promotes proteasomal degradation of APOBEC3G protein (Sheehy et al., 2003; Kamura et al., 2004; Yu et al., 2004). Here, we report that the Vif-ECS also targets key components of the IFN-α JAK/STAT pathway. We demonstrate that HIV-1-Vif associates with STAT1 and STAT3 and promotes their ubiquitination and MG132-reversible proteasomal degradation, which is reliant on the SOCS box binding motifs of Vif and expression of the E3 ligase proteins, Cul5 and RBX2. Interestingly, while several HIV immune evasion processes are likely to be "at play" in vivo, compared to controls, we observed less IFN-α-mediated STAT1 and STAT3 phosphorylation and ISG induction in PBMCs infected in vitro with HIV-1 IIIB and in PBMCs from HIV patients, highlighting a functional signalling reduction in this anti-viral pathway. In fact, we also observed that T-lymphoblast cells pre-treated with IFN-α were less resistant to infection with IIIBΔVif (measured by p24 levels), than the WT IIIB clone, indicating that the presence of Vif functionally inhibits IFN-α's anti-viral protection. Together, these findings are an insight into the suppressive capabilities of HIV-1 on the IFN-α JAK/STAT pathway and may even provide some explanation for the failure of endogenous and therapeutic IFN-α to mediate control of HIV-1 replication in patients.

2. Materials and Methods

2.1. Cell Culture

HEK293T and Huh7 cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) or ThP-1 and CEM cells were cultured in (Roswelle Park Memorial Institute) RPMI supplemented with 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 humidified atmosphere. Cells were treated with 1000 U/ml human IFN-α (Roferon) and 1 μM MG132 (Sigma).

2.2. PBMC Isolation

PBMCs were isolated from 15 to 30 ml of blood by centrifugation (900*g* × 20 min) over Ficoll and washed ×2 in RPMI. Cells were treated with 1000 U/ml human IFN-α (Roferon).

2.3. Transfection

HEK293T cells were transfected with Mirus TransIT-2020 transfection reagent at a ratio of 1 μl transfection reagent: 1 μg DNA with HIV-1 Vif (a kind gift from Prof. Michael Malim, King's College London School of Medicine, UK) or the pCMV Empty Vector (EV) control for 48 h. HEK293T cells were also transfected with HIV-1 provirus pIIIB or HIV-1 provirus pIIIBΔVif (kind gifts from Prof. Reuben Harris, University of Minnesota), HIV-1 provirus YU-2 (a kind gift from Prof. Greg Towers, University College London) or an empty vector control. HEK293T cells were also transfected with HA-tagged STAT1 (a kind gift from Dr. Antonis Koromilas, McGill, Montreal), STAT2, myc-tagged STAT3 (a kind gift from Dr. Valeria Poli, University of Turin, Italy), HA-tagged Ubiquitin, or empty pcDNA3.1 using Mirus TransIT-2020 at the same ratio of transfection reagent: DNA. HEK293T cells were also transfected with constructs encoding hairpin sequences specific for human Cul5 (1637–1657), RBX2 (161–181), and EGFP (Clontech) mRNAs (kind gifts from Keiichi I. Nakayama, Kyushu University, Japan) for 48 h

using Mirus TransIT-2020. HEK293T cells were also transfected with constructs encoding Vif or VifΔSLQ and the corresponding empty pMEP4 vector using Mirus TransIT-2020. 24 h post transfection the cells were stimulated with 1 μM $CdCl_2$ for 24 h. Huh7 cells were transfected with HIV-1 Vif or empty pCMV using Lipofectamine 2000 transfection reagent (Invitrogen, USA) at a ratio of 2 μl transfection reagent: 1 μg DNA for 48 h.

2.4. Infection

HEK293T cells were transfected with HIV-1 IIIB, IIIBΔVif or YU-2 infectious molecular clones. 48 h later virus containing supernatants were removed. Supernatants from EV transfected cells were used for mock infections. ThP-1, CEM cells or PBMCs were spinoculated with HIV YU-2, HIV IIIB or IIIBΔVif at 1200*g* for 90 min at 25 °C.

2.5. Immunoblotting

Cells were lysed in HEPES lysis buffer (50 mM HEPES, 150 mM NaCl, 2 mM EDTA, 1% NP40 and 0.5% sodium deoxycholate) or RIPA buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA pH 8, 1% TRITON-X and 0.5% SDS) supplemented with 1 mM PMSF, 1 mM Na_3VO_4, 5 μg/ml leupeptin and 1 mM DTT and analysed by immunoblotting using antibodies against p-STAT1, p-STAT3, STAT1, HA (Cell Signaling Technology), STAT2, STAT3, p65 (Santa Cruz Biotechnologies), HIV-Vif (Abcam), p24 (NIH AIDS program) and β-actin (Sigma) and HRP-linked secondary anti-mouse or anti-rabbit antibodies (Invitrogen) and visualised using Biorad ChemiDoc MP imaging system. Blots were analysed using Image Lab software (Bio-rad laboratories).

2.6. Immunoprecipitation

Cells were lysed in HEPES lysis buffer (50 mM HEPES, 150 mM NaCl, 2 mM EDTA, 1% NP40 and 0.5% sodium deoxycholate) supplemented with 1 mM PMSF, 1 mM Na_3VO_4, 5 μg/ml leupeptin and 1 mM DTT. For ubiquitination studies, lysates were treated with 1% SDS and boiled at 95 °C for 5 min to dissociate interacting proteins. Lysates were immunoprecipitated with STAT1 (Cell Signaling Technology), STAT2, STAT3 (Santa Cruz Biotechnologies), myc-tag or HA-tag (Cell Signaling Technology) antibodies and protein A/G agarose beads (Santa Cruz Biotechnologies), before immunoblotting for HA (Cell Signaling Technology), HIV-1 Vif (Abcam), myc, STAT1 (Cell Signaling Technology), STAT2 or STAT3 (Santa cruz).

2.7. RT-PCR

RNA was isolated from cells using TRI Reagent following the manufacturer's instructions (Sigma). RT-PCR was performed using SensiFAST reverse transcriptase (Bioline). qRT-PCR was performed using SYBR-green (Biorad) at cycling parameters; 35 cycles of 95 °C, 59 °C and 72 °C for 30 s using primers specific for human RSP15 FP CGGACCAAAGCGATCTCTTC, RP CGCACTGTACAGCTGCATCA,

β-actin FP GGACTTCGAGCAAGAGATGG RP, AGCACTGTGTTGG CGTACAG,

STAT1 FP TGATGGCCCTAAAGGAACTG, RP AGGAAAACTGTCGCC AGAGA,

STAT2 FP CACACTATGCATGGTATCACAAACA, RP CTGAAGATTTC CATTGGCTCAGT,

STAT3 FP GAGAAGGACATCAGCGGTAAGAC, RP GCTCTCTGG CCGACAATACTTT,

MxA FP GGTGGTGGTCCCCAGTAATG, RP ACCACGTCCACAACC TTGTCT,

MxB FP AAGCAGTATCGAGGCAAGGA, RP TCGTGCTCTGAACA GTTTGG,

ISG15 FP TCCTGCTGGTGGTGGACAA, RP TTGTTATTCCTCACC AGGATGCT,

CUL2 FP GGCAGAGGAGGACGATTGTT, RP GGGTTCAGGATAG GCCACAC,

CUL5 FP TGCGCCCGATTGTTTTGAAG, RP ATTGCTGCCCTGTT TACCCAT,

RBX1 FP CGATGGATGTGGATACCCCG, RP CTGTCGTGTTTT GAGCCAGC or

RBX2 FP GTCCAGGTGATGGTGGTCTG, RP GCCTTTGTAGGG CACTGGAT.

2.8. Sub-cloning

Vif and VifΔSLQ (kind gifts from Prof. Michael Malim, King's College London School of Medicine, UK) were amplified from pCMV vector using the following primers;

FP AGCTGCTAGCAAGCTATGGAAAACAGATGGCAGG,

RP TATCATGTCTGGATCCTAGTGTCCATTCATTGTATG using CloneAmp HiFi PCR Premix (Clontech) and inserted into *Bam*HI and *Hind*III (NEB) digested pMEP4 vector by In-Fusion Cloning (Clontech).

2.9. Stable Cell Line

HEK293T cells were seeded in 10 cm dishes and grown to 90% confluency. Cells were transfected with 8 μg pMEP4 construct encoding Vif using Lipofectamine 2000 transfection reagent (Invitrogen). pMEP4 transfected cells were selected using 300 μg/ml Hygromycin and treated with 1 μM $CdCl_2$ for 24 h to induce Vif expression.

2.10. Patients

Blood from 16 patients (age range 27–58 years) on antiretroviral treatment for HIV and 5 healthy volunteers were included in this study. All individuals gave written and informed consent. Ethical approval was granted by the Tallaght Hospital and St James's Hospital (Dublin, Ireland) Joint Research Ethics Committee.

3. Results

3.1. STAT1 and STAT3 Protein Expression is Significantly Reduced Upon Expression of HIV-Vif

Since therapeutic IFN-α has only a modest impact on HIV-1 infection (Lane, 1991), we wondered if the virus could block its anti-viral activity by degrading components of the JAK/STAT signalling pathway. Since several viruses target STAT proteins for degradation and HIV-Vif degrades APOBEC3G, we initially investigated the effect of Vif upon STAT1-3 protein levels. HEK293T cells were transiently transfected with a DNA construct encoding HIV-Vif or the EV control. STAT1, STAT2 and STAT3 proteins were measured by Western blotting and quantified by densitometric analysis. Upon expression of Vif, STAT1 and STAT3 protein levels were significantly reduced, while STAT2 expression was unaffected (Fig. 1A–C). To determine if Vif degrades "specific" proteins, we also analysed expression of the NF-κB subunit, p65. As with STAT2, Vif had no significant effect on p65, indicating targeted specificity (Fig. 1D).

Having observed a loss of STAT1 and STAT3 protein in the presence of Vif, we next investigated whether their loss was mediated at the transcriptional level. mRNA expression of STAT1-3 was quantified by qRT-PCR in Vif transfected HEK293T cells. Vif had no significant effect on the mRNA of STAT1-3 (Suppl. Fig. 1A), indicating that the reduction of STAT1 and STAT3 protein was mediated at a post-translational stage. Vif expression also significantly suppressed IFN-α-induced pSTAT1 and pSTAT3 (Suppl. Fig. 1B), revealing a functional loss of normal IFN-α signalling the presence of Vif.

As an additional experimental means of Vif expression, we also stably transfected HEK293T cells with a $CdCl_2$-inducible Vif construct. Using this $CdCl_2$-induction of Vif, we again observed a reduction of

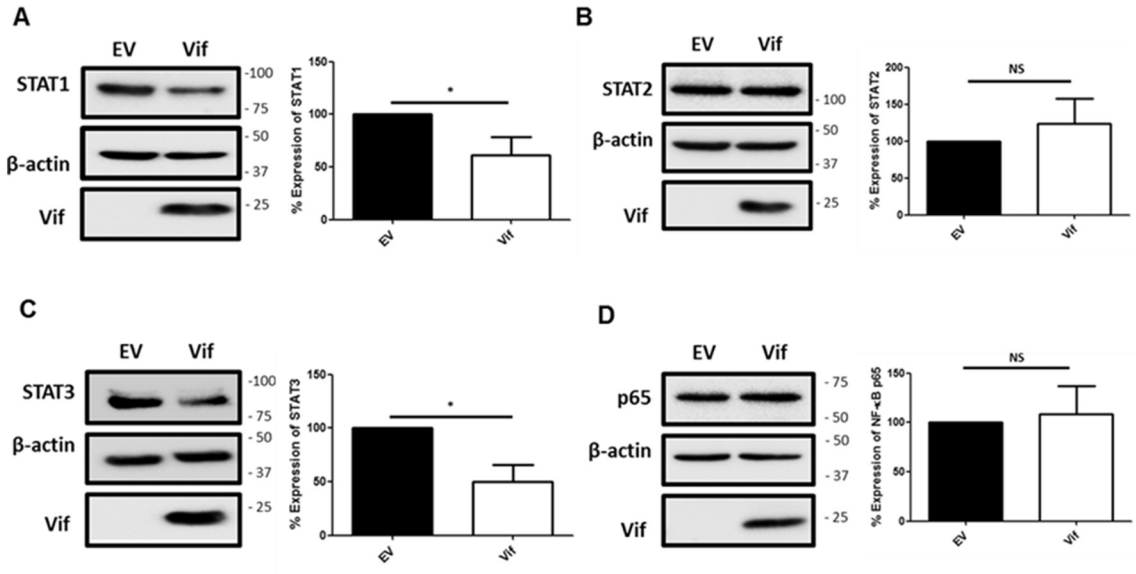


Fig. 1. STAT1 and STAT3 protein are specifically reduced upon expression of HIV-Vif. HEK293T cells were transfected with EV or HIV-Vif before cells were lysed and subjected to Western blotting using (A) STAT1, (B) STAT2, (C) STAT3 and (D) p65 antibodies (kDa ladder weights included on right of blots). All blots were re-probed for HIV-Vif and β-actin. Densitometric values of STATs and p65 were calculated relative to β-actin and compared to EV, which was normalised to 100%. All results are the average ±SD of (B, C, D) three or (A) four independent experiments. $^{*}P < 0.05$; Non-Significant (NS), (Paired *t*-test).

STAT1 and STAT3 protein levels (Suppl. Fig. 2A–B and E–F) and IFN-α-induced pSTAT1 and pSTAT3 (Suppl. Fig. 2A–D), providing further support for Vif's role in suppressing the anti-viral IFN-α pathway.

3.2. STAT1 and STAT3 Protein Expression is Significantly Reduced Upon Expression of Full Length HIV-1 IIIB, but not IIIBΔVif

Having observed a loss of STAT1 and STAT3 protein in the presence of Vif, we next analysed STAT protein expression in cells expressing the full length HIV IIIB infectious molecular clone or a IIIB version with a 230 base pair deletion in the region coding for Vif (IIIBΔVif). HEK293T cells were transfected with either of these HIV-1 clones before STAT1, STAT2 and STAT3 protein expression was examined using immunoblotting. HIV expression was confirmed by assessing p24 levels, using a p24 antibody (Simm et al., 1995). Expression of the full length HIV IIIB infectious molecular clone significantly reduced STAT1 and STAT3 protein levels, while STAT2 expression was not significantly affected (Fig. 2A–C). However, neither STAT1 nor STAT3 protein levels were significantly reduced in the absence of Vif (IIIBΔVif clone) (Fig. 2A and C), again suggesting a role for Vif in their cellular reduction.

3.3. Infection of Myeloid ThP-1 Cells With HIV-1 Reduces STAT1 and STAT3 Protein Expression, Along With ISG15 Induction

Having observed a reduction of STAT1 and STAT3 protein in 293 T cells transfected with Vif or the full length HIV IIIB clone, we next examined the effect of HIV IIIB infection in myeloid, monocytic ThP-1 cells. Compared to mock infected cells, infection with IIIB significantly reduced STAT1 and STAT3 protein levels, but IIIBΔVif infection did not (Fig. 3A & B), further indicating that HIV-Vif plays a significant role in targeting these STAT proteins. Similarly, infection of ThP-1 cells with another HIV strain (HIV-1 YU-2), also significantly reduced STAT1 and STAT3 protein expression (Suppl. Fig. 3), revealing these Vif-mediated effects are not limited to a single HIV strain.

To investigate functional consequences of Vif-mediated STAT reduction, we next analysed downstream ISG induction. ThP-1 cells were infected with HIV IIIB or IIIBΔVif, before being stimulated with IFN-α for 0 or 2 h. HIV IIIB, but not IIIBΔVif, infection significantly impaired IFN-α-mediated induction of ISG15 (Fig. 3C). HIV IIIB infection had no effect on IFN-α-induced MxA expression (Fig. 3D), highlighting that Vif's effect upon the JAK/STAT pathway has a differential outcome for specific ISG expression.

3.4. Cellular HIV-p24 Protein Expression is Inhibited by IFN-α Pre-treatment Only in the Absence of Vif (HIV IIIBΔVif)

Having observed that HIV infection promoted the degradation of essential IFN-α pathway components, we subsequently investigated if there was a functional consequence of this upon HIV "permissivity" (as measured by p24 protein levels). CEM T lymphoblastoid cells were pre-treated for 4 h with IFN-α, before infection with either HIV IIIB or IIIBΔVif for 24 h. We observed that IFN-α pre-treatment reduced the expression of p24 protein in the absence of Vif (IIIBΔVif), but was unaffected by IFN-α in cells infected with the Vif-containing IIIB molecular clone (Fig. 3E). These findings indicate that Vif may have a role in protecting HIV from IFN-α's anti-viral activity. Indeed, these results support a hypothesis that the loss of STAT1 and STAT3 in Vif-expressing cells, may reduce IFN-α's ability to block HIV infection and/or replication, possibly revealing a previously unknown HIV immune evasion mechanism.

3.5. Infection of Primary Human PBMCs With HIV IIIB Infectious Clone Reduces IFN-α-Induced STAT1 and STAT3 Phosphorylation and ISG Induction in a "Partially" Vif-dependent Manner

Having observed that infection of myeloid ThP-1 cells with HIV IIIB significantly reduced STAT1 and STAT3 protein expression and IFN-α-induced ISG15, we wondered if HIV infection also reduced expression of phosphorylated STAT1 and STAT3 in primary human immune cells. In order to investigate this, PBMCs were isolated from healthy volunteers and infected with either HIV IIIB or the Vif deletion mutant, before stimulating with IFN-α for 15 min. Even though this in vitro "acute" infection of PBMCs with HIV did not reduce total STAT1 and STAT3 levels, we found that infection with full length HIV IIIB significantly decreased both pSTAT1 and pSTAT3 levels (Fig. 4A & B). Interestingly, while pSTAT1 and pSTAT3 levels in HIV IIIBΔVif infected PBMCs were not significantly reduced, there was less phosphorylation compared to mock

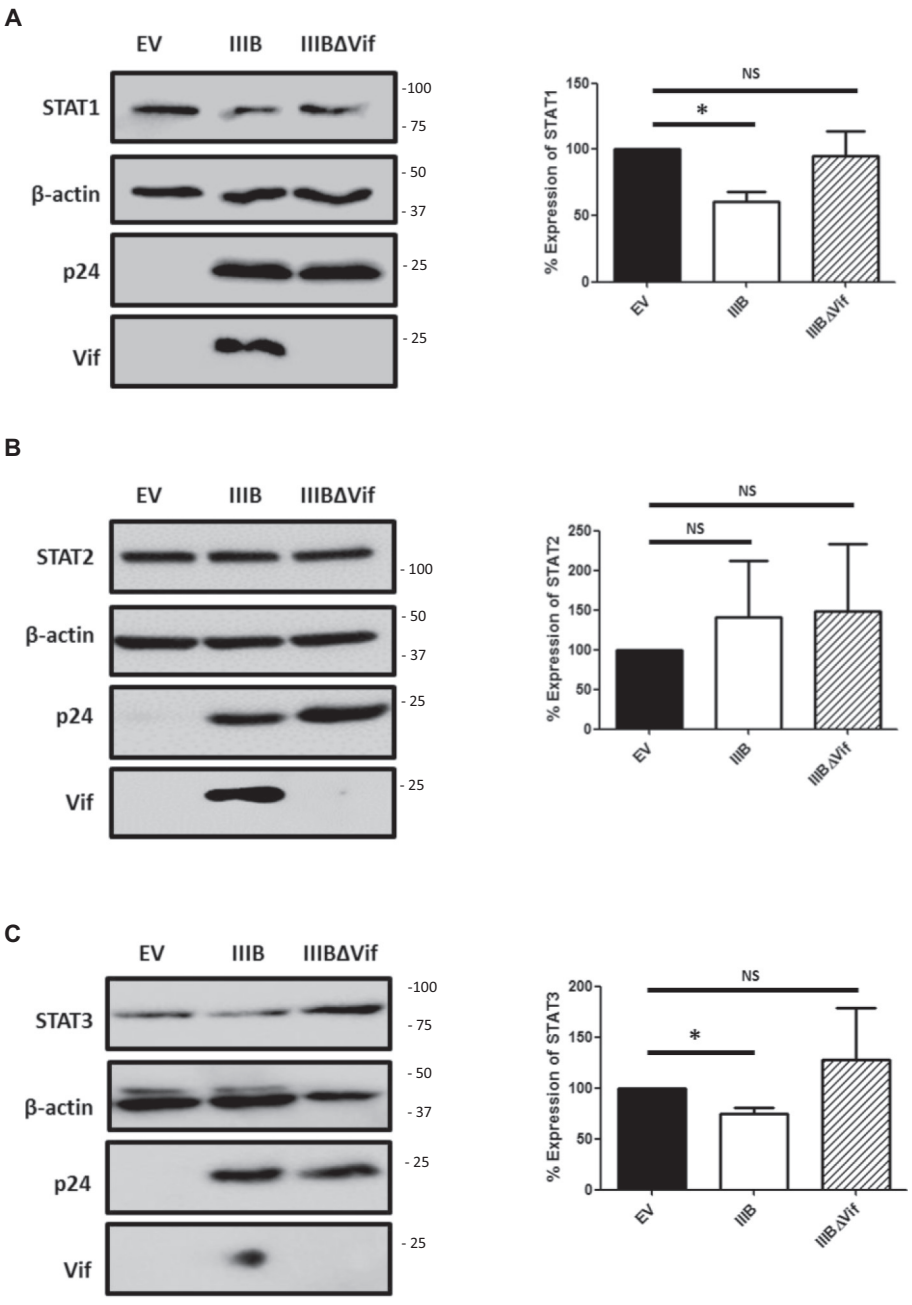


Fig. 2. Expression of HIV-1 in HEK293T cells decreases STAT1 and STAT3 protein levels. HEK293T cells were transfected with HIV IIIB, HIV IIIBΔVif or EV control. 24 h post transfection the cells were lysed and subjected to immunoblotting using (A) STAT1, (B) STAT2 and (C) STAT3 antibodies (kDa ladder weights included on right of blots). All blots were re-probed for β-actin, HIV-p24 and HIV-Vif. Densitometric analysis was performed using image lab software. Densitometric values of STATs were calculated relative to β-actin and compared to EV, which was normalised to 100%. Graphs are the average ±SD of three independent experiments $^{*}P < 0.05$; NS (Paired *t*-test).

infected (Fig. 4A & B), indicating that while Vif has a significant impact upon IFN-α signalling, there may be other viral processes that target IFN-α signalling. Together these results indicate that Vif significantly contributes to a decrease in IFN-α signalling. Having observed this reduction in active STAT1 and STAT3, we next investigated the effect of Vif further downstream of STATs. PBMCs from healthy volunteers were infected with either IIIB or IIIBΔVif, before treating with IFN-α for 2 h and measuring the induction of ISGs: ISG15, MxA and MxB. Compared to uninfected PBMCs treated with IFN-α, infection with either full length IIIB or IIIBΔVif, impaired the induction all three ISGs (Fig. 4C–E). Interestingly, the IFN-α-mediated induction of both ISG15 and MxB was significantly higher in PBMCs infected with IIIBΔVif, compared with those infected with the Vif–containing virus, indicating a partial restoration of these ISGs in the absence of Vif. However, as in ThP-1 cells, the removal of Vif had no significant affect on MxA induction (Fig. 4E). Together these results support the hypothesis that Vif suppresses the JAK/STAT pathway, thus reducing the induction of specific ISGs, but highlights that other viral immune evasion mechanisms must also play a role in subverting these essential anti-viral responses.

3.6. PBMCs From HIV-Infected Patients Have Lower STAT1 and STAT3 Protein Levels, Impaired IFN-α-Mediated Phosphorylation and Reduced ISG15 Induction

Having observed the effect of "acute" in vitro HIV infection upon primary human PBMCs, we next analysed the effect of HIV upon IFN-α

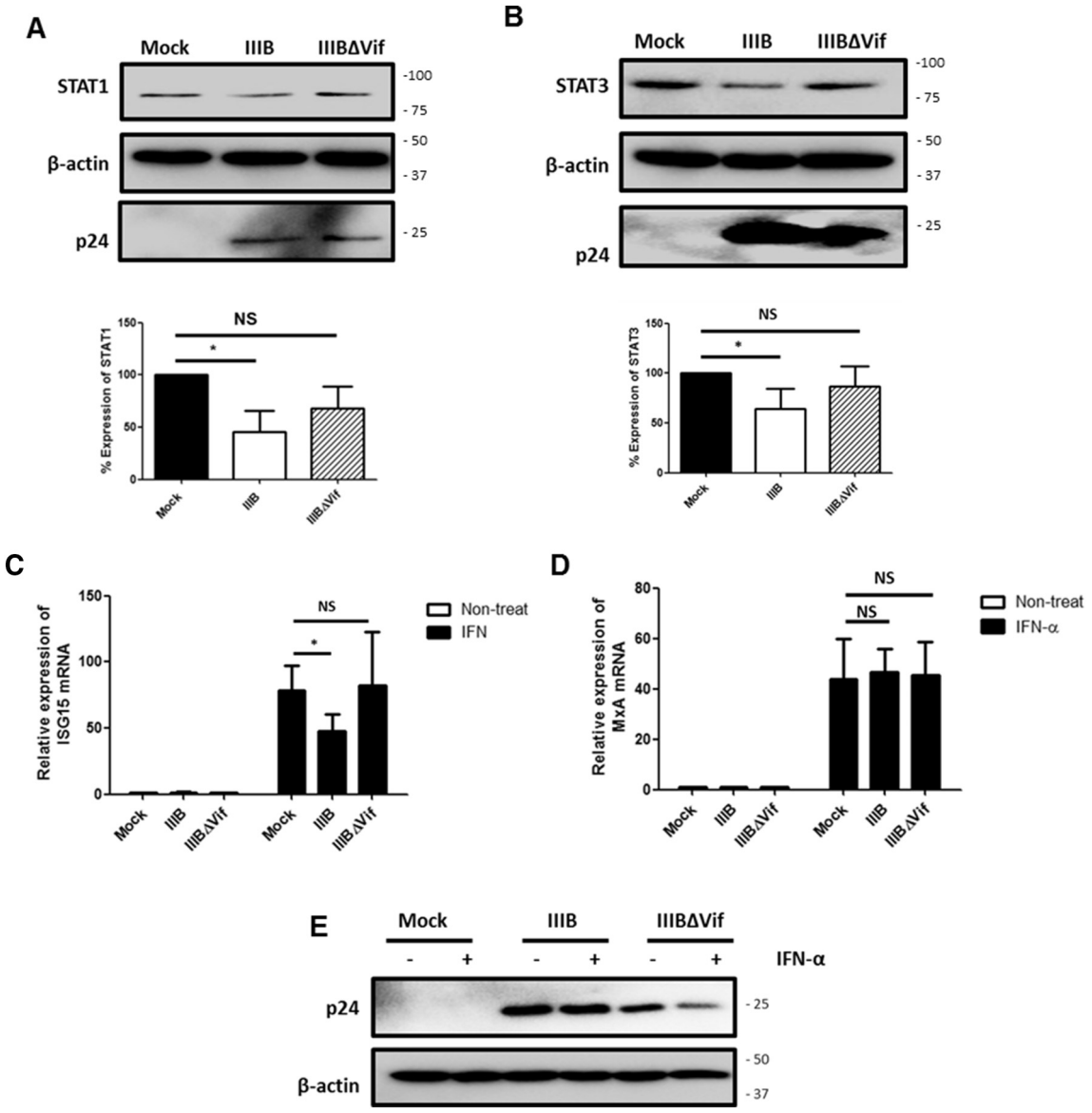


Fig. 3. HIV IIIB infection suppresses STAT1 and STAT3 protein levels and ISG15 induction in a Vif-dependent manner and only infectivity of IIIBΔVif is sensitive to IFN-α. ThP-1 cells were mock infected or infected with IIIB or IIIBΔVif for (A) 2 or (B) 6 days. Thereafter, lysates were generated and subjected to immunoblotting using STAT1, STAT3, β-actin and p24 antibodies (kDa ladder weights included on right of blots). Densitometric analysis was performed using Image Lab software and values for STATs were calculated relative to β-actin and compared to the mock infected control, which was normalised to 100%. Graphs are the mean ± SD of three independent experiments. $^{*}P < 0.05$; NS (Paired *t*-test). ThP-1 cells were also infected for 6 days and then stimulated with IFN-α for 2 h before measuring (C) ISG15 and (D) MxA gene expression by qRT-PCR. Gene expression was calculated relative to the RPS15 housekeeping gene and IFN-stimulated samples were compared to unstimulated, which were normalised to 1. Graphs are the mean ± SD of four independent experiments $^{*}P < 0.05$; NS (unpaired *t*-test). (E) CEM cells were treated with IFN-α for 4 h before being infected with IIIB and IIIBΔVif for 24 h. Lysates were then generated and subjected to immunoblotting using p24 and β-actin antibodies (kDa ladder weights included on right of blots). n = 3.

responses in ex vivo PBMCs from "chronic" HIV-infected patients. We isolated PBMCs from HIV-infected patients and healthy volunteers and treated them for 0 and 15 min with IFN-α, before analysing tyrosine phosphorylation and total protein levels of STAT1 and STAT3. PBMCs from HIV-infected individuals had significantly decreased levels of pSTAT1, when compared to PBMCs from healthy controls (HC) (Fig. 5A and B). Total STAT1 was also significantly reduced in PBMCs from HIV-infected patients, compared with healthy controls (Fig. 5A and C). Interestingly, while the mean densitometric value of pSTAT3 and STAT3 protein in PBMCs from HIV+ patients was less than that of healthy controls, the difference was not statistically significant (Fig. 5A, D and E). It should be noted that these patients were on anti-retroviral treatment, therefore it was not surprising that the inhibitory effects of HIV-1 upon pSTAT3 previously observed in primary PBMCs was not as "pronounced". To analyse the IFN-α pathway further downstream of STATs, PBMCs from HIV-infected patients or healthy controls were treated with IFN-α for 2 h, before ISG15 mRNA was measured by qRT-PCR. Interestingly, we observed impaired induction of ISG15 in HIV patient immune cells, compared to healthy controls, revealing a functional reduction in this anti-viral ISG's expression (Fig. 4F).

3.7. HIV-Vif Protein Interacts With Both STAT1 and STAT3

Given that Vif expression reduced STAT1 and STAT3 protein (Fig. 1A and C), but not their mRNA (Suppl. Fig. 1A), we hypothesised that, as had previously been observed for APOBEC3G (Sheehy et al., 2003), Vif might target these proteins for ubiquitin-mediated proteasomal degradation. To investigate this we first analysed if STAT1, STAT2 or STAT3 interacted with Vif. Lysates from HEK293T cells expressing Vif and

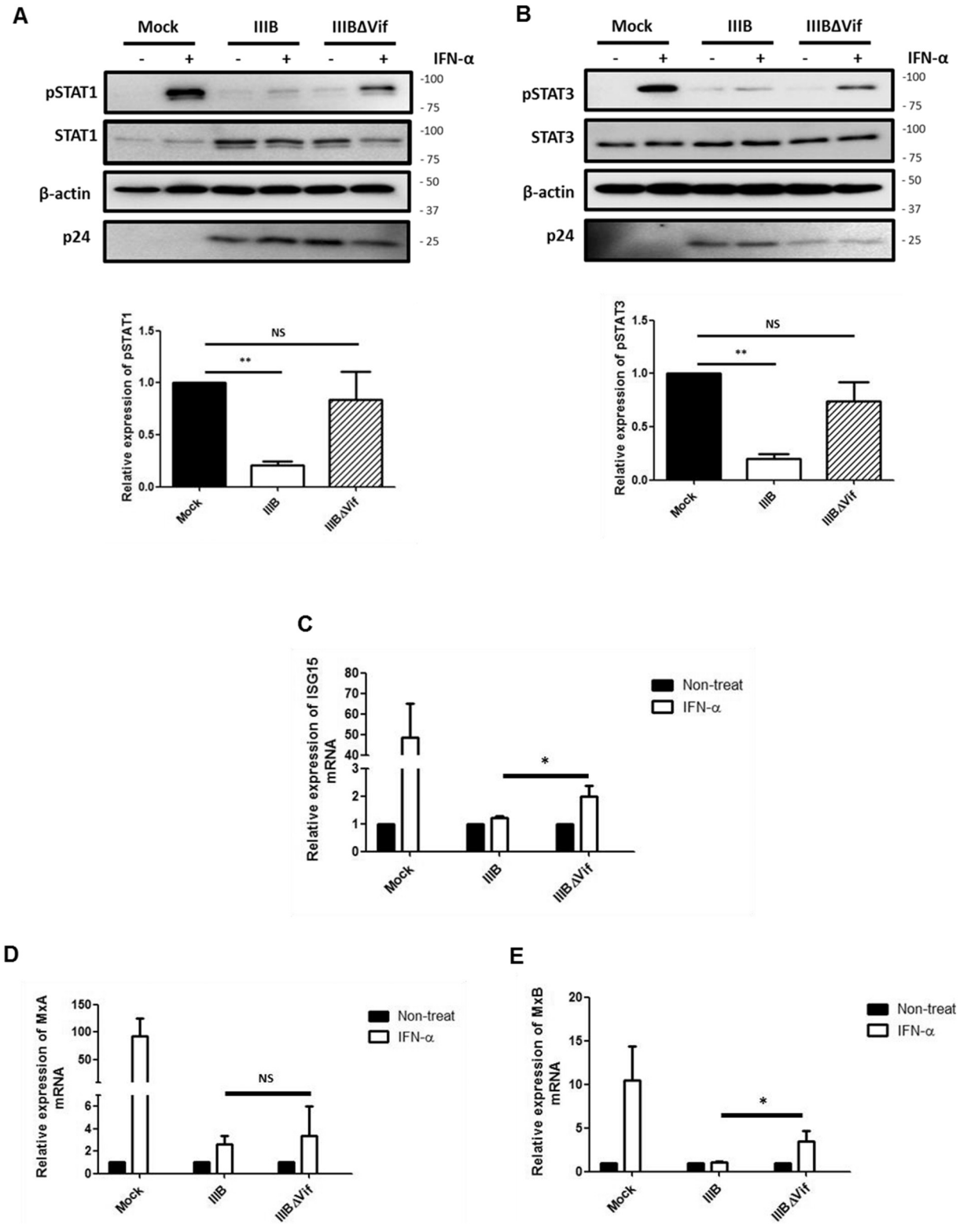
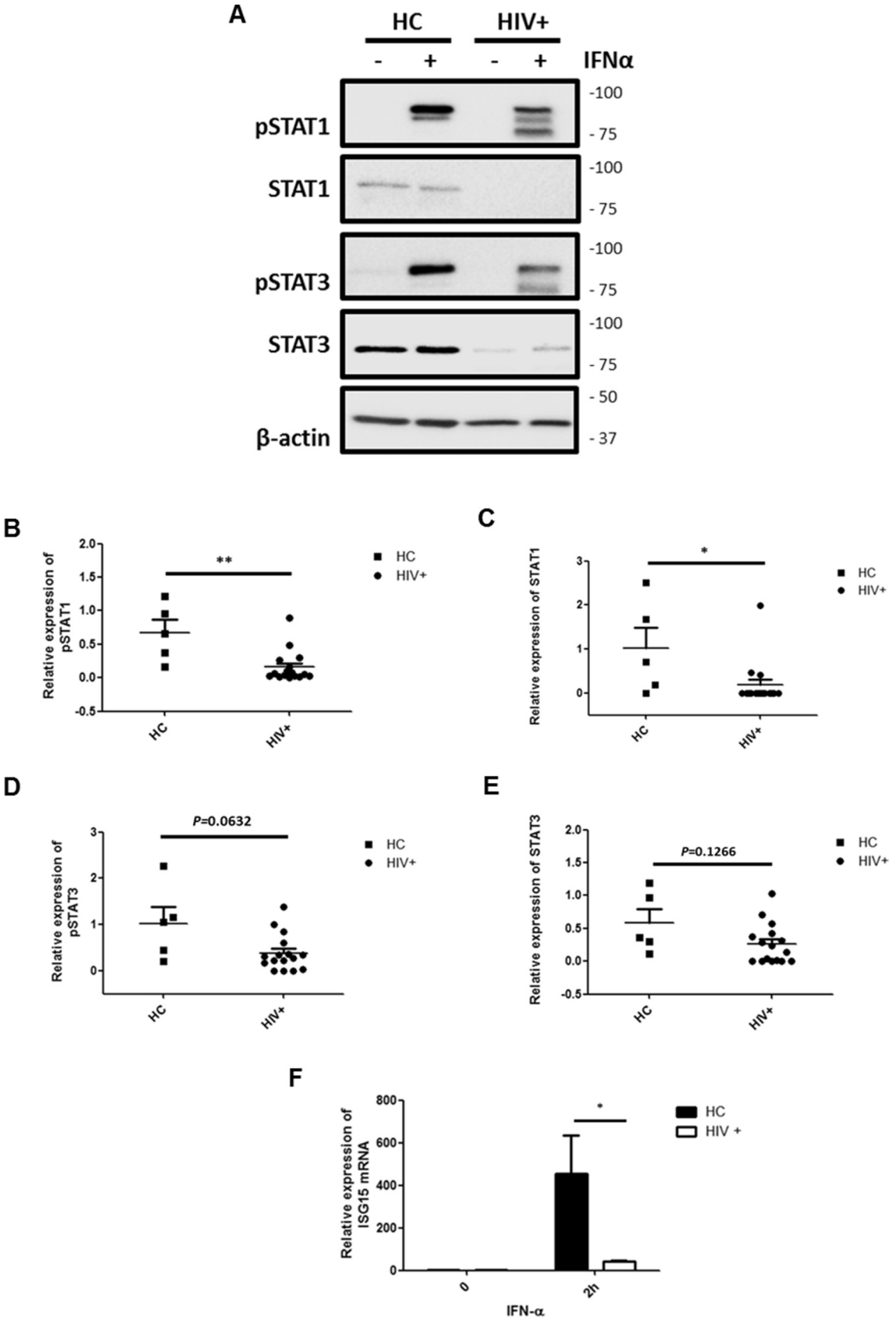


Fig. 4. "Acute" in vitro infection of healthy PBMCs with HIV-1 IIIB impairs IFN-α-mediated STAT1 and STAT3 phosphorylation and ISG induction, while removal of Vif in IIIBΔVif-infected PBMCs partially restores induction of pSTAT1, pSTAT3, ISG15 and MxB, but not MxA. PBMCs were isolated from healthy volunteers. They were mock infected or infected with IIIB or IIIBΔVif for 48 h and stimulated with IFN-α for 15 min. Thereafter, lysates were generated and subjected to immunoblotting using (A) pSTAT1, STAT1, (B) pSTAT3, STAT3 antibodies. All blots were also probed with β-actin and p24 antibodies. Densitometric analysis was performed using Image Lab software and values for pSTATs were calculated relative to β-actin and compared to the mock infected control, which was normalised to 1. All graphs are the mean ± SEM of three independent experiments. $^{**}P < 0.01$; NS (Paired *t*-test). PBMCs were isolated from healthy volunteers before infecting with mock or IIIB or IIIBΔVif for 48 h and then stimulating with IFN-α for 2 h. Total RNA was extracted and converted to cDNA which was used as a template to measure levels of (C) ISG15, (D) MxA and (E) MxB by qRT-PCR. Gene expression was calculated relative to RPS15 and compared to unstimulated cells, which were normalised to 1. Graphs are the mean ± SEM of five independent experiments. $^{*}P < 0.05$; NS (Mann-Whitney).

STAT1-3, revealed that Vif interacts with STAT1 (Fig. 6A) and STAT3 (Fig. 6B). However, STAT2 did not co-immunoprecipitate with Vif (Fig. 6C). This was not surprising, given that we previously observed that Vif did not degrade STAT2 protein (Fig. 1B). These findings show that Vif can interact with STAT1 and STAT3, which may facilitate proximal ubiquitination and proteasomal degradation.



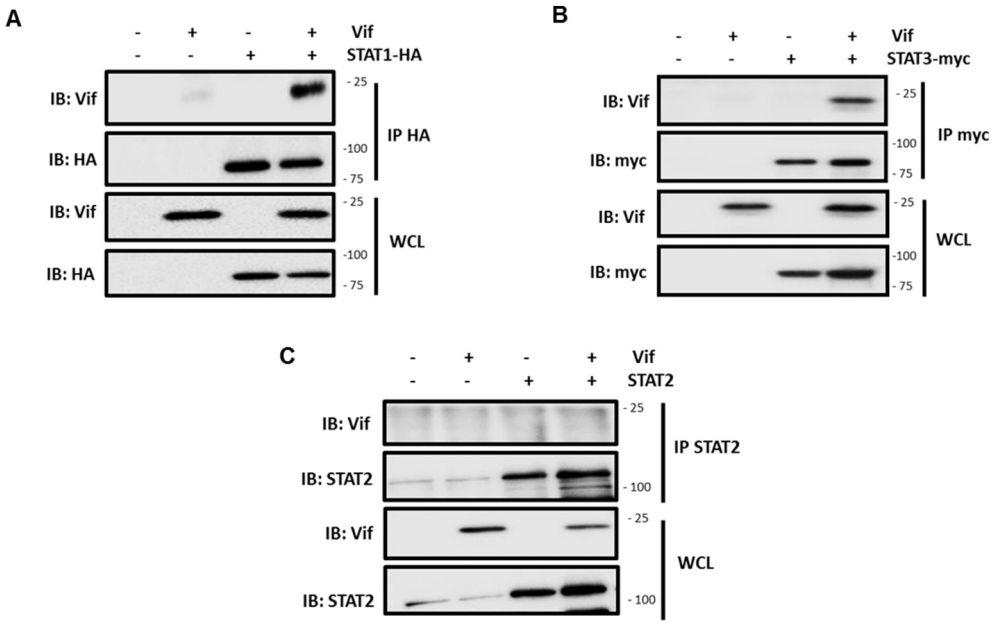


Fig. 6. HIV-Vif interacts with STAT1 and STAT3, but not STAT2. (A) HEK293T cells were transfected with STAT1-HA and HIV-Vif or corresponding EV controls. Lysates were generated and immunoprecipitated using an HA antibody. Immunoprecipitates (IP) and whole cell lysates (WCL) were subjected to Western blotting using Vif and HA antibodies. (B) HEK293T cells were transfected with STAT3-myc, HIV-Vif or EV controls. Cells were lysed and immunoprecipitated using a myc antibody. IP and WCL were subject to Western blotting using myc and Vif antibodies. (C) HEK293T cells were transfected with STAT2, HIV-Vif or EV controls. Cells were lysed and immunprecipitated using a STAT2 antibody. IP and WCL were subjected to Western blotting using STAT2 and Vif antibodies. All blots are representative of three independent experiments and kDa ladder weights are included on right of blots.

3.8. HIV-Vif Protein Promotes STAT1 and STAT3 Ubiquitination and Proteasomal Degradation

To further investigate the mechanism by which Vif may reduce STAT1 and STAT3 protein, we analysed the role of the proteasome using a proteosomal inhibitor, MG132. In HEK293T cells expressing Vif, treatment with MG132 restored STAT1 (Fig. 7A and Suppl Fig. 4A) and STAT3 (Fig. 7B and Suppl Fig. 4B) protein expression, indicating that Vif promotes their degradation through the proteasome. In support of these findings, MG132 treatment also prevented Vif-mediated STAT1 and STAT3 degradation in the human hepatocyte cell line, Huh7, revealing a HIV-mediated immune evasion mechanism functional in different cell types (Suppl. Fig. 4C–E). We also found that in HEK293T cells, transfected with HA-tagged Ubiquitin, STAT1 and STAT3 had enhanced ubiquitination in the presence of Vif, demonstrating the process of Vif-mediated STAT1 and STAT3 ubquitination "in action" (Fig. 7C & D). Together these results indicate an immune evasion mechanism by which Vif promotes the ubiquitination and subsequent degradation of STAT1 and STAT3 protein, through the MG132-sensitive 26S proteasome.

3.9. The Vif SOCS-Box Motif (SLQXLA) is Essential for Vif-Induced STAT1 and STAT3 Degradation

Vif is known to use its SOCS-box motif to hydrophobically bind ElonginB/C and mutation of its Ser-Leu-Gln residues to Ala-Ala-Ala (SLQ ➔ AAA) prevents this association (Yu et al., 2004). Having observed that Vif interacted with STAT1 and STAT3 and promoted their proteasomal degradation, we hypothesised that it mediates this process via its SOCS-box motif (SLQXLA). To investigate if the Vif-ECS motif was required for Vif-mediated STAT1 and STAT3 degradation, HEK293T cells were transiently transfected with $CdCl_2$-inducible Vif, VifΔSLQ or EV. The expression of Vif significantly reduced STAT1 (Fig. 8A) and STAT3 (Fig. 8B) protein expression, while VifΔSLQ reduced neither STAT1 (Fig. 8A), nor STAT3 (Fig. 8B), indicating that the formation of the functional ECS-type E3 ligase complex, via Vif's SOCS-box, enables STAT1 and STAT3 degradation.

3.10. Vif-Mediated STAT Degradation Requires Cul5 and RBX2 E3 Ligase Proteins

Having found that the Elongin C binding site within Vif's SOCS-box was essential for STAT1 and STAT3 degradation, we next analysed, using shRNA knockdown, if the ECS scaffold proteins, Cul5 and RBX2 were also required for Vif-mediated STAT degradation. Cul5 shRNA successfully suppressed Cul5 mRNA expression (Fig. 9A). The expression of fellow family member, Cul2, was not reduced by Cul5 shRNA, which ensured knockdown specificity (Suppl. Fig. 5A). While the expression of Vif significantly degraded STAT1 and STAT3 in the presence of the control (CT) shRNA, suppression of Cul5 impaired the ability of Vif to degrade STAT1 and STAT3 proteins (Fig. 9C & E), indicating that Cul5 is required for their degradation. To determine if RBX2 was also required for Vif-mediated STAT degradation, endogenous RBX2 expression was suppressed using specific shRNA. RBX2 shRNA successfully suppressed its mRNA expression (Fig. 9B), while the expression of fellow family member, RBX1, was unaffected, ensuring knockdown specificity (Suppl. Fig. 5B). As we observed with Cul5 knockdown (Fig. 9C & E), suppression of RBX2 expression also impaired Vif-mediated degradation of STAT1 and STAT3 protein (Fig. 9D & F), indicating that RBX2 is

Fig. 5. PBMCs from HIV-infected patients have reduced STAT1 and STAT3 protein levels, IFN-α-induced STAT activation and lower levels of ISG15 induction. PBMCs were isolated from 16 HIV-infected patients and 5 healthy control volunteers. Cells were treated with IFN-α for 15 min, before being lysed in RIPA buffer. (A) Cell lysates were subjected to immunoblotting probing with phospho-STAT1, STAT1, phospho-STAT3, STAT3 and β-actin antibodies. Densitometry analysis represents the levels of (B) pSTAT1, (D) pSTAT3 detected in cell lysates from IFN-α-stimulated cells and (C) STAT1 and (E) STAT3 protein detected in the cell lysates from unstimulated cells, which are presented as a ratio to β-actin. (F) PBMCs were isolated from 12 HIV infected patients and 5 healthy controls, before treatment for 2 h with IFN-α. Total RNA was extracted and then converted to cDNA, which was used as a template to measure mRNA levels of ISG15 using qRT-PCR. Gene expression was calculated relative to the housekeeping gene RPS15. IFN-stimulated values were compared to unstimulated, which were normalised to 1. All graphs are the mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ (Mann-Whitney).

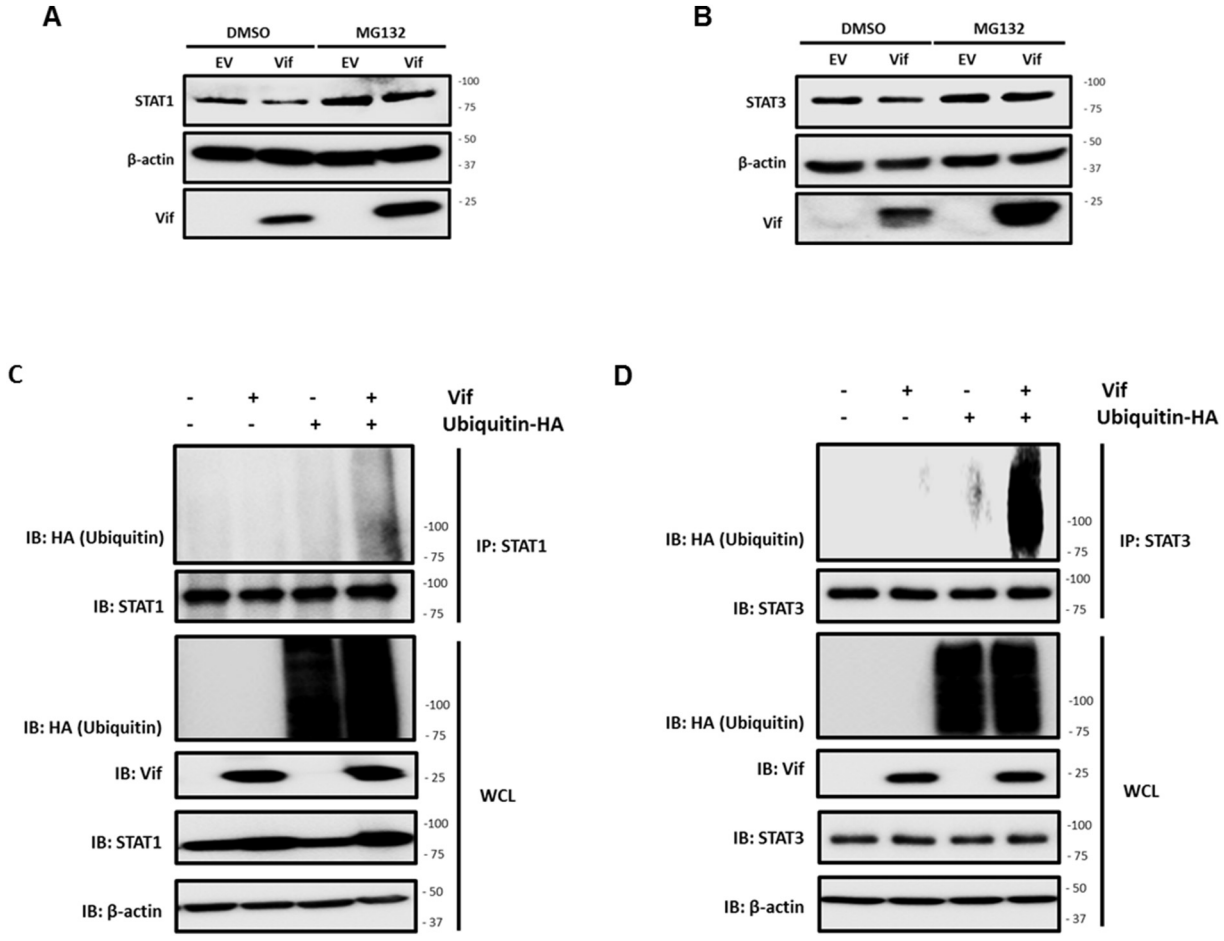


Fig. 7. Loss of STAT1 and STAT3 in the presence of Vif is mediated by ubiquitination and proteasomal degradation. HEK293T cells were transfected with HIV-Vif or EV control for 24 h before cells were treated with 1 μM MG132 for 16 h. Lysates were subjected to Western blotting using (A) STAT1 or (B) STAT3 antibodies. All blots were re-probed with Vif and β-actin antibodies. (C) HEK293T cells were transfected with STAT1-flag, HIV-Vif and Ubiquitin-HA or EV control for 24 h. Cells were then treated with 1 μM MG132 for 16 h before lysates were generated and immunoprecipitated using an anti-STAT1 antibody. (D) HEK293T cells were transfected with HIV-Vif and Ubiquitin-HA or the corresponding EV controls for 24 h. Cells were then treated with 1 μM MG132 for 16 h before lysates were immunoprecipitated using an anti-STAT3 antibody. IP and WCL were subject to Western blotting using a HA antibody. Blots were re-probed for (C) STAT1, (D) STAT3, Vif and β-actin. All blots are representative of three independent experiments and kDa ladder weights included on right of the blots.

also required for their degradation. Together these results demonstrate that Vif uses the "classical" ECS-E3 ligase complex to promote STAT1 and STAT3 degradation.

4. Discussion

The ubiquitin-proteasome system, found in all cells types, is required for the recycling of damaged, dysfunctional or redundant proteins (Hershko and Ciechanover, 1998). Several viral species hijack this basic biological mechanism to promote their own survival. By targeting cellular proteins required for anti-viral immunity, viruses evade endogenous defence mechanisms and increase the amino acid pool for synthesis of their own proteins. This immune evasion process is elegantly demonstrated through the actions of HCV, RSV and Mumps, which target the Type I IFN JAK/STAT pathway proteins for degradation (Sheehy et al., 2003; Stevenson et al., 2013; Elliott et al., 2007; Ulane et al., 2003). The HIV-protein Vif also uses this ubiquitin-proteasome system to degrade the cytidine deaminase, APOBEC3G (Sheehy et al., 2003). Furthermore, Vif and Viral protein r (Vpr), have been shown to target IRF3 for proteasomal degradation (Okumura et al., 2008). Whereby the effect of Viral protein u (Vpu), upon IRF-3 is less clear, with one group reporting its proteolytic degradation (Doehle et al., 2012) and another showing no Vpu-mediated degradation (Hotter et al., 2013). Vpu has also been shown to promote proteolytic cleavage of IRF3, resulting in a fragmented IRF3, that no longer contains its essential DNA binding domain (Park et al., 2014). Interestingly, the HIV-1 Protease is also thought to inhibit IRF-3 phosphorylation and decrease expression of both IFN and ISGs, by targeting RIG-I to the lysosomes (Solis et al., 2011). Collectively, this spectrum of HIV immune evasion mechanisms that target detection, IFN production and ISG expression, highlight the importance of the type 1 IFN anti-viral process in clearing HIV infection (Bourke et al., 2017). This current study indicates that HIV-Vif targets STAT1 and STAT3 proteins for degradation, indicating an effective immune evasion technique used by HIV-1 to block the anti-viral effects of Type I IFNs. Type I IFN expression is induced on detection of viral PAMPs (Pathogen Associated Molecular Patterns), by PRRs, which subsequently activates the JAK/STAT pathway (Perry et al., 2005). The anti-viral activities of the JAK/STAT pathway makes its components obvious targets for viral immune evasion. Since several viruses have already been shown to promote STAT protein degradation (Banks et al., 2003), we wondered if HIV-Vif also uses its E3 ligase activity to block this essential pathway. Indeed, we found that Vif not only catalysed the ubiquitination and proteasomal degradation of the classical anti-viral protein, STAT1, but also targeted the multi-functional protein, STAT3, presenting a mechanism by which HIV subverts immune responses. The anti-viral role of STAT1 during IFN-α signalling is well established (Fu et al., 1990), making it a prime target for Vif-mediated degradation that acts to block downstream ISG induction. In contrast, while STAT3 is phosphorylated by IFN-α (Yang et al., 1998; Stevenson et al., 2013), its anti-viral role remains less clear. However, several

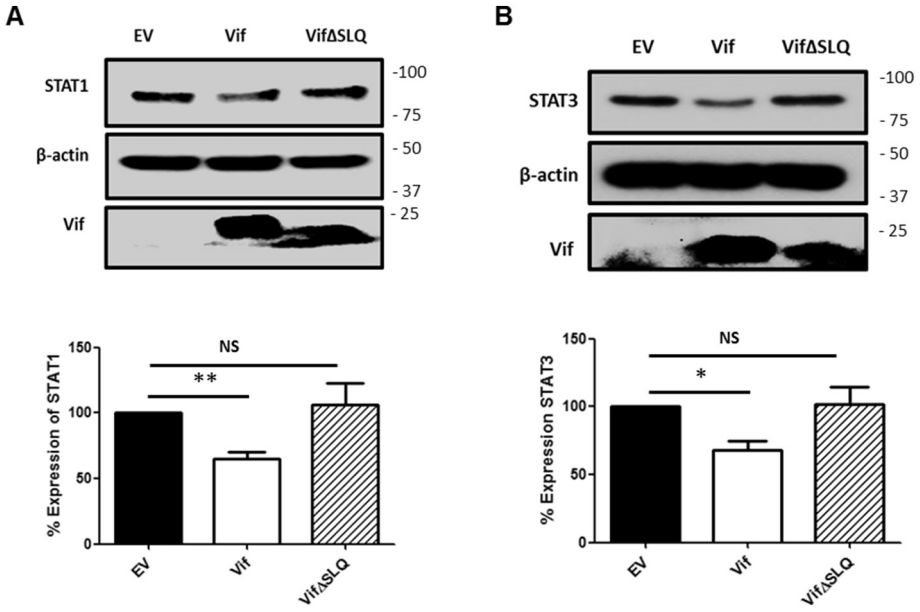


Fig. 8. Vif's Elongin C SOCS-box binding site is required for Vif-induced STAT1 and STAT3 degradation. HEK293T cells were transiently transfected with $CdCl_2$-inducible DNA constructs encoding Vif-WT, VifΔSLQ or EV control for 24 h. Cells were treated with $CdCl_2$ for 24 h before cell lysates were generated. Immunoblot analysis was performed using (A) STAT1 or (B) STAT3 antibodies. All the blots were re-probed for Vif and β-actin. Densitometric analysis was performed using image lab software and levels of STAT1 or STAT3 were calculated relative to β-actin and compared to the EV control, which was normalised to 100%. Graphs are the average ± SD of three independent experiments and kDa ladder weights are included on right of the blots. $^{*}P < 0.05$; $^{**}P < 0.01$ (Paired *t*-test).

viruses, including Measles, Mumps and HCV, target STAT3's signal transduction (Stevenson et al., 2013; Ulane et al., 2003; Palosaari et al., 2003) and we have shown STAT3 to be essential for anti-viral activity against Vaccinia and Influenza (Mahony et al., 2017), pointing towards a significant role for STAT3 in anti-viral immunity. Indeed, HIV-Vif-mediated STAT3 degradation further emphasises its anti-viral potential.

Type 1 IFNs are highly pleiotropic innate cytokines, contributing directly to HIV-1 control via upregulation of ISGs, and indirectly via the activation of other innate and adaptive anti-viral responses. IFN-α JAK/STAT signalling mediates induction of ISGs and can regulate cell activation, function, proliferation or survival (Der et al., 1998b). In ThP-1 cells we studied the effects of Vif-mediated STAT degradation upon IFN-α induction of two ISGs, with diverse anti-viral effects. We found that the induction of ISG15, but not MxA, was significantly suppressed, revealing a differential, but specific immune evasion mechanism for Vif. Since we observed a Vif-mediated reduction in STAT1 and STAT3 expression, it was no surprise to observe that IFN-α induction of ISG15 was significantly reduced. Indeed, ISG15 has an essential role inhibiting HIV-1 virion release (Okumura et al., 2006; Pincetic et al., 2010; Woods et al., 2011), which makes it a likely immune evasion target for HIV. Interestingly, STAT3 has been shown to have a role in the upregulation of ISG15 in response to IFN-α which may explain why Vif has evolved to target it for proteasomal degradation (Mahony et al., 2017). In contrast to ISG15, MxA has no currently known role in the control of HIV-1 replication. Interestingly, Ribavirin, which was until recently, used in combination with IFN-α for the treatment of HCV, enhances IFN-α-mediated MxA induction in Huh7 cells, but fails to enhance IFN-α induced PKR, OAS or CXCL10 (Stevenson et al., 2011), highlighting the complexity of signal transduction involved in specific ISG expression. Indeed, HIV can upregulate MxA independently of IFN-α in monocytes, further revealing the unique nature of MxA expression and an area of research that warrants further exploration (Baca et al., 1994). We found that IFN-α induced significantly higher levels of ISG15 in primary human PBMCs infected with a IIIB Vif deletion mutant (IIIBΔVif), compared to cells infected with the full length virus; and similar to our findings in ThP-1 cells, the presence of Vif had no significant effect on MxA induction. MxB, unlike MxA, has been shown to have an inhibitory effect upon HIV-1 (Goujon et al., 2013; Haller and Kochs, 2011; Kane et al., 2013; Liu et al., 2013). In line with MxB's important role against HIV, we found that compared to PBMCs infected with full length HIV-1 IIIB, IIIBΔVif-infected cells treated with IFN-α also produced significantly higher levels of MxB in response to IFN-α. Indeed these results highlight that, unlike MxA, MxB induction is stunted in the presence of Vif. Together these findings provide an insight into how Vif protein creates a favourable environment for HIV-1 replication.

In agreement with our findings in primary PBMCs from HIV-infected patients, Hardy et al., reported that monocytes from HIV-infected patients had reduced levels of pSTAT1 and anti-viral gene induction (Hardy et al., 2009). It is worth noting that they also discovered that the effect of HIV infection upon IFN-α signalling was independent of IFNα/β levels (Hardy et al., 2009), indicating that, as we have now discovered, HIV infection attenuated the IFN-α signalling pathway. While our studies showed that "acute" infection of primary human PBMCs with HIV-1 stunted pSTAT1 and pSTAT3 expression, we did not observe a reduction of total STAT1 and STAT3 protein. However, compared to healthy controls, both phosphorylation and total STAT protein levels were reduced in PBMCs from "chronically" infected patients. Indeed, it is well known that "acute" infection of HIV leads to a cytokine "storm" (Stacey et al., 2009), and since cytokines induce the expression of STATs (Lehtonen et al., 1997; Narimatsu et al., 2001), we hypothesise that cytokine induction of STAT proteins during "acute" infection of PBMCs may indeed "mask" the Vif-mediated reduction of STAT proteins. Of course, while HIV-Vif may have a significant role in regulating IFN-α responses via STAT degradation, we should note that other HIV immune evasion strategies are also "at play" in vivo. Indeed, compared to controls, infection of PBMCs with both HIV-1 IIIB and IIIBΔVif decreased IFN-α-mediated ISG15, MxA and MxB induction, highlighting the presence of additional HIV immune-suppressive activity.

Importantly, our studies also indicate that Vif's attenuation of the IFN-α signalling pathway affects HIV infectivity. In fact, upon pre-treatment of CEM cells with IFN-α, there was reduction in p24 protein levels in IIIBΔVif infected cells, but not in IIIB infected cells, indicating that Vif protects HIV from innate anti-viral IFN-α responses. Similarly to Vif, the V proteins of simian virus 5 and human parainfluenza virus type 2 target

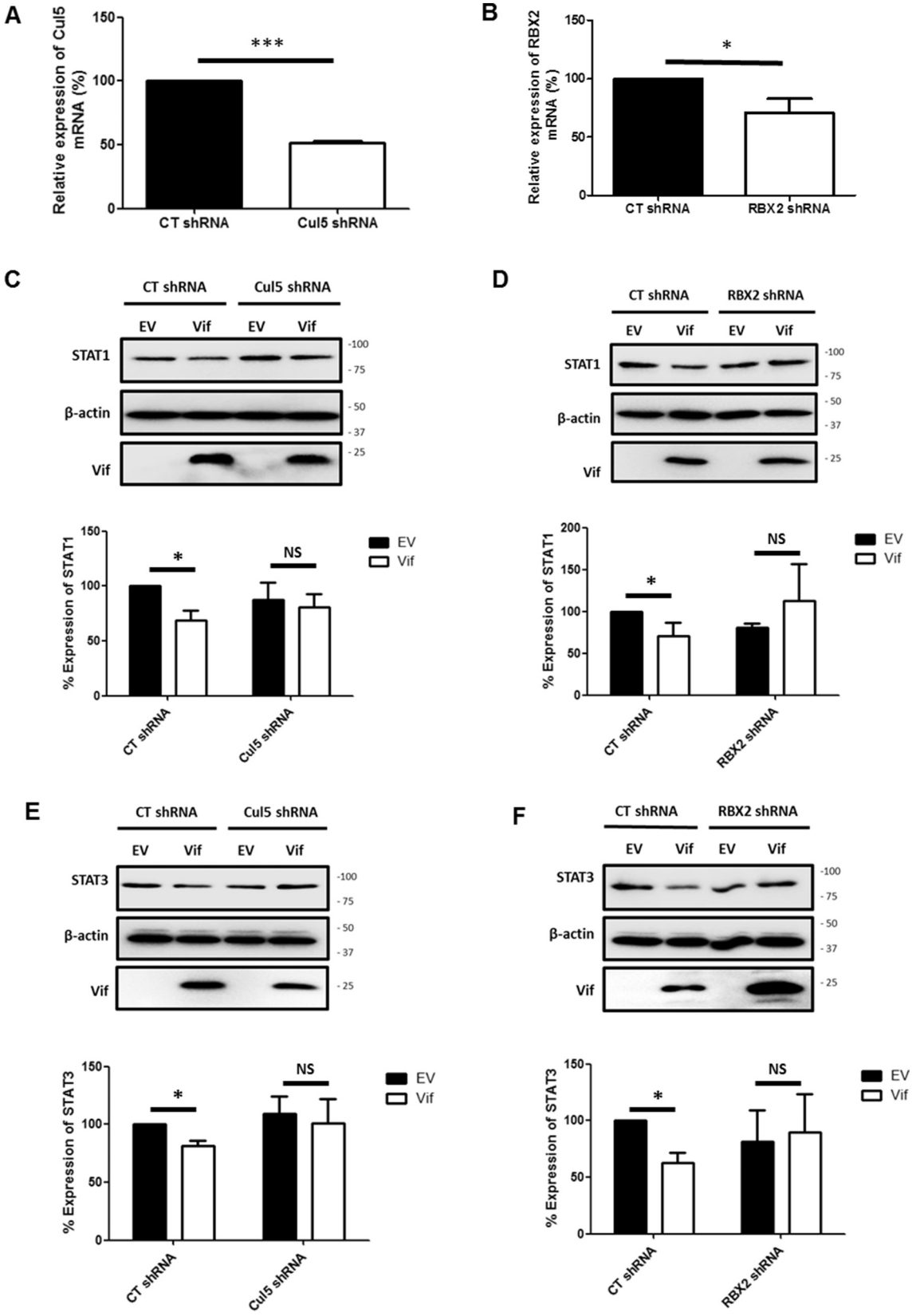


Fig. 9. The E3 ligase proteins, Cul5 and RBX2, are essential for Vif-mediated STAT degradation. HEK293T cells were transfected with shRNA specific for (A) Cul5, (B) RBX2 shRNA or control (CT) shRNA before mRNA of (A) Cul5 or (B) RBX2 were measured by qRT-PCR. RNA was calculated relative to the house-keeping gene, RPS15, and compared to CT shRNA, which was normalised to 100%. Graph is the average ± SD of (A) three or (B) four independent experiments. HEK293T cells were transfected with HIV-Vif or EV control along with shRNA specific for (C, E) Cul5 or (D, F) RBX2 or control shRNA. Protein lysates were subjected to immunoblotting using (C, D) STAT1 or (E, F) STAT3 antibodies. All blots were re-probed for HIV-Vif and β-actin. Densitometric analysis was performed using image lab software and levels of STAT1 and STAT3 were calculated relative to β-actin and compared to CT shRNA/EV controls, which were normalised to 100%. kDa ladder weights included on the right of the blots. Graphs are the average ± SD of (C, D, F) three or (E) four independent experiments. $^{*}P < 0.05$; $^{***}P < 0.001$; NS (Paired *t*-test).

STAT proteins for degradation, which also prevents the induction of an anti-viral state in V protein expressing cell lines (Andrejeva et al., 2002). Together these findings highlight the crucial antiviral activity of STAT proteins.

IFN-α's broad anti-viral effects made it an obvious choice for treatment for HIV infection in the early 1980s. However, responses to treatment were variable, with higher baseline CD4 T cell counts being associated with better clinical response and greater decline in viral load. While these clinical benefits were modest and some decline in viral load was observed in several studies, the decrease was limited in magnitude (typically 0.5 to 1 log), and was not sustained (Katabira et al., 1998; Vento et al., 1993; Soriano et al., 1994; Sperber et al., 1993; Fischl, 1991; Skillman et al., 1996; Hatzakis et al., 2001; Asmuth et al., 2010; Manion et al., 2012). More recent studies have tested the effects of pegylated-IFN-α upon HIV control during structured ART interruptions. While enhanced HIV control was observed in some studies, viremia eventually rebounded in patients who remained off ART (Dianzani et al., 2008; Azzoni et al., 2013; Lane et al., 1988; Goujard et al., 2012). Together, these findings suggest that IFN-α treatment can improve the control of HIV (particularly when administered in early infection, or to patients treated with ART), but unknown viral immune evasion mechanisms have limited its efficacy. The results of our study reveal a mechanism that may help explain this therapeutic failure. We have found that HIV-Vif has a functional role in degrading STAT1 and STAT3, demonstrating a mechanism by which HIV blocks specific anti-viral gene induction. Restoration of anti-viral responsiveness to IFN-α may enhance HIV clearance and pave the way towards a new therapeutic approach for HIV, that could be used alongside ART and/or employed together with latency-reversing agents, as part of a much needed HIV cure strategy.

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Conflicts of Interest

None.

Author Contributions

All authors analysed and interpreted data; assisted in drafting the article and approved the version to be submitted. SG, SA, RM, CB, SN also carried out experiments that acquired the data. NJS also conceived and designed the study.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.03.006.

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