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Characterizing Immune Response to HIV-1 Infection in BICD2-Knockout Cells

Omar Abdel-Rahim
Loyola University Chicago

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LOYOLA UNIVERSITY CHICAGO

CHARACTERIZING IMMUNE RESPONSE TO HIV-1 INFECTION IN BICD2-KNOCKOUT
CELLS

A THESIS SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
MASTER OF SCIENCE

PROGRAM IN INFECTIOUS DISEASES AND IMMUNOLOGY

BY

OMAR ABDEL-RAHIM

CHICAGO, ILLINOIS

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LIST OF ABBREVIATIONS

| | |
|----------------------------------|-------------------------------------|
| HIV-1 | Human Immunodeficiency Virus Type 1 |
| NC | Nucleocapsid |
| MA | Matrix Protein |
| CA | Capsid Protein |
| RTC | Reverse Transcription Complex |
| NVP | Nevirapine |
| NPC | Nuclear Pore Complex |
| AIDS | Acquired Immunodeficiency Syndrome |
| ART | Antiretroviral Therapy |
| SIV | Simian Immunodeficiency Virus |
| BICD2 | Bicaudal D Homolog Protein 2 |
| VLP | Virus-Like Particles |
| IFN | Interferon |
| ISG | Interferon-Stimulated Gene(s) |
| rhTRIM5 α | Rhesus Macaque TRIM5-alpha |
| TRIM5 α /huTRIM5 α | Human TRIM5-alpha |
| cGAS | Cyclic GMP-AMP Synthase |
| qRT-PCR | Quantitative Real-Time PCR |
| RNA-Seq | RNA Sequencing |

ABSTRACT

Human immunodeficiency virus (HIV-1) is an intracellular pathogen that has infected over 37 million people worldwide, with over 1 million deaths annually. Although incidence has decreased over time, it remains a persistent global health concern. This is especially the case in young, sexually active adults and in Sub-Saharan Africa, where antiretroviral therapies are not readily available. The virus is known for its ability to infect and kill host immune cells (such as T cells and macrophages), causing progressive loss and dysregulation of the host immune response.

The HIV-1 infection cycle begins when the extracellular virion binds to host surface receptors and fuses with the cellular membrane. A viral ‘core’, a capsid housing an RNA genome and viral enzymes, is released into the cytoplasm of the host cell. The viral core attaches to the host microtubule network through cargo adaptors, hijacking conventional transport systems to travel through the cytoplasm and reach the nucleus.

As the core traffics in the cytoplasm, the viral RNA genome is reverse transcribed into DNA, coordinated with capsid uncoating. Upon arrival at the host nucleus, the capsid helps facilitate the import of viral DNA through a mechanism that remains incompletely understood. The viral DNA is then integrated into the host genome, where it can either be expressed to produce viral progeny or remain latent within the host genome. This latency can last years and is a major reason why HIV-1 can be difficult to effectively combat with currently available therapies.

Prior to the integration necessary to establish latency, one of the first major intracellular interactions the viral core must navigate is attachment to the host microtubule network, crucial

for transport to the nucleus. It has already been shown that numerous viruses, including HIV-1, can utilize host motor proteins and cargo adaptors to effectively traffic through the cytoplasm. In doing so, viruses may be able to evade immune detection, potentially reaching the nucleus before immune alarms can be tripped.

Without this attachment to the host microtubule network, proper viral translocation is disrupted, and an immune response can be initiated. However, the connection between this interruption of trafficking and the induction of an immune response remains unclear and is the topic of recent study. If the relationship between the virus and host cargo adaptors it attaches to could be further investigated, therapies to interfere with this interaction and elicit an immune response in infected cells could potentially be developed. This could provide an alternative means of combating HIV-1 infection without using current treatment methods, which require lifelong therapy and high levels of treatment adherence to avoid resistance, and can be associated with adverse effects over time.

To investigate the elevated immune response observed in the absence of proper viral translocation, we utilized cell lines lacking an important cargo adaptor for HIV-1 trafficking, BICD2. We hypothesized that, in the absence of BICD2, a key viral determinant was being detected by a host restriction factor to trigger the host immune response. We aimed to investigate what viral component was responsible as well as what host factors were involved in detection and larger immune activity. We found that the viral capsid appears to induce the elevated immune response in the absence of BICD2, after apparent detection by capsid sensor TRIM5 α , and that the broader host immune response involved both innate and adaptive immune pathways.

CHAPTER ONE

BACKGROUND

Literature Review

Human Immunodeficiency Virus (HIV-1).

There is a far greater quantity of research on HIV-1 than can be covered here. In the context of my research, it is important to consider HIV-1 in terms of structure, infection cycle, and interaction with the host immune system.

In its extracellular form, the HIV-1 virion is composed of an outermost lipid layer which contains the HIV-1 envelope proteins gp41 and gp120 which protrude from the lipid bilayer [1]. There is a layer of matrix proteins underneath this lipid layer, and an internal HIV-1 'core'. The viral core is comprised of a conical protein capsid shell that houses viral RNA, necessary enzymes (reverse transcriptase [RT], integrase, etc.), and viral nucleocapsid proteins [1, 2].

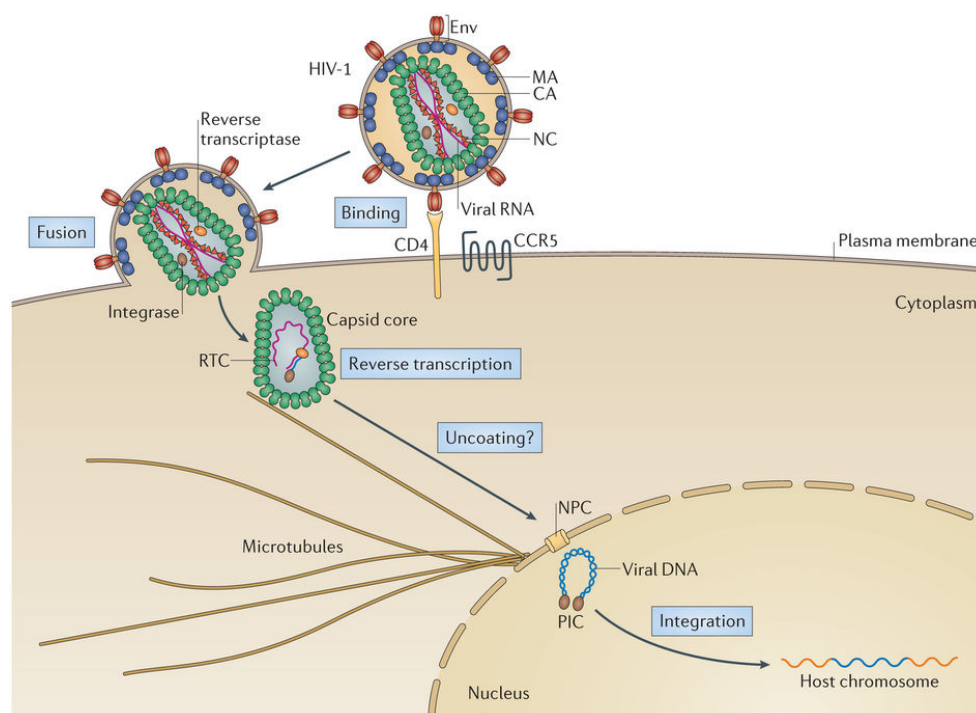
To begin the process of viral entry, the HIV-1 envelope binds to extracellular receptor proteins (such as CD4). Co-receptor proteins, such as CXCR4 or CCR5, are recruited and help facilitate this entry [1]. The target cells of HIV-1 infection are cells of the immune system which express CD4, such as CD4+ T-cells or macrophages. Following this receptor interaction, the HIV-1 virion fuses with the host membrane and releases the viral core into the host cell. The core then attaches to the host microtubule via cargo adaptors and begins translocation towards the host cell nucleus [3-5].

As the core traffics through the cytoplasm and towards the host nucleus, the viral RNA genome is reverse transcribed to DNA, utilizing the reverse transcriptase enzyme and nucleotides

found within the host cell. Additionally, while in the cytoplasm, the HIV-1 capsid undergoes uncoating, the process by which the capsid CA protein which comprised the viral core is shed from the rest of the ribonucleoprotein complex [2]. However, the coordination between these processes is crucial, as the uncoating of the capsid can expose the viral genome to several host restriction factors [6].

If properly timed, the viral core will arrive at the host nucleus as the capsid has uncoated enough to allow for the passage of the viral DNA from the core and into the nucleus. This is done through interaction with nuclear pore (NUP) proteins lining the nucleus such as NUP358 [2, 7]. After successful entry, the DNA is integrated into the host genome, with the help of another key enzyme brought along by the virus, integrase [8].

To facilitate integration, integrase catalyzes the attack of 3' phosphodiester bonds on double-stranded DNA, forming overhangs on either side [8, 9]. After processing and removal of overhang bases, integrase then facilitates the strand transfer, incorporating viral DNA into the host genome. Subsequent steps to finalize and complete this integration are catalyzed by host DNA repair enzymes. This completes the first half of the HIV-1 infection cycle (Fig. 1).



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Figure 1. HIV-1 Infection Cycle. A simplified cartoon of the first half of the HIV-1 infection cycle. It begins with the attachment of the virion to extracellular host receptors, followed by fusion, viral core entry & microtubule attachment, translocation, nuclear import, and proviral integration. Adapted from Campbell & Hope (Nature 2015) [2].

After integration into the host genome, the viral DNA can be transcribed into viral RNA genome and viral mRNA, both of which are processed and exported to the cytoplasm [10, 11]. From there, the mRNA is translated into Gag and Gag-Pol polyprotein precursors, viral envelope glycoproteins, and accessory viral proteins (that contain numerous viral proteins such as CA, RT, protease and integrase) that then traffic to the plasma membrane. These Gag, Gag-Pol and Env proteins can then travel to the plasma membrane, where the Gag and Gag-Pol polyprotein precursors can begin to multimerize.

During this process, the viral RNA genome is encapsidated into the assembling virion. Envelope glycoproteins are incorporated, and with the help of the endosomal sorting complex required for transport (ESCRT) protein machinery the virion buds from the infected cell. Once

budding is complete, the virion is released, though it is still in immature form. The virion transitions to a mature form as its Gag and Gag-Pol precursor polyproteins are cleaved by the viral protease into several Gag proteins, including CA (that is used in assembly of the capsid core). The mature virion, with the conical capsid core, can then begin the infection cycle over again [10]. Over decades of research, as knowledge of the HIV-1 infection cycle has grown, new treatments have been developed to combat infection.

Conventional Treatment of HIV-1 Infection.

Treatment of HIV-1 infection was developed to combat acquired immunodeficiency syndrome (AIDS), the set of illnesses resulting from advanced HIV-1 infection. Earliest therapies involved administration of azidothymidine (AZT), a reverse transcriptase inhibitor [12, 13]. AZT is an analog of a common nucleoside, thymidine triphosphate, and effectively terminates the synthesis of viral DNA, as subsequent nucleosides cannot bind to the nitrogen group present on AZT 5-triphosphate.

Though effective in limiting the production of HIV-1 DNA, AZT has a number of side effects and limitations [14]. These include partial obstruction of host mitochondrial DNA polymerase activity, as well as suppression of red blood cell production in the bone marrow, leading to anemia [15]. Additionally, HIV-1 is capable of developing resistance to AZT by mutation of its viral reverse transcriptase [16, 17]. Though AZT and other more recently approved antiretroviral medications are active against HIV, it is generally insufficient to treat HIV-1 with a single drug due to the development of resistance in the setting of monotherapy. In order to better treat HIV-1 infected patients, combination antiviral drug treatment known as ART (antiretroviral therapy) is employed [18].

As a combination of multiple different antiviral drugs administered over an extended period, ART has been shown to be effective at limiting productive HIV-1 replication and transmission [18, 19]. To do so, effective ART regimens typically include some combination of protease inhibitors, integrase inhibitors, and nucleoside and non-nucleoside reverse transcriptase inhibitors. In a comprehensive effort to combat viral infection, ART remains a way to target the HIV-1 infection cycle at multiple steps.

Though current ART is much more effective than past treatments, it does not completely abolish HIV-1 infection in the host [18, 20, 21]. In patients receiving ART treatment, HIV-1 remains integrated into the host genome, allowing latently infected cells to persist and leading to ongoing viral replication in tissues which may not be detectable in blood. As HIV-1 is known for its capacity to mutate, particularly in the setting of nonadherence to ART, patients can develop antiviral resistance that may require switches to alternate, more complex ART therapies [22].

In addition to viral resistance, ART itself can have adverse side effects, including increased risk of hypersensitivity responses, lipid disorders, toxicity to the liver, kidneys, and gastrointestinal tract, and possibly cardiovascular disease [23]. As current treatment is intended for life, ART also bears a significant financial cost [24]. In short, though effective, ART has a number of drawbacks.

In recent studies, the effectiveness of replacing ART with interferon monotherapy has been assessed [25]. In one sample study, HIV-infected patients who had been receiving ART were assigned to have pegylated interferon added to their ART regimen. After 5 weeks ART was interrupted, but IFN monotherapy was continued for 12 or 24 weeks, and viral load and levels of HIV-1 DNA integration were assessed. Compared to past historical studies on the effects of ART interruption, a comparatively higher proportion of individuals had decreased levels of HIV-1

DNA integration. Additionally, a significant proportion of individuals had lower viral loads than ‘virologic failure’ benchmarks despite ART interruption, further suggesting that administration of interferon can have a suppressive effect in the absence of ART.

The benefits of interferon administration have also been shown in combating relatives of HIV-1 infection [26]. In a study of rhesus macaques infected with simian immunodeficiency virus (SIV), treatment with interferon was found to induce expression of antiviral genes, reducing the levels of systemic infection. Indeed, blockage of the IFN receptor crucial to this immune response led to lower levels of antiviral gene expression, increases in the size of SIV reservoirs and more rapid progression to AIDS. Taken together, these results suggest that the administration of interferon offers significant promise in combating HIV-1 infection.

Despite its benefits, the major limitations of systemic interferon treatment are adverse response to the treatment itself and a substantial drop-off in effectiveness observed over time. Though successful in combating early viral infection, the initial suppression of virus by interferon is not sustainable for extended periods in infected individuals [26-28]. Eventually the viral load climbs, and CD4 T-cell depletion is accelerated. Additionally, the constitutively active state of the host immune system has a number of clinical consequences, including an increased risk of heart complications [29]. This eventual desensitization of the host poses an obstacle to the common administration of interferon as a means of HIV-1 treatment, despite its potential to boost the host immune response.

Host Factor Detection of HIV-1 & Immune Response.

Though HIV-1 does not usually elicit an effective immune response, the host cell has multiple factors that can detect different viral determinants, including the genome and capsid,

and induce an immune response. Two of the most well-studied host sensors for HIV-1 are the nucleic acid sensor cGAS and the capsid sensor TRIM5 α .

Cyclic GMP-AMP synthase (cGAS) is an enzyme specialized for the detection of viral DNA and is capable of initiating an antiviral response [6, 30-32]. Upon detecting viral DNA, cGAS catalyzes the synthesis of cyclic guanosine adenosine monophosphate (cGAMP) that acts as a second messenger molecule and initiates the activation of the endoplasmic reticulum protein stimulator of interferon genes (STING) [31, 32]. STING then acts as an innate signaling hub, coordinating multiple downstream immune responses (Fig. 2).

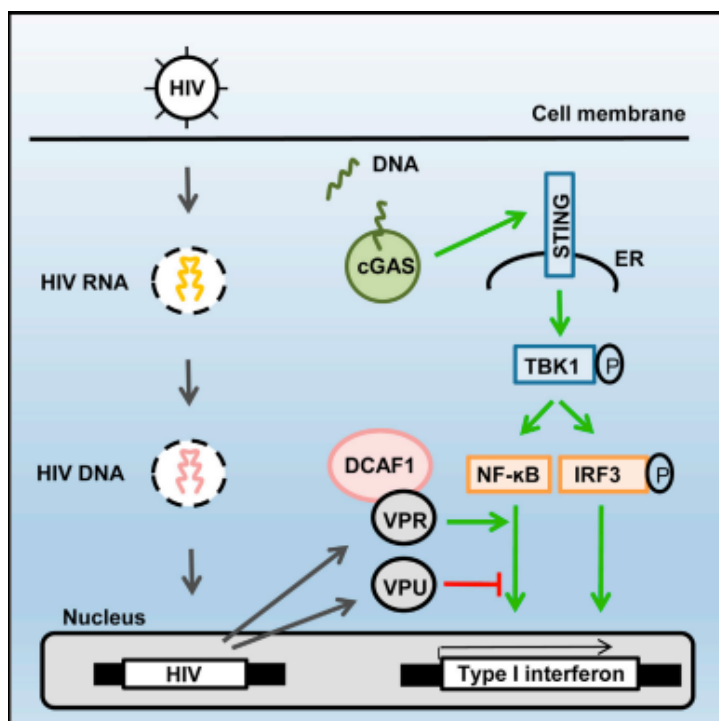


Figure 2. cGAS Activity. The basic mechanism of action for cGAS activity in response to HIV-1. Viral DNA (produced after reverse transcription of the viral RNA genome) can be detected, inducing the production of cGAMP. cGAMP then activates the STING receptor, that can activate TBK1, leading to downstream activation of NF κ B and IRF3, that stimulate the innate immune response. This can be inhibited by viral factors like Vpu. Adapted from Vermeire et. al (Cell Reports 2016) [32].

The major outcomes of cGAS detection of viral genome include interferon production and the activation of antiviral transcription factors such as IRF3 and NFkB [31, 32]. This leads to the initiation of an innate immune response. Impairing cGAS activity, by viral factors such as Vpu targeting IRF3 for proteolytic degradation, has been shown to reduce levels of type 1 interferon production and decrease innate immune signaling, leading to a higher susceptibility to infection [32, 33].

As the capsid is such a central component to HIV-1 infection, it is unsurprising that there are host factors capable of detecting it as well. This includes the cytoplasmic capsid sensor TRIM5 α [34]. Initially discovered in rhesus macaques resistant to SIV infection, TRIM5 α is now one of the most studied antiviral factors in the current literature. Although rhesus macaque TRIM5 α can more efficiently restrict HIV-1 [34-36], numerous studies support the notion that human TRIM5 α can inhibit HIV-1 infection to some degree or in some cell types [35, 37-40].

After viral entry, TRIM5 α recognizes the HIV-1 capsid, and forms a hexameric lattice around the viral core [34, 41]. From there, the capsid is fragmented (known as ‘premature uncoating’), by a mechanism that has not been fully established [36]. This disrupts the delicate coordination between uncoating and reverse transcription [30]. Additionally, the dimerization of RING domains on TRIM5 α catalyzes the synthesis of K63-linked ubiquitin chains that can both mark TRIM5 α for degradation and activate the TAK1 kinase complex that activates downstream immune factors such as AP-1 and NF-kB [34, 42]. This leads to both the acceleration of uncoating and the induction of a host immune response (Fig. 3).

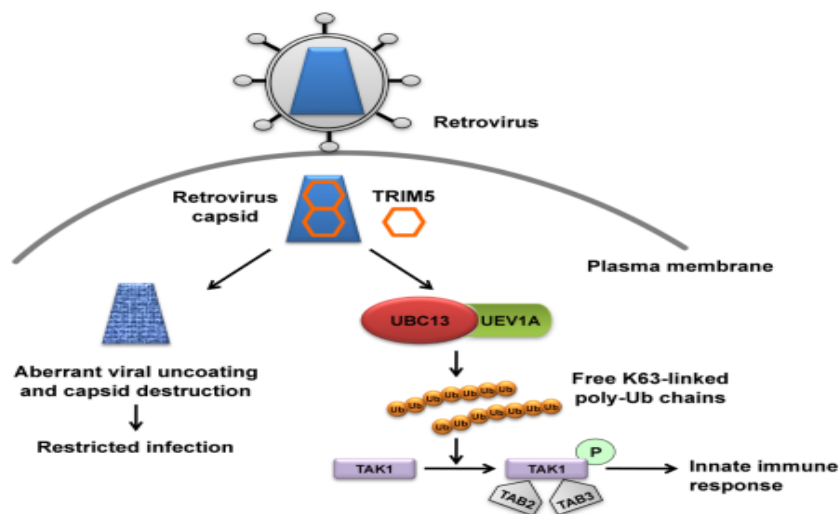


Figure 3. TRIM5 α Activity. The basic mechanism of action for TRIM5 α in response to HIV-1. The viral capsid is detected, followed by formation of a hexameric lattice, disruption of the capsid structure and activation of the TAK1 signaling pathway through K63 polyubiquitin chain synthesis (catalyzed by UBC13 & UEV1A enzyme complex). Adapted from de Silva & Wu (Viruses 2011) [43].

In considering the ability of human TRIM5 α to restrict HIV-1, mutations in human TRIM5 α that alter susceptibility to HIV-1 infection have been well characterized. For example, infected individuals with a mutation in the TRIM5 gene at position 43 such that the amino acid encoded changes from a histidine to a tyrosine (H43Y) may have reduced TRIM5 α restrictive ability and an accelerated disease progression into AIDS [37, 44]. In contrast, a mutation at position 136 to change the encoded amino acid from an arginine to a glutamine (R136Q) appears to confer a protective effect against an HIV-1 variant as well as a potential decreased risk of infection [37, 45]. However, there remains substantial disagreement about whether some of these polymorphisms are actually responsible for altering the ability of human TRIM5 α to restrict HIV-1, and the effect these polymorphisms have on disease progression [44, 46, 47]. The functional importance of different TRIM5 α polymorphisms thus remains a contentious topic for investigation.

Higher expression of TRIM5 α has been associated with a reduced risk of HIV-1 infection, as seronegative individuals in high-risk areas were found to have higher expression levels of TRIM5 α compared to seropositive individuals [38]. Additionally, stabilizing the levels of TRIM5 α (that is normally ubiquitinated and degraded) has been found to improve host protection from HIV-1 infection [39]. The same can be said of overexpression of TRIM5 α , which has been found to improve host HIV-1 restriction. This suggests that the modest restrictive ability of human TRIM5 α for HIV-1 may be dependent on the levels of expression.

In addition to cGAS & TRIM5 α , there are other antiviral host factors involved in restriction of HIV-1 infection. This includes sensors for the same viral particles, such as IFI16 which is capable of viral DNA detection [48], and immune system mechanisms for detecting different HIV-1 particles entirely, such as B-cells recognizing gp120 envelope proteins [49]. Collectively, the host immune system utilizes both intracellular and extracellular factors to combat viral infection and initiate an immune response.

These immune responses often include the production of interferon molecules. These are small signaling molecules frequently made after the intracellular detection of a virus [29]. Interferon (IFN) molecules bind to host cell receptors, triggering phosphorylation cascades and activation of transcription factors. These transcription factors then promote the expression of 'Interferon-Stimulated-Genes' (ISGs), genes specialized for an antiviral response [50, 51].

ISGs have a variety of effects both intracellular and extracellular, helping contribute to a cohesive antiviral response by interfering with viral activity at every stage of the life cycle [50, 51]. For example, ISG20 impairs the production of viral RNA, while members of the IFIT family can disrupt viral protein synthesis [52, 53]. ISG products can affect the entry and exit of the HIV-1 virion into the host cell, as well as disrupting replication, reverse transcription, translation

and more. Collectively, the ISG response from IFN production appears to be critical to combating viral infection.

HIV-1 Immune Suppression.

To suppress the host immune response, HIV-1 utilizes numerous viral proteins. For example, Nef can reduce signaling through the T-cell receptor (TCR), by disrupting necessary cytoskeletal remodeling processes important to TCR activation [54]. Vpu suppresses the cGAS-mediated IFN response to HIV-1 infection [32]. Vif induces the ubiquitination and degradation of host factor APOBEC3G, that would otherwise deaminate viral DNA produced from reverse transcription [55, 56]. On the whole, such viral factors can impair the host capacity to combat HIV-1 infection.

On the cellular level, HIV-1 suppresses the host immune system by infecting and killing immune cells important to the cell-mediated response. Two major players in the host antiviral response are CD4⁺ and CD8⁺ T-cells, respectively [57]. CD4⁺ T-cells normally coordinate a host of immune functions, including activation of pro-inflammatory Th1 cells and production of interferon gamma to activate CD8⁺ T-cells. CD8⁺ cytotoxic T-cells identify and kill target infected cells, through release of granzymes and other cytolytic compounds [58, 59]. With such important roles, the infection and killing of CD4⁺ T-cells characteristic of HIV-1 is especially detrimental to the host [60-62]. Impairment of the host's cell-mediated immune response significantly reduces the capacity of the host to fight HIV-1 infection, allowing the virus to persist, and allowing the development of AIDS.

HIV-1 Immune Evasion.

Though there are numerous host factors capable of detecting different viral determinants, the fact remains that HIV-1 does not normally elicit an effective immune response upon host cell

infection. This is due at least in part to the ability of HIV-1 to evade immune detection [63, 64]. For example, as viral RNA is converted to DNA through reverse transcription, excess DNA in the cytoplasm is degraded by the host exonuclease TREX1, preventing it from being detected and an immune response being induced [65]. Once the viral DNA integrates into the host genome, it is able to avoid detection by host factors, as the viral DNA can remain latent for long periods of time [66].

To further evade detection and immune response, various components of the HIV-1 virion can demonstrate significant antigenic variation and sequence diversity. Changing the specific sequences of different viral components, such as the viral reverse transcriptase or Gag protein epitopes, can alter the susceptibility of the virus to immune factors and antiviral therapies [16, 18, 67].

Outside of the interplay between viral proteins and different host factors, one recently explored possibility could be that HIV-1 evades the immune system simply by efficiently translocating to the nucleus [4]. As the cytoplasm is not fluid, but rather retains significant viscosity, directed motion of the viral core could be imperative for access to the host nucleus and integration into the host genome [68]. The primary means by which HIV-1 travels intracellularly is by attachment to molecular motors and host cargo adaptors, allowing for trafficking along the host microtubule network [3-5, 69].

Virus Attachment to the Microtubule Network.

Made up of long, dynamic, polymers of tubulin proteins, the microtubule network is the backbone of cellular structure and organization [3, 69]. Through a large, winding microtubule cytoskeleton, cells maintain organization of organelles, retain overall cell structure, and traffic

molecules intracellularly. The cargo that can move along the microtubule network includes whole organelles, proteins, nucleic acids, and even viral particles [3-5].

To facilitate this intracellular travel, attached to the microtubule network are a large quantity of motor proteins, such as kinesin and dynein, that are utilized for anterograde and retrograde (outwards and inwards relative to the nucleus) transport, respectively [69-71]. These proteins can be incorporated into larger motor protein structures, such as the dynein/dynactin complex. Through ATP-mediated conformational changes, these motor protein complexes attach and travel along the microtubule network.

Viral hijacking of the host microtubule network is not specific to HIV-1 but has been demonstrated with multiple viruses [3-5, 72]. Interaction with the host microtubule network for efficient trafficking through the cytoplasm is often an integral step in the viral infection cycle.

Many transported cargoes, including viruses, attach not to motor protein complexes directly, but rather to cargo adaptors [4, 73, 74]. These are proteins that have specific ends for binding to both the motor protein complex and to cargo, respectively. Cargo adaptor interaction with the host motor protein complex increases processivity, allowing for efficient trafficking of cargo along the host microtubule network [74].

Bicaudal D Homolog Protein 2 (BICD2).

As previously described, in order for HIV-1 to effectively infect host cells, it must reach the nucleus, doing so by attaching to host cargo adaptors and travelling along the microtubule network [4, 5]. If the cargo adaptors are removed, then this trafficking is impaired and the viral core is left stranded in the cytoplasm, without means of directed motion to the nucleus [4]. This can significantly impair the productive infection of the host cell.

One cargo adaptor that HIV-1 can attach to is Bicaudal D Homolog Protein 2 (BICD2), a retrograde dynein adaptor protein primarily used for transport between the Golgi apparatus and the endoplasmic reticulum [4, 69]. In the context of HIV-1, BICD2 was identified in a genome-wide shRNA screen of factors that are important for viral replication [75]. It has been shown to attach via specialized binding domains to the HIV-1 viral core and the dynein/dynactin complex, and begin retrograde trafficking to the nucleus via a dynein motor.

When BICD2 is depleted, not only is this trafficking impaired, but an elevated level of ISG expression has been observed, leading to a decrease in productive infection [4]. Further, the uncoating process of the HIV-1 capsid (necessary to allow the passage of viral genome into the host nucleus) is perturbed upon depletion of BICD2. Unsurprisingly, the nuclear import of the viral genome is also disrupted, significantly hampering integration into the host genome. Collectively, this suggests that the attachment between the viral core, BICD2, and the dynein motor are essential in HIV-1 infection. However, this interaction has not been extensively investigated, with much of the specifics of detection and potential for immune response remaining unclear.

Aims and Hypothesis

In considering HIV-1 motility, previous work from our lab has shown that the dynein motor adaptor Bicaudal 2 Homolog Protein (BICD2) is a relevant factor. When BICD2 is depleted in cells, the transport of the viral core along the host microtubule network is significantly impaired. Subsequently, levels of viral infection fall and an elevated immune response is elicited, including increased production of interferon and expression of antiviral interferon-stimulated genes (ISGs).

Though the elevated ISG response is an important contributor to the impairment of HIV-1 infection observed in the absence of BICD2, the specific mechanisms behind this induction (as well as the relevant host factors) are not well known. The goal of this project is to characterize the viral determinants and host factors involved in this immune response elicited in the absence of BICD2.

In AIM 1, I hypothesize that there is a specific component of the HIV-1 core that is responsible for eliciting the elevated ISG expression observed in the absence of BICD2. The viral core has several components (capsid, viral RNA, viral DNA) that could be detected by host factors. Our goal is to investigate which are especially important for triggering the heightened immune response observed.

In AIM 2, I work to identify what cellular factors may be involved in both the detection of the HIV-1 viral particles in the absence of BICD2 as well as the subsequent immune response. The host cell has a variety of well-known factors specialized in the detection of HIV-1 capsid, genome, and other particles. My goal is to identify which are important in recognizing the viral core and initiating the elevated immune response observed.

Significance

Since the discovery of HIV-1 as the precursor to AIDS, numerous advances have been made in combating the virus at different stages of the life cycle. Though the virus is difficult to eradicate, progress has been made in improving the health of HIV-1 infected patients. HIV-1 is most commonly treated through administration of antiretroviral therapy (ART), a combination of antiviral compounds that inhibit viral replication at multiple steps of the life cycle [18]. As an investigated alternative to ART, it has been shown that treatment with pegylated interferon can upregulate antiviral gene expression, leading to an immune response capable of suppressing viral replication in the short-term [25, 26].

However, the major issue with current HIV-1 therapy and researched alternatives arises with long-term patient health. Though both ART & IFN treatment have their immediate benefits, ART cannot effectively abolish latent HIV-1 populations [20, 21], and sustained IFN leads to eventual desensitization [27, 29]. With alternative HIV-1 therapies an ever-growing field, an additional avenue to explore could be disrupting the function of cargo adaptors that HIV-1 utilizes to traffic to the host nucleus.

If therapeutics could be developed that interfere with the interaction between the intracellular viral core and the host microtubule network, this could significantly impair viral infection. This could be especially effective in patients receiving therapy as part of a 'kick and kill' strategy, where HIV-1 is roused from latency to be targeted [76, 77]. Upon reactivation, HIV-1 in the host receiving this novel therapy could potentially be less productively infectious, as the attachment step of the infection cycle is disrupted. This could lead to induction of an ISG response to combat viral infection that would be localized to infected cells where this HIV-BICD2 attachment would be occurring.

Additionally, treatments to disrupt the virus-microtubule network interaction could hypothetically elicit a localized ISG response without employing interferon monotherapy. This could potentially avoid the comprehensive desensitization and immunocompromise associated with systemic administration of interferon, while still combating infection. To begin considering treatment in this context, it is essential to further understand how the host responds to HIV-1 infection in the absence of viral attachment to cargo adaptors and the host microtubule network.

CHAPTER TWO
MATERIALS & METHODS

Cell Lines Used

THP1 and HEK293T (hereafter referred to as '293T') cells were obtained from ATCC. 293T cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 10% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin and 10ug/mL Ciprofloxacin Hydrochloride. THP1 cells were cultured in RPMI 1640 medium (Cellgrow) supplemented with 10% Gibco characterized FBS, 1% penicillin/streptomycin and 10 µg/ml Ciprofloxacin Hydrochloride.

Generation of Stable Knockout Cell Lines

CRISPR-Cas9 gene editing was used to generate BICD2 and TRIM5-knockout cell lines. CRISPR guide RNA (gRNA) oligonucleotide sequences were designed and ordered through Thermo Fisher Scientific. The forward and reverse gRNA oligonucleotides were annealed together via overnight PCR (37°/30min., 95°/5min., 70°/30min, 65°/30min, 60°/30min., 55°/30min., 50°/30min, 45°/30min., 40°/30min., 35°/30min., 30°/30min, 25°/30min, 22°/∞). Annealed products were digested with BsmB1 (NEB) and ligated into a LENTICRISPRV2 backbone (Addgene) via a mixture of annealed product, the backbone of interest, 10X ligation buffer (Promega), T4 DNA Ligase (Promega), and molecular grade water (Thermo Scientific).

The ligation product was transformed into STBL3 *E.coli* cells, plated onto LB-Ampicillin plates, and incubated overnight at 37 degrees Celsius. Colonies were picked from transformation plates, inoculated into LB-Amp tubes, and incubated in a shaker overnight at 37 degrees Celsius.

The following morning, DNA was purified via Mini-prep (Qiagen) according to the

company protocol. The Mini-prep DNA product was digested with Kpn1 & Age1 (NEB) and run on a 10% SDS-PAGE gel to confirm the successful ligation of the insert into the backbone of interest.

After confirmation of the insertion, the DNA was transfected into HEK293T cells. At 48 hours, vector was harvested and added onto plated THP1 cells. The cells were centrifuged for 2 hours at 2,000 rpm, followed by a media change. 48 hours later, drug selection was initiated onto the THP1 cells, which were left under selection for 72-96 hours. A density-gradient separation using Lymphocyte Separation Medium (Corning) was performed to purify live cells that were transferred to a new plate. The knockout of the gene of interest in the stable cell lines was confirmed via Western blot.

Western Blotting

Cell lysates were prepared by lysing cells with lysis buffer containing protease inhibitor cocktail (PIC), 100mM TRIS pH 8.0, 1%NP-40, and 150mM NaCl for 30 minutes on ice. Lysates were spun down at 13,000 rpm for 10 min and supernatant was collected. Protein concentration of the lysates was measured using Pierce BCA protein assay kit (Thermo Scientific), and equal amounts of protein (with Laemmli 2X Sample Loading Buffer) was loaded into and ran on an 10% SDS-PAGE gel.

After SDS-PAGE separation, the proteins were wet-transferred to a nitrocellulose membrane (Bio-Rad). Membranes were probed using specific primary antibodies and then with secondary antibodies conjugated to Horseradish Peroxidase (HRP) (Thermo Scientific). Antibody complexes were detected using SuperSignal™ West Femto Chemiluminescent Substrate (Thermo Scientific), and chemiluminescence was detected using the Bio-Rad ChemiDoc™ Imaging System.

HEK293T Transfection & Virus Generation

HEK293T cells were maintained in 10cm plates in Dulbecco's Modified Eagle's Medium (DMEM) with 10% FBS, 1% Penicillin/Streptomycin, and 10ug/mL Ciprofloxacin Hydrochloride. Once confluent, the 293T cells were transfected by addition of a mixture of DNA of interest, polyethylenimine (PEI), and oxidized DMEM. At 48 hours, the virus was harvested and filtered through 45mm filters (Millex-HA).

HIV-1 viral particles pseudotyped with JRFL were generated by transfecting 293T cells with 4ug R7-GFP/6ug JRFL. HIV-1 viral particles pseudotyped with VSVg were generated by transfecting 293T cells with 7ug R7-GFP/3ug VSVg. VLPs (Virus-Like-Particles) were generated by transfecting 293T cells with 4ug PSPAX/6ug JRFL. SIV (Simian Immunodeficiency Virus) viral particles pseudotyped with VsVg were generated by transfecting 293T cells with 7ug SIV3+/3ug VSVg.

THP1 Differentiation & Virus Infection

THP1 cells were counted via hemocytometer and plated at a density of 1.0×10^6 cells/mL with phorbol 12-myristate 13-acetate (PMA) to differentiate them into monocyte-derived-macrophages (MDMs). Media was changed after 24 hours with media w/o PMA addition. After 48 hours, virus (HIV/VLP) was harvested from transfected HEK293T cells and equal amounts added onto the differentiated MDM cells, either with or without addition of Nevirapene. The plate was centrifuged at 2,000rpm for 2 hours, followed by a media change and incubation overnight. 24 hours after spinoculation, the MDM cells were harvested by trypsinization (Corning, 0.05% trypsin/0.53mM EDTA/1x Sodium Bicarbonate) and stored on ice until RNA purification.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was purified from cell lysate 24 hours post-infection using NucleoSpin RNA extraction kit (Macherey-Nagel). The RNA was converted into cDNA using the GoScript™ RT System (Promega). Quantitative Real-Time PCR (qRT-PCR) was performed using gene specific primers and SYBR green master Mix (Bio-Rad), following the Bio-Rad ISG protocol. GAPDH primers were used as a housekeeping gene for normalization.

The following primer pairs were used:

GAPDH Fwd 5'- GCACCGTCAAGGCTGAGAAC-3'

GAPDH Rvr 5'- GGCATGGACTGTGGTCATGAG-3'

STAT1 Fwd 5'-CCGTTTTTCATGACCTCCTGT-3'

STAT1 Rvr 5'-TGAATATTCCCCGACTGAGC-3'

IFI27 Fwd 5'-TCTGGCTCTGCCGTAGTTTT-3'

IFI27 Rvr 5'-GAACTTGGTCAATCCGGAGA-3'

IFIT1 Fwd 5'-CAACCATGAGTACAAATGGTG-3'

IFIT1 Rvr 5'-CTCACATTTGCTTGGTTGTC-3'

IFIT2 Fwd 5'-AATTGAGGTGGCAACATAGTTTGA-3'

IFIT2 Rvr 5'-CCCGTCGCTTCTAGCTATGTATC-3'

IP10 Fwd 5'-TGAAATTATTCCTGCAAGCCAATT-3'

IP10 Rvr 5'-CAGACATCTCTTCTCACCCCTTCTTT-3'

HIV-1 Infectivity

THP1 cells were plated with PMA at a density of 3.0×10^5 cells/mL into a 24-well plate. At 48 hours, the MDM cells were pre-treated with SIV (generated by 293T transfection) addition for 4 hours. After pre-treatment HIV-1 (R7-GFP/VSVg) was harvested from HEK293T

transfection and added onto the MDM cells. The plate was centrifuged for 2 hours at 2,000 rpm, followed by a media change and incubation overnight. After 48 hours, the cells were harvested and re-suspended in a mixture of 10% paraformaldehyde & 90% 1X PBS, followed by flow cytometry on the CANTOIII machine in the Loyola University Chicago Flow Cytometry Core. The samples were gated for GFP fluorescence and percentage values were recorded and analyzed with FlowJo.

RNA-Seq & Sequencing Analysis

THP1 cells were plated with PMA at a density of 1.0×10^6 cells/mL into a 12-well plate. Media was changed at 24 hours. At 48 hours, HIV-1 (R7/JRFL) was harvested from transfected HEK293T cells and added onto the MDM cells. The plate was centrifuged at 2,000rpm for 2 hours, followed by a media change and incubation overnight. After 24 hours, the MDM cells were harvested by trypsinization (Corning) and stored on ice until RNA purification.

Total RNA was purified from cell lysate 24 hours post-infection using NucleoSpin RNA extraction kit (Macherey-Nagel). The RNA was checked for quality and concentration via NanoDrop 1000 (Thermo Scientific) and confirmed for phenotype of interest via qRT-PCR. The 30uL samples were delivered to the University of Chicago Genomics Core for RNA-Seq analysis. The data files returned were processed via Galaxy, and with the assistance of the Loyola University Chicago Genomics Core. Pathway analysis was performed using EnrichR & DAVID. Heatmaps were generated using Microsoft Excel & RStudio.

CHAPTER THREE

RESULTS

Investigating Relevant HIV-1 Viral Particles

To assess the importance of the viral DNA genome, we treated MDM cells with Nevirapene (NVP) concomitantly with HIV-1 infection. NVP is a non-nucleoside inhibitor that blocks viral reverse transcriptase activity, preventing the conversion of viral RNA to DNA (Fig. S4). If viral DNA was the determinant triggering the elevated ISG phenotype observed in the absence of BICD2, then NVP treatment would mitigate this induction.

However, we found that treatment with NVP after HIV-1 infection had no detectable effect on the induction of an ISG response (Fig. 4), with no significant difference in the levels of ISG expression after NVP treatment compared to untreated samples. This suggests that the viral DNA genome may be not directly involved in host factor detection and immune activation in the absence of BICD2.

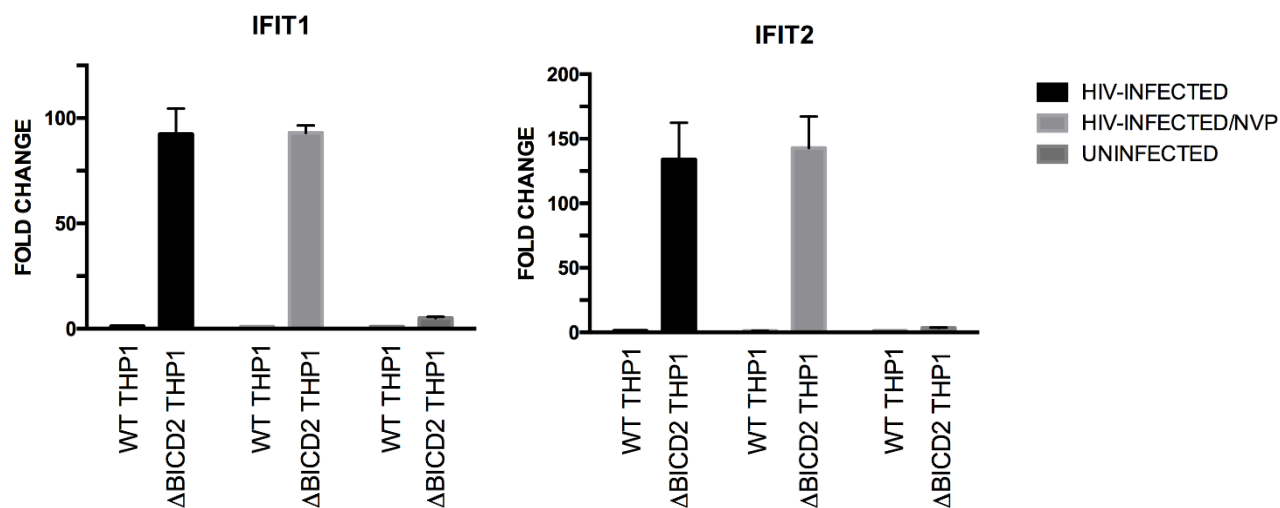


Figure 4. Analysis of Impact of NVP Treatment on ISG Response. qRT-PCR analysis comparing ISG mRNA levels between WT and BICD2-knockout THP1 cells, after R7/JRFL HIV-1 infection and with or without treatment with the non-nucleoside analog RT inhibitor Neverapene (NVP). Results are normalized against UNINFECTED WT THP1 levels of mRNA. Error bars are standard error.

We next investigated if the viral capsid is key to the host response in BICD2-depleted cells. We infected MDM cells with either HIV-1 or Virus-Like-Particle (VLP) structures that retain HIV-1 capsid and envelope proteins but lack viral genome (Fig. S5). If the capsid is the viral component involved in triggering the elevated immune response observed, then we would expect that infection of BICD2-deficient cells with VLP structures would result in a substantial ISG response.

We found that VLP infection, similar to HIV-1 infection, induced significantly higher levels of ISG expression in BICD2-knockout cells compared to WT cells (Fig. 5). This suggested that the HIV-1 capsid may be the more relevant viral particle detected to trigger elevated ISG expression.

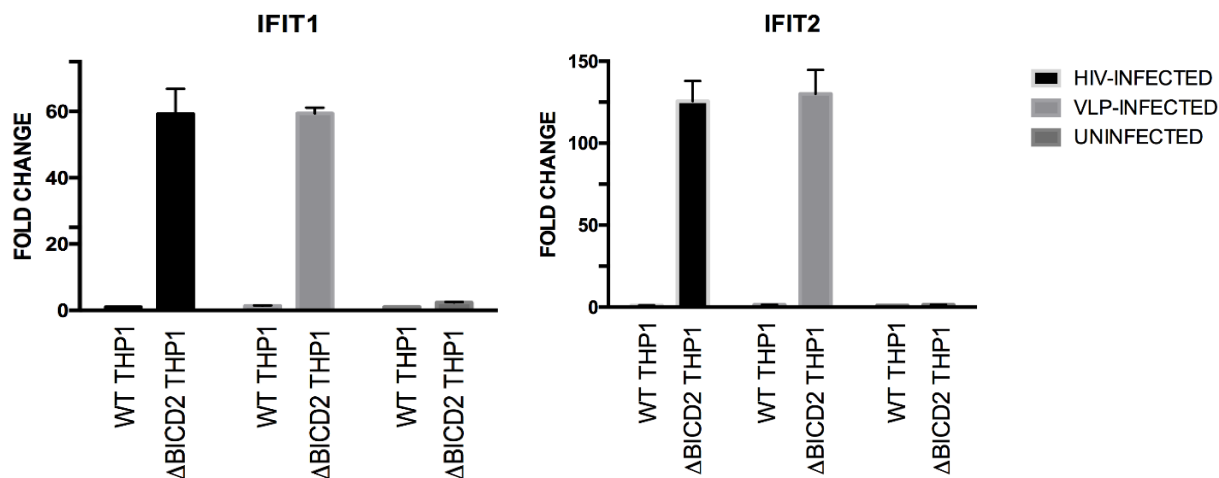


Figure 5. Analysis of Impact of VLPS on ISG Response. qRT-PCR analysis comparing the levels of ISG mRNA levels between WT and BICD2-knockout THP1 cells, after infection with either R7-JRFL HIV-1 or PSPAX-JRFL VLPs. Results are normalized to UNINFECTED WT THP1 levels of mRNA. Error bars are standard error.

Characterizing Relevant Host Restriction Factors

As our results indicate that the capsid appears to be the more important viral determinant driving the immune response in the absence of BICD2, we began investigating relevant host factors with known capsid sensor TRIM5 α . TRIM5 α has the capacity to recognize and bind to the HIV-1 capsid, accelerating the uncoating process and triggering an immune response [34, 36].

To clarify its role in the absence of BICD2, we assessed the impact of removing TRIM5 α on the host ISG response to HIV-1 infection. If the viral capsid is being detected in the absence of BICD2, and TRIM5 α is important for this detection and immune response, then we would expect that knocking out TRIM5 α would mitigate the elevated ISG expression usually observed in the absence of BICD2.

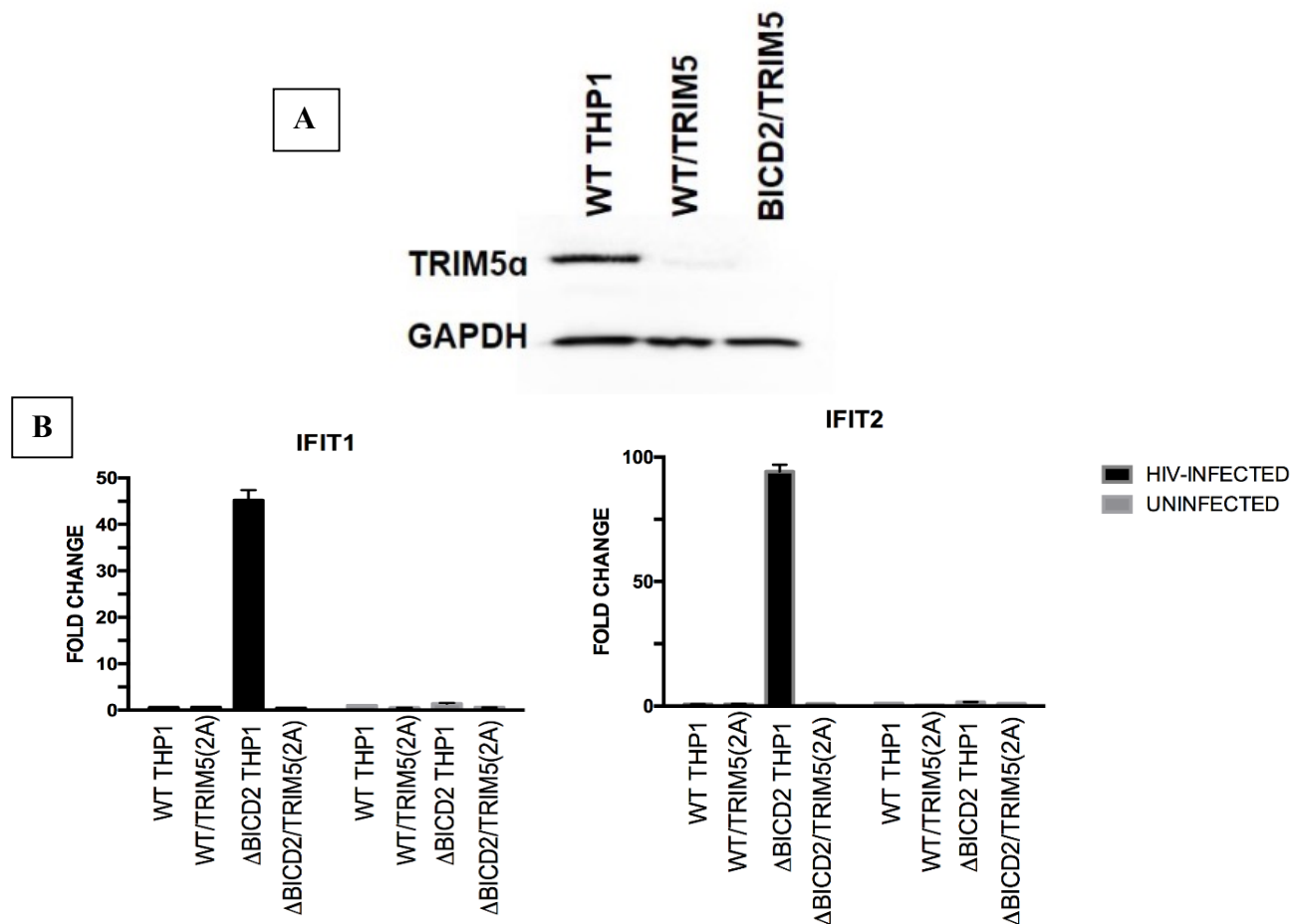
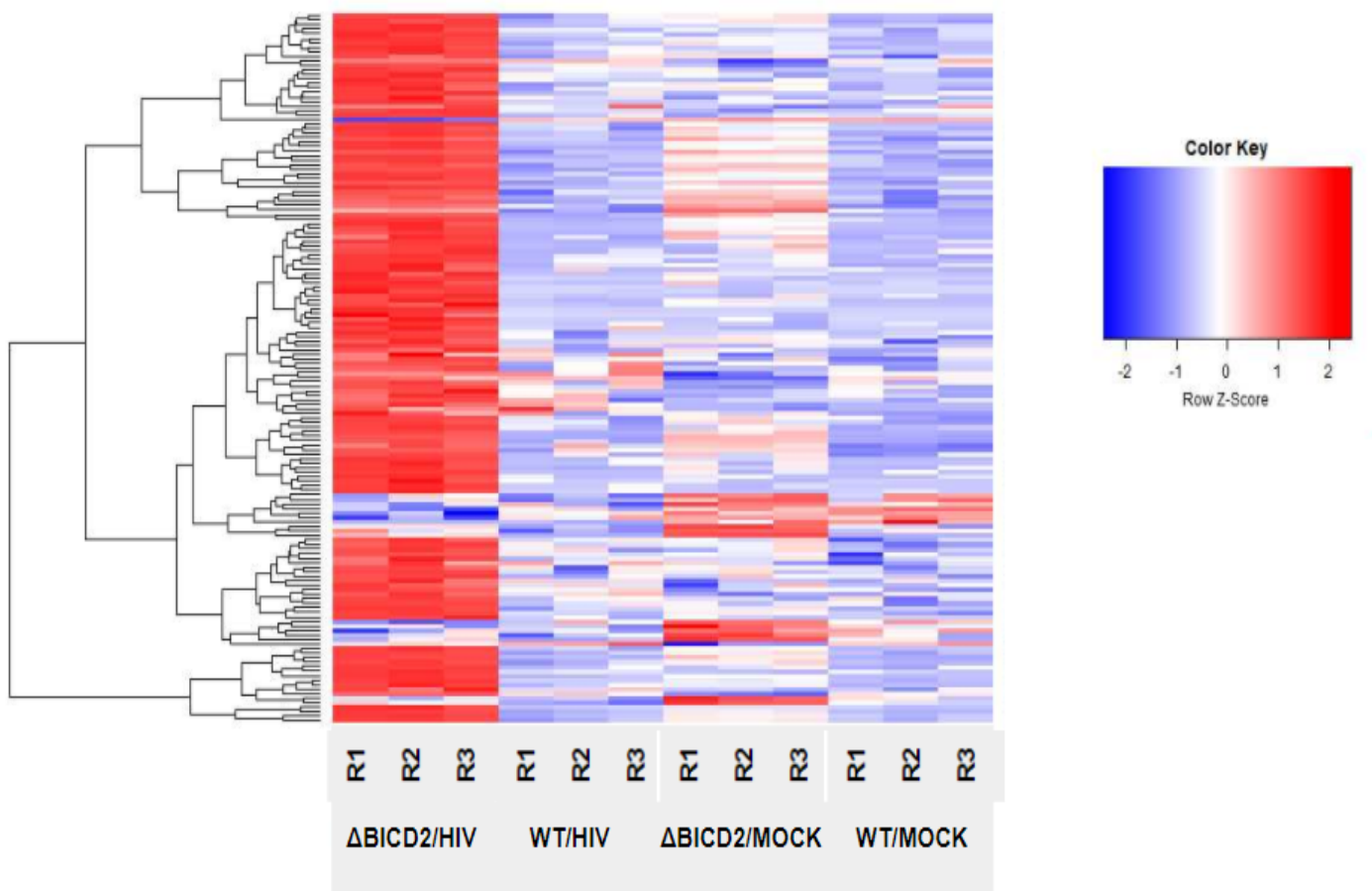


Figure 6. Importance of TRIM5 α in ISG Response in Absence of BICD2. A) Western Blot showing successful knockout of TRIM5 α in WT THP1 & BICD2-Knockout cells. B) qRT-PCR analysis comparing levels of ISG mRNA between WT, TRIM5 α -knockout, BICD2-knockout, and BICD2/TRIM5 α double-knockout cells after R7/JRFL HIV-1 infection. Results are normalized against UNINFECTED WT THP1 mRNA levels. Error bars are standard error.

We found that knocking out TRIM5 α in BICD2-depleted cells significantly reduced the levels of ISG expression observed (Fig. 6). BICD2/TRIM5 α double-knockout cells had drastically reduced levels of ISG expression compared to BICD2-knockout cells. This suggests that TRIM5 α may be involved in the detection of the viral capsid and downstream induction of the elevated immune response in the absence of BICD2.

RNA-Seq - Gene Expression Profile

After analyzing the impact of knocking out TRIM5 α on the ISG response elicited in the absence of BICD2, we wanted to investigate additional host immune factors involved. To do this, we compared the gene expression profiles after HIV-1 infection between WT and BICD2-knockout cells through RNA-Seq analysis, and visualized these differences via heatmap (Fig. 7).



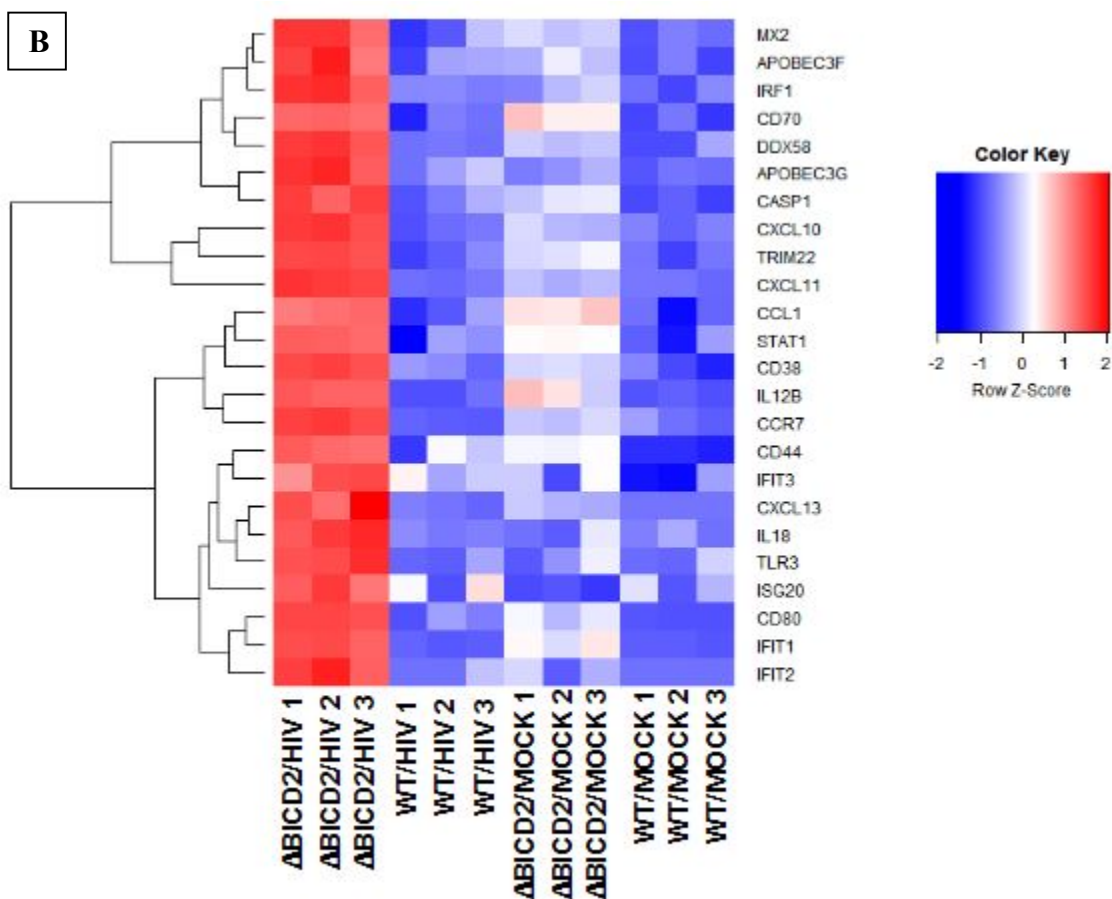


Figure 7. RNA-Seq Gene Expression Visualization Heatmap. A) The complete heatmap of all 157 differentially expressed genes between our four sample types (BICD2/HIV-1, WT/HIV-1, BICD2/MOCK, WT/MOCK). B) Specific antiviral genes and their differential expression between the four sample groups. Color keys with z-score scales are provided. Significance $p < 0.05$.

We found that after HIV-1 infection, BICD2-knockout cells had 157 total genes differentially expressed, including several key factors involved in host antiviral response (Fig. S8). Consistent with prior findings, we saw comparatively increased expression of ISGs like IFIT1 and IFIT2. Additionally, factors important to T-cell activation were upregulated, such as surface presentation antigens CD70 and CD80, and the Th1 activation cytokine IL-12.

Numerous restriction factors were also upregulated in the absence of BICD2 upon HIV-1 infection, including TRIM22, MX2, and APOBEC3G. These factors have been implicated in the inhibition of the HIV-1 infection cycle at various stages, including reverse transcription, uncoating, and nuclear import [56, 78-81]. The increased expression of these factors suggests that there are additional pathways involved in the detection and immune response to HIV-1 in the absence of BICD2 in addition to TRIM5 α activity.

RNA-Seq – Pathway Analysis

After investigating what factors were upregulated in BICD2-knockout cells in response to HIV-1 infection, we visualized them in the context of immune pathways. To do this, we utilized online softwares DAVID & EnrichR, pinpointing specific involvements (Fig. S9) and creating network clusters of related pathways (Fig. 8).

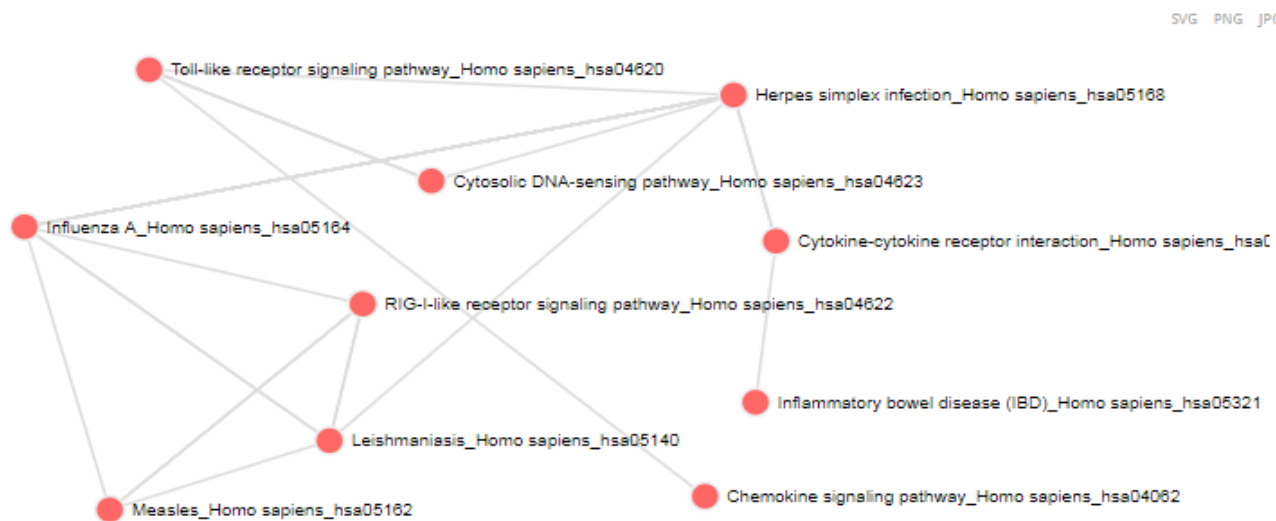


Figure 8. EnrichR Network Pathway Analysis. A diagram of the known immune response pathways that correspond to the differentially expressed factors identified via DeSEQ2 analysis, visualized in network form.

The antiviral factors upregulated in BICD2-knockout cells were involved in a variety of pathways in both the innate and adaptive immune response. This included the cytosolic DNA-

sensing pathway, possibly used to detect excess HIV-1 DNA in the cytoplasm [31, 32], as well as robust chemokine activity, possibly to attract additional immune cells to amplify the response.

Additional pathways involved included TLR signaling and apoptosis, as well as a number of ubiquitous antiviral response pathways similar between HIV-1 and other viruses, such as HSV and IAV. Collectively, the pathways upregulated in BICD2-depleted cells upon HIV-1 infection are likely part of a larger antiviral state, spanning innate and adaptive immune pathways (Fig. 9).

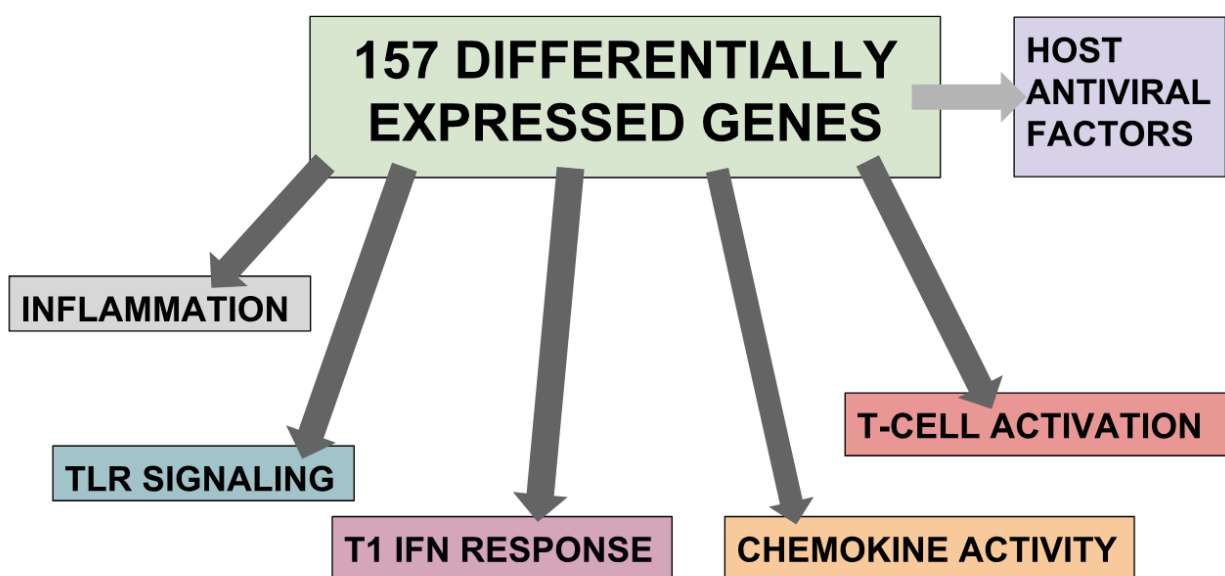


Figure 9. Pathway Analysis Summary. Across DAVID and EnrichR, the conserved pathways that upregulated factors were involved in are summarized. Four of the most upregulated pathways included apoptosis, T1 IFN response, Th1 activation, and chemokine activity. Additional pathways are not shown.

CHAPTER FOUR
SUMMARY & DISCUSSION

Summary

Taken together, we have been able to further investigate and characterize the elevated host immune response to HIV-1 infection observed in the absence of BICD2. Our results suggest that the viral capsid, rather than the viral genome, is the component responsible for the elicitation of the host ISG response. While levels of ISG expression were not mitigated by blocking the production of viral DNA, naked capsid structures lacking viral genome induced substantial expression of ISGs.

Based off the apparent detection of the viral capsid, we also investigated what host sensors could be involved. By knocking out TRIM5 α in BICD2-knockout cells, we were able to mitigate the elevated ISG response normally observed in the absence of BICD2 [4]. This suggests that, after accumulation of viral capsid in the cytoplasm of BICD2-deficient cells, TRIM5 α may be directly involved in the detection and initiation of an immune response. This is in accordance with the idea that human TRIM5 α is able to modestly restrict HIV-1 infection [37-40].

In addition to TRIM5 α , we found many other host factors involved in the elevated host immune response observed in the absence of BICD2. This includes chemokines, surface presentation antigens, interferons and other restriction factors, all of which have well-established connections to the host antiviral response and were involved in pathways across both the innate and adaptive immune system.

Discussion

Protection Against Viral Reinfection.

From heatmap and pathway RNA-Seq analysis across WT and BICD2-knockout cells either infected with HIV-1 or left uninfected, it appears that an elevated immune response across both innate and adaptive arms of the immune system is elicited after HIV-1 infection in BICD2-depleted cells. This includes the production of interferon, ISG expression, induction of apoptosis, and activation of T-cells, all of which are not exclusive to the HIV-1 response. Rather, these pathways are part of larger host antiviral activity and immune activation. Whether this comprehensive immune response protects cells against reinfection with other viruses would merit further study. Specifically, it would be worth investigating if the host antiviral defenses raised to counter HIV-1 infection in the absence of BICD2 would also apply to inhibition of other viruses.

Role of TRIM5 α .

Based on our findings that the viral capsid is the more relevant viral particle, it appears that TRIM5 α , rather than other host sensors such as cGAS, is more directly involved in detection and immune response. This is also in line with our finding that knocking out both BICD2 and TRIM5 α reduces the levels of ISG expression in response to HIV-1 infection, impairing the host antiviral response and potentially allowing for more productive infection.

Previous studies have shown that human TRIM5 α is less able to restrict HIV-1 infection compared to counterparts in rhesus macaques. However, despite this comparatively lower restrictive ability, polymorphisms in huTRIM5 α can lead to either an impaired or enhanced capacity to fight infection [37, 44, 45]. This notion of human TRIM5 α playing a role in restricting HIV-1 infection is supported by studies that have found that the expression level of

TRIM5 α is connected to the host ability to combat HIV-1 infection [38]. Transduction of huTRIM5 α into cells has been shown to convey a modest capacity to restrict HIV-1 infection [35]. Additionally, people in high-risk populations who have been resistant to HIV-1 infection have been found to have higher levels of TRIM5 α expression [38]. And stabilizing human TRIM5 α expression (as it naturally self-degrades more than rhesus TRIM5 α) has been shown to bolster the host response to HIV-1 infection [39].

Taken together, this suggests that though inferior to rhesus TRIM5 α , human TRIM5 α does retain modest ability to inhibit HIV-1 infection. The extent to which this restrictive capacity changes after impairment of host factors normally hijacked for viral infection (such as BICD2) remains unclear. Whether removing the ability of the HIV-1 core to traffic effectively through the cytoplasm allows human TRIM5 α an expanded window of time to recognize the viral core and initiate an immune response would be an interesting avenue to explore further. This could lend support to the hypothesis that HIV-1 avoids immune sensors via its efficient translocation to the nucleus [4].

HIV-1 Capsid Epitopes.

As our results indicate that the viral capsid is the component detected to elicit the ISG response, it would be interesting to investigate what capsid epitopes are directly involved. The role that different capsid epitopes may play in the context of attachment to host cargo adaptors and the microtubule network is unclear.

By further investigating the specific capsid-cargo adaptor connection sites, potential therapies could also be developed to recognize the specific capsid epitopes and prevent their binding to cargo adaptors. Inhibiting this key interaction could disrupt the HIV-1 infection cycle, while also triggering host immune response to combat infection.

HIV-1 Therapy Development.

The importance of the elevated immune response in the absence of BICD2 may also be valuable to consider in the context of therapy for HIV-1. The commonly administered treatment for HIV-1 is antiretroviral therapy (ART) [18, 19]. This treatment regimen includes drugs that inhibit HIV-1 replication at various stages of the viral life cycle, including reverse transcription, protein processing, and integration.

The goal of ART is to suppress the levels of HIV-1 replication and viral load accumulation, slowing the rate at which HIV-1 infects and kills CD4 T-cells. However, long-term administration of ART has a number of consequences at both the cellular and clinical level [23]. ART is not able to completely abolish HIV-1 infection, as a latent reservoir of HIV-1 remains integrated into the genomes of infected cells, and these latently infected cells can still replicate [20, 21, 76, 82]. Additionally, long-term ART has been associated with minor toxicities that can manifest as clinical symptoms including nausea, vomiting, and muscle exhaustion [23]. Due to these known consequences, alternative therapies to HIV-1 infection are being extensively investigated.

One alternative to ART that has been studied is interferon monotherapy. Administering interferon leads to the upregulation of ISGs, and various other immune defenses in the host. This has shown some promise as a means of combating viral infection. In cases where HIV-infected patients on ART had IFN first added to their treatment regimen but later had ART interrupted, continuation of IFN treatment alone was able to suppress viral progression at a higher level than past studies on ART interruption would suggest [25]. In addition, IFN administration in rhesus macaques infected with SIV lead to upregulation of antiviral genes, resulting in lower levels of

systemic infection [26]. This further corroborates the idea that IFN monotherapy has the potential to boost the host immune system, helping combat viral infection.

However, interferon therapy holds significant long-term consequences. This includes persistent immune activation that can lead to inflammation and tissue damage [29]. Additionally, the benefits of interferon therapy decrease significantly over time, as a desensitization effect occurs and the hosts' ability to combat viral infection falls [26-29].

With both ART & interferon treatments displaying short-term benefits but long-term consequence, new therapies for HIV-1 are a prominent research avenue. In our research, we have shown that attachment to the cargo adaptor BICD2 and trafficking along the microtubule network are important for HIV-1 infection, and that in the absence of this attachment an elevated immune response is observed. As such, development of drugs specific to the inhibition of virus-microtubule attachment and trafficking may offer valuable pursuits for potential therapies.

Drug therapies to target and disrupt components of the host cell microtubule network are already well-established in the cancer research field [83]. Primarily, this includes compounds that destabilize host tubulin polymers and disrupt dynamic reorganization, killing target cells. However, relatively little research exists on the development of drugs that inhibit the activity and interactions of cargo adaptors in the context of viral infection.

By treating the host with compounds that specifically interfere with the interaction between the viral core and the microtubule network (by disrupting viral connection to cargo adaptors), infected cells could be specifically targeted. This could be done in conjunction with treatments that force HIV-1 out of latency, as part of a 'shock-and-kill' approach [76, 77]. If HIV-1 could be reactivated from a latent state in the presence of an inhibitor of the interaction between the viral core and the microtubule network, then the infection cycle could be disrupted

and an immune response triggered. This spatial control of immune response in infected cells could potentially be more beneficial to the host than the systemic immune response (and eventual desensitization) observed in patients receiving interferon monotherapy [27, 29]. Further, such a therapy could inhibit the levels of productive HIV-1 infection, while also potentially reducing the latent reservoir in infected cells (Fig. 10).

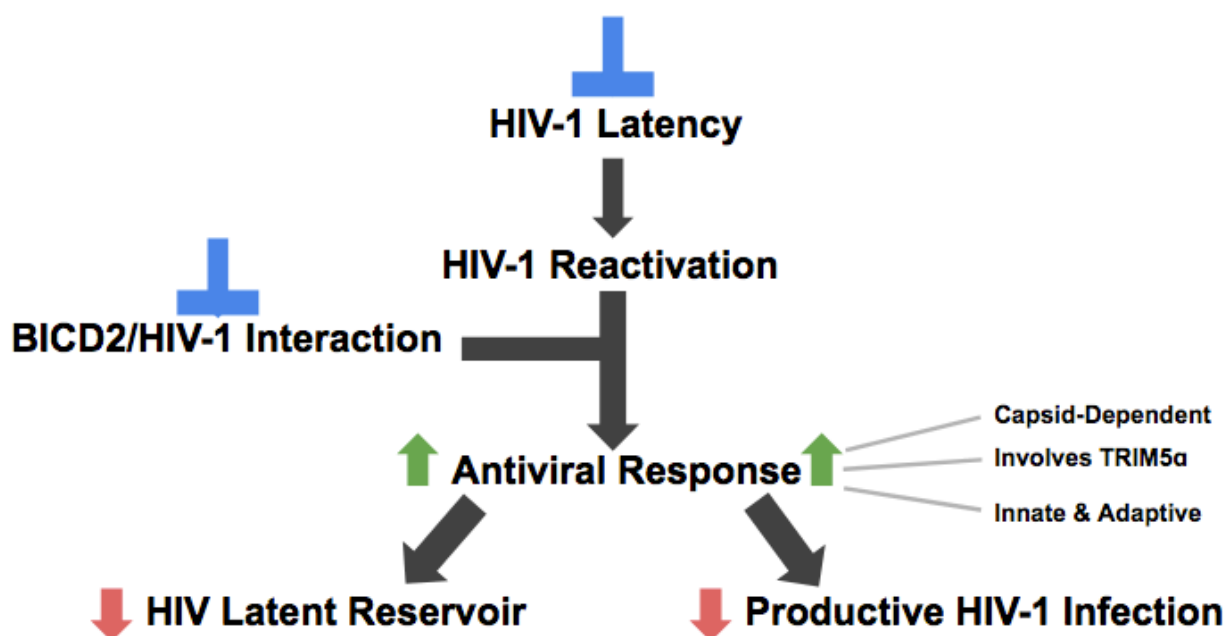


Figure 10. Potential Therapy Rationale & Summary Diagram. An outline of the rationale behind a potential therapy to inhibit the interaction between BICD2 and HIV-1, coinciding with therapies to induce HIV-1 out of latency. Together, this inhibition of the infection cycle leads to an elevated immune response to reactivated HIV-1, potentially inhibiting productive infection and reducing the latent viral reservoir. Additionally, a summary of the investigation into the elevated immune response is provided, as it appears to be capsid-dependent and utilize capsid sensor TRIM5, while also involving innate and adaptive pathways. Blue represents drug therapies, green represents an increase, red represents a decrease.

APPENDIX
SUPPLEMENTAL FIGURES

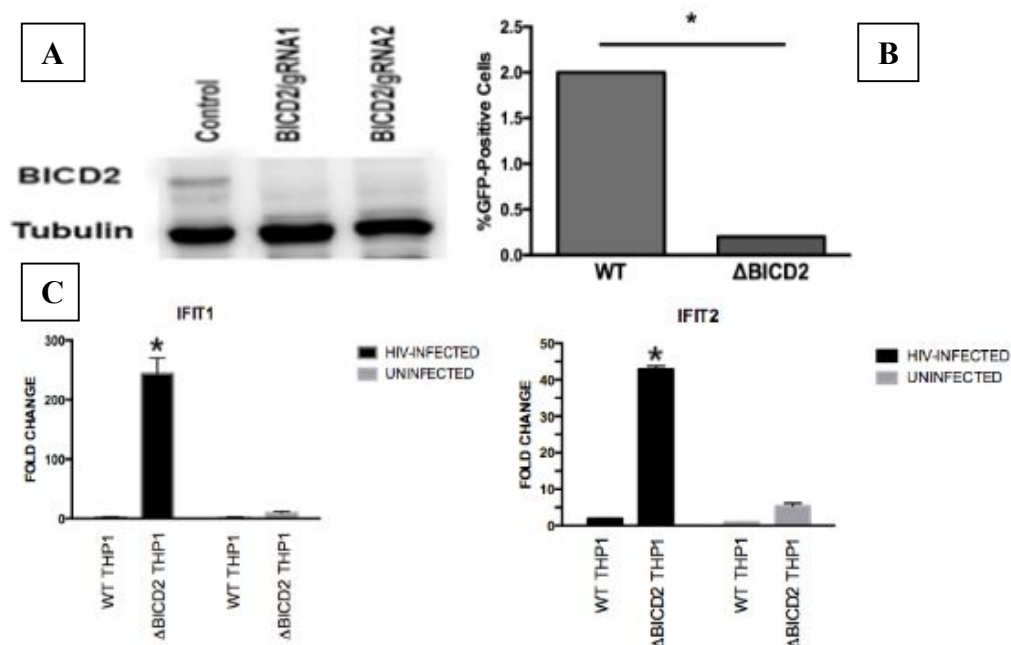


Figure S1. Elevated ISG phenotype in BICD2-knockout cells. A) Western Blot showing the successful knock-out of BICD2 in WT THP1 cells. CRISPR gRNA sequences (BICD2gRNA1 5'-GATCTTCTCACGCGTGGTCT-3' BICD2gRNA 2 5'-GCTGCAGGACTACTCGGAAC-3'). B) Percent GFP-positive cells compared between WT & BICD2-knockout cells after infection with R7-VSVg. C) qRT-PCR analysis of ISG mRNA levels after HIV-1 infection, comparing WT and BICD2-knockout THP1 cells. Results are normalized against UNINFECTED WT THP1 cells. Error bars are standard error.

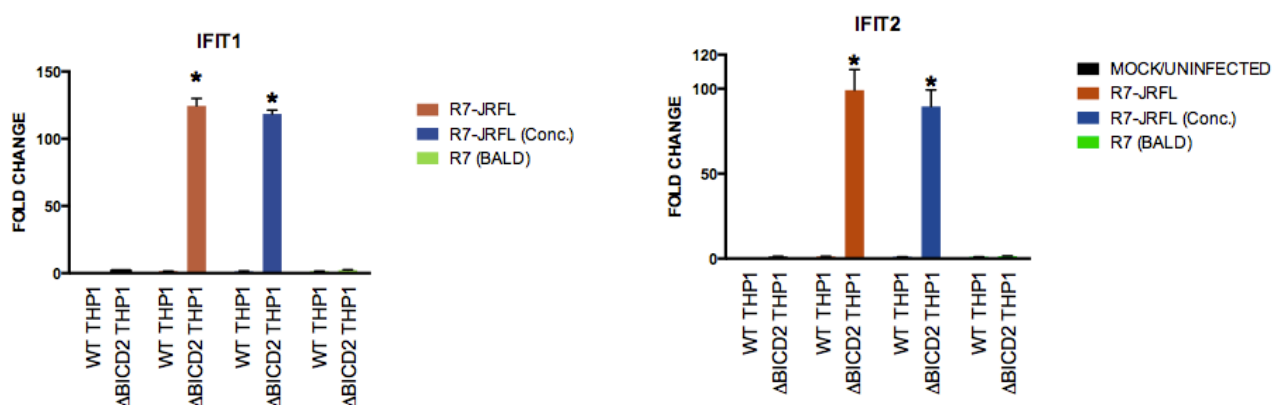


Figure S2. 'Bald' Virus Analysis. qRT-PCR data of ISG mRNA levels between WT & BICD2-knockout cells showing that 'bald' virus (lacking any viral envelope or capsid, just the DNA encoding the viral genome) is incapable of eliciting an ISG response, disproving the notion that possible LPS contamination from DNA purification could be responsible for ISG induction observed. For reference, they are compared to HIV-1 R7/JRFL virus, in both concentrated and un-concentrated forms. Error bars are standard error.

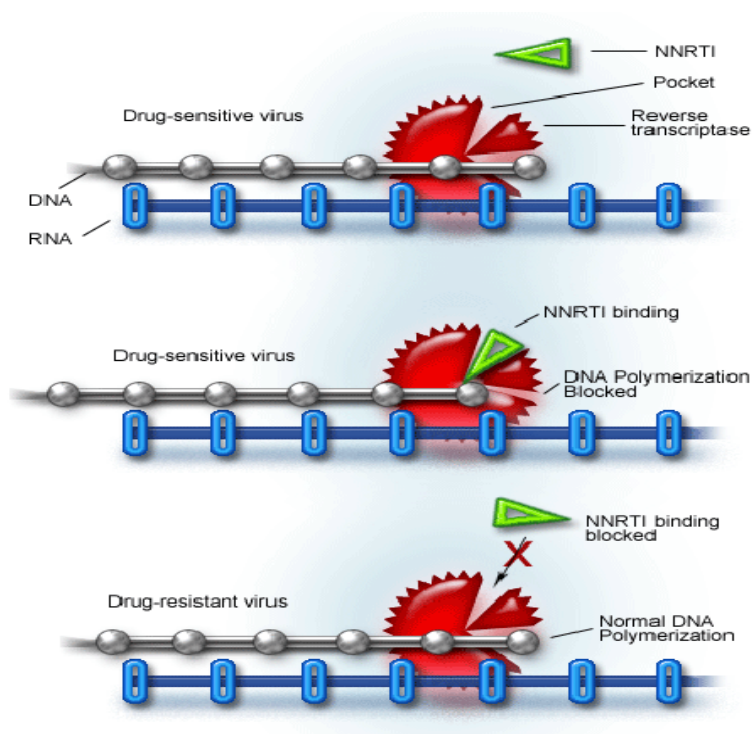


Figure S3. NVP Mechanism. Mechanism of action of Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), a class of drugs that includes Nevirapine.

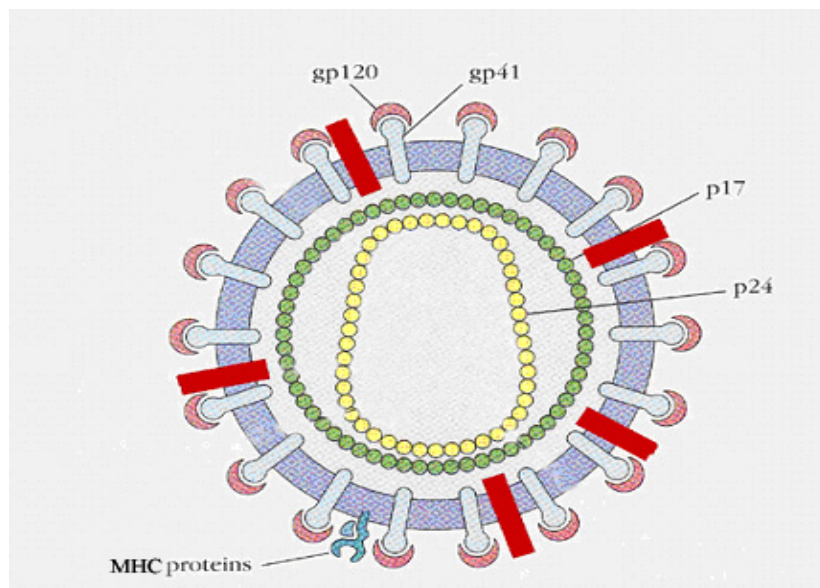


Figure S4. VLP Structure. Diagram of the structure of a VLP mimicking HIV-1 virion, but lacking viral genome.

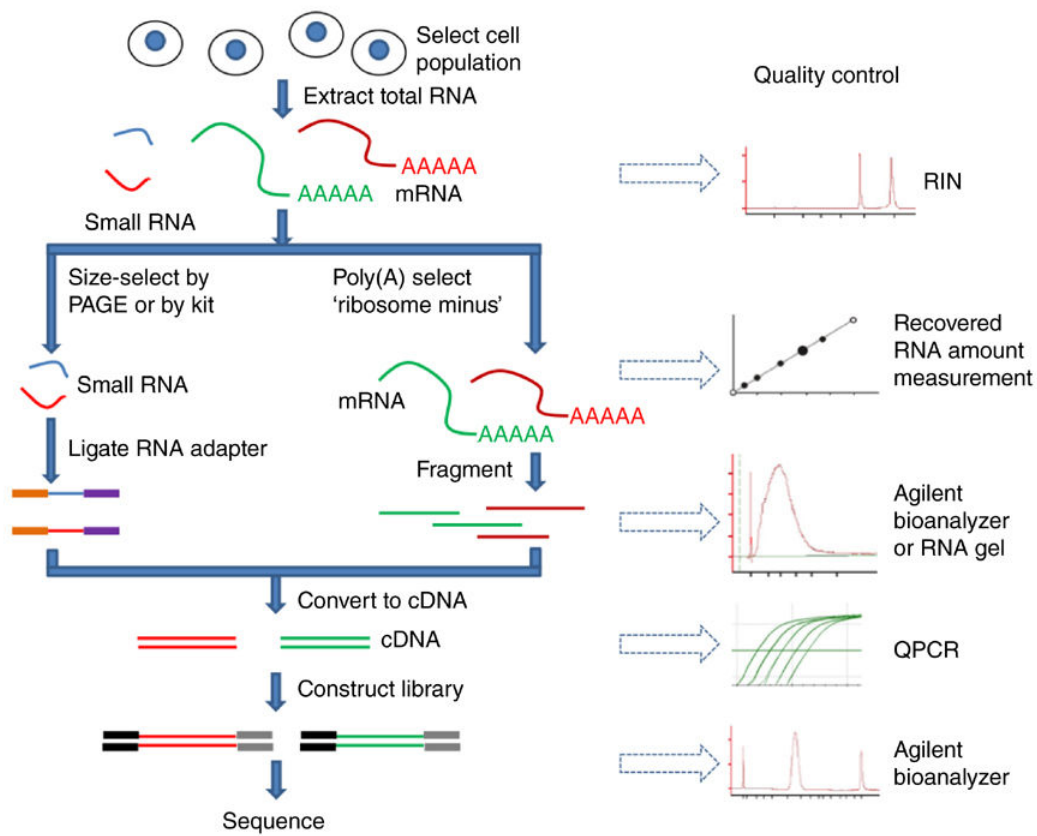


Figure S5. RNA-Seq General Outline. An overview of the library generation and single-end sequencing done in the RNA-Seq analysis performed at the University of Chicago Genomics Core. Adapted from Zeng & Mortazavi (Nature Immunology 2014).

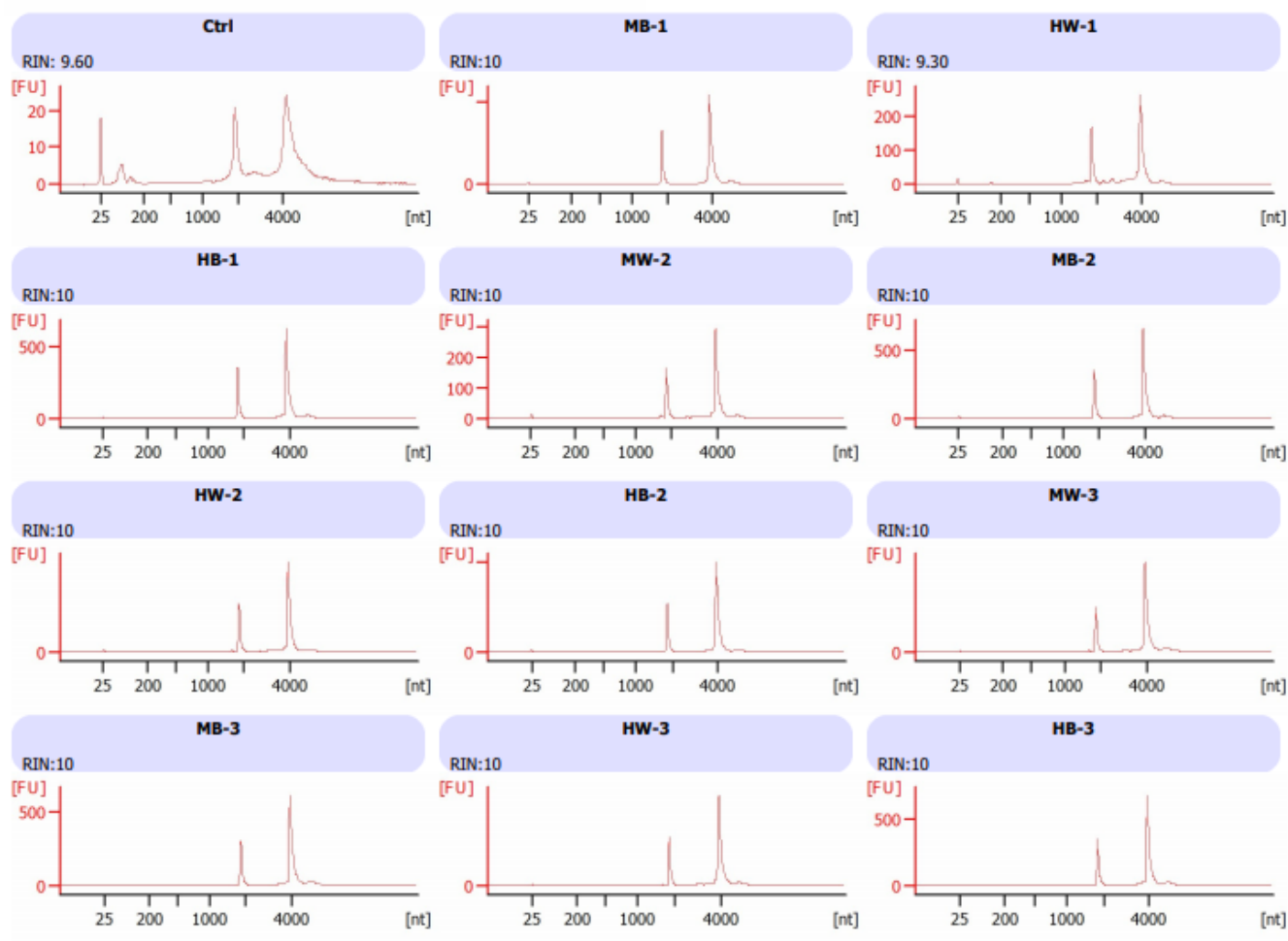


Figure S6. Quality Assurance of RNA Samples. Bioanalyzer analysis & RIN scores for the RNA samples sent to the University of Chicago Genomics Core for RNA-Seq Analysis. The peak at 1500 represents 18s rRNA and the peak at 4000 represents 28s rRNA. RIN scores are a measure of RNA purity and durability, on a scale from 1-10. Ctrl/MW2/MW3 = MOCK/WT. MB1/MB2/MB3 = MOCK/ Δ BICD2. HW1/HW2/HW3 = HIV/WT. HB1/HB2/HB3 = HIV/ Δ BICD2.

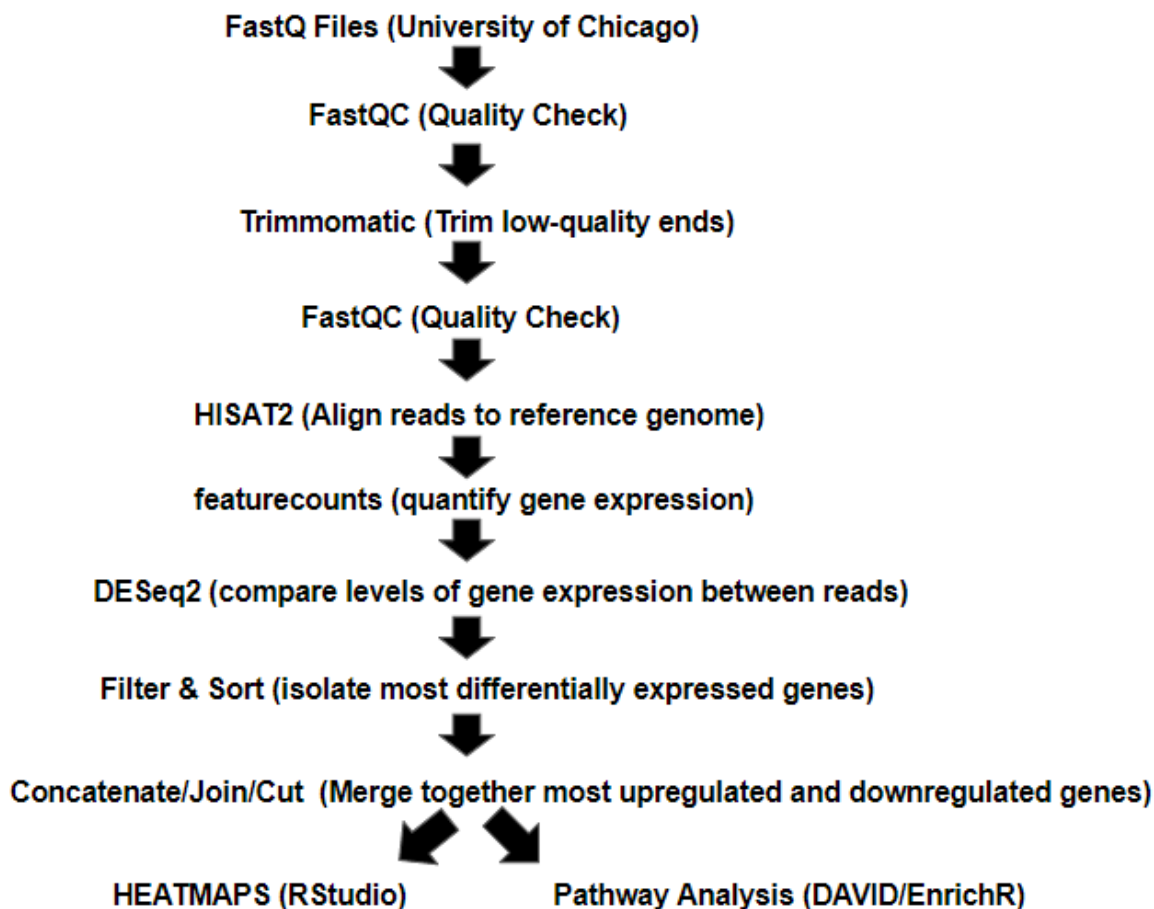


Figure S7. RNA-Seq Analysis Workflow. After receiving the FastQ files from sequencing from the University of Chicago, the sequences were checked for quality via FastQC (a tool in online bioinformatics analysis software Galaxy). Any low quality ends of the reads were trimmed, and the sequences were re-checked for quality before being aligned to a human reference genome. The levels of gene expression were then compared between the different sequencing reads (corresponding to the different samples), and the genes significantly different in expression were isolated. These genes were merged together into a CSV file in Microsoft Excel. Heatmaps were then constructed using HEATMAP tool in RStudio, and pathway analysis was performed by online softwares DAVID & EnrichR.

| | | | | | |
|---------------|----------|---------------|---------------|---------------|---------------|
| EPHA7 | IFITM3 | EXT1 | C5ORF56 | VPS54 | RP11-121C2.2 |
| SP110 | SAMD9 | ISG20 | DUSP5 | MIR3945HG | FSTL1 |
| PRRX1 | GALNT18 | BTC | EDN1 | KIAA1644 | SQRDL |
| GCA | TRANK1 | KIF18B | RUBCN | STAP1 | RP5-1195D24.1 |
| CD70 | CISD1 | CTD-2201E18.3 | GABBR2 | PBK | PTGS2 |
| RSAD2 | SAMD9L | GNB5 | PAX5 | CD38 | IFIH1 |
| MX2 | HERC5 | CARD16 | PKDREJ | CASP1 | GPR132 |
| PLEKHA4 | RASD2 | PGR | IL18 | CCR7 | PNMA2 |
| CRIM1 | GBP1 | LINC01235 | SSTR2 | SLC18A1 | TAGAP |
| CXCL10 | ATXN7L1 | ATF5 | USP30-AS1 | TBX1 | GCNT4 |
| CXCL11 | GBP3 | EXOC3L1 | IRF1 | ZNF462 | ITGB8 |
| IFI44L | GBP2 | AC015969.3 | RP11-517I3.2 | IL15RA | CCL1 |
| CXCL12 | IL12RB1 | JADE2 | RP11-434H6.6 | STAT1 | TRIM22 |
| SCD | GBP5 | RORB | TLR3 | ERAP2 | FADS1 |
| NEIL3 | TBC1D17 | IFIT2 | LTB4R | GPR31 | |
| CXCL13 | MSR1 | SLC30A10 | CLIC5 | AC113189.5 | |
| RP11-533E19.5 | SDS | IFIT1 | SLC25A3 | MCOLN2 | |
| AXL | APOBEC3F | RP11-129M16.4 | RP11-236B18.5 | ROR1-AS1 | |
| NUPR1 | APOBEC3G | IFIT3 | GMPR | SERPINB9 | |
| XRN1 | DDX58 | HELZ2 | TNFAIP6 | SOD2 | |
| CD300E | FAM26E | AF127936.5 | SLC35F5 | P2RX7 | |
| ANKRD22 | FAM26F | HCAR2 | ZNF43 | ADAM19 | |
| TMCC3 | ARRDC3 | RP11-79H23.3 | ENDOD1 | ADAM15 | |
| TNFSF8 | C7ORF49 | HESX1 | ADAR | CMPK2 | |
| NCF1 | XRCC2 | IL12B | SIRPB1 | CD44 | |
| OPTN | PARP14 | TNFSF10 | AC002511.3 | BLZF1 | |
| PCDHB8 | EXT1 | RP11-449D8.1 | SOCS1 | NRP2 | |
| IFITM3 | ISG20 | C5ORF56 | RASGEF1B | RP11-218M11.1 | |

Figure S8. List of Differentially Expressed Genes. From the DeSEQ2 analysis in Galaxy, the complete list of genes differentially expressed in the Δ BICD2/HIV THP1 cells infected with HIV-1 compared to WT/HIV, Δ BICD2/MOCK, and WT/MOCK samples.

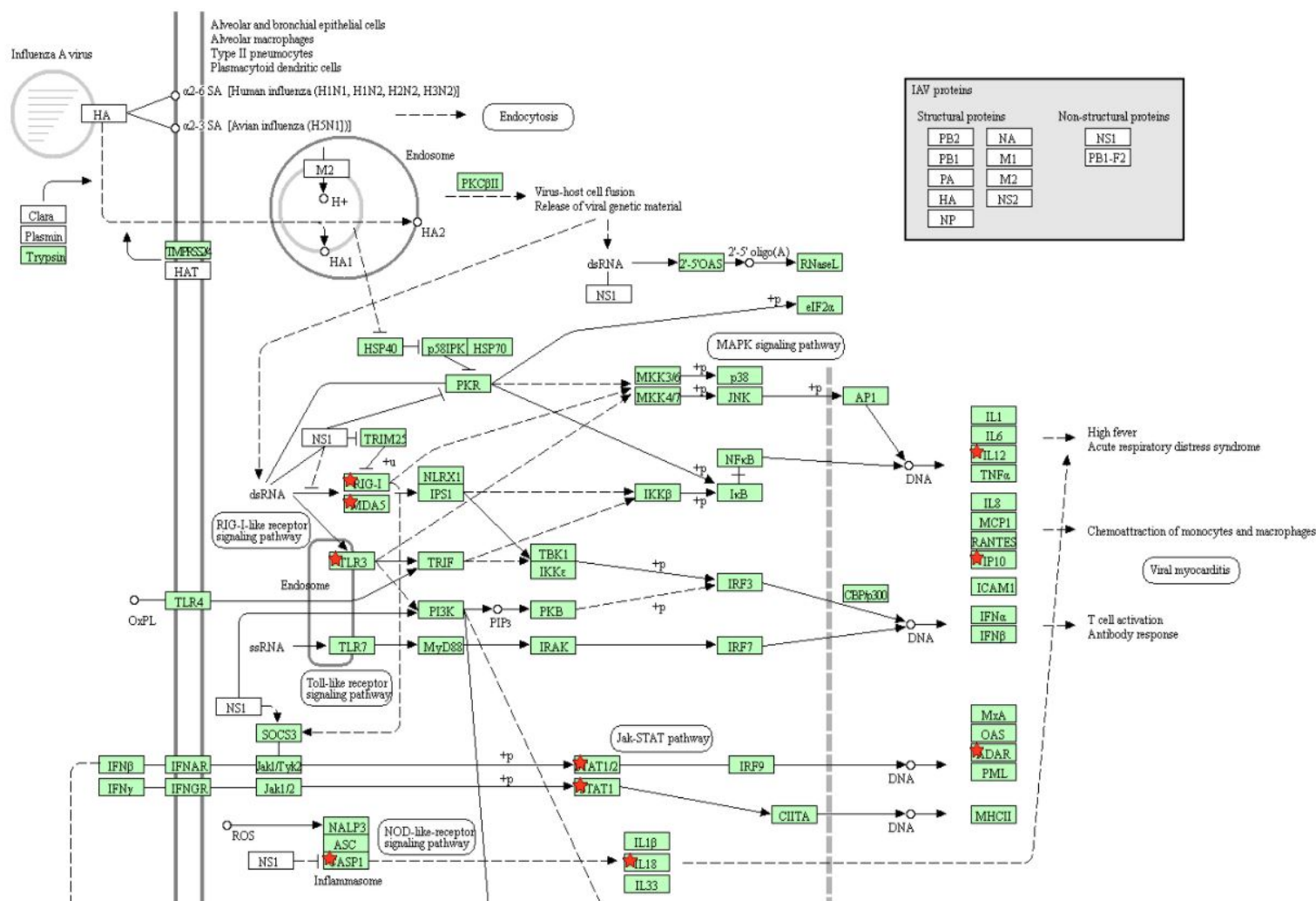
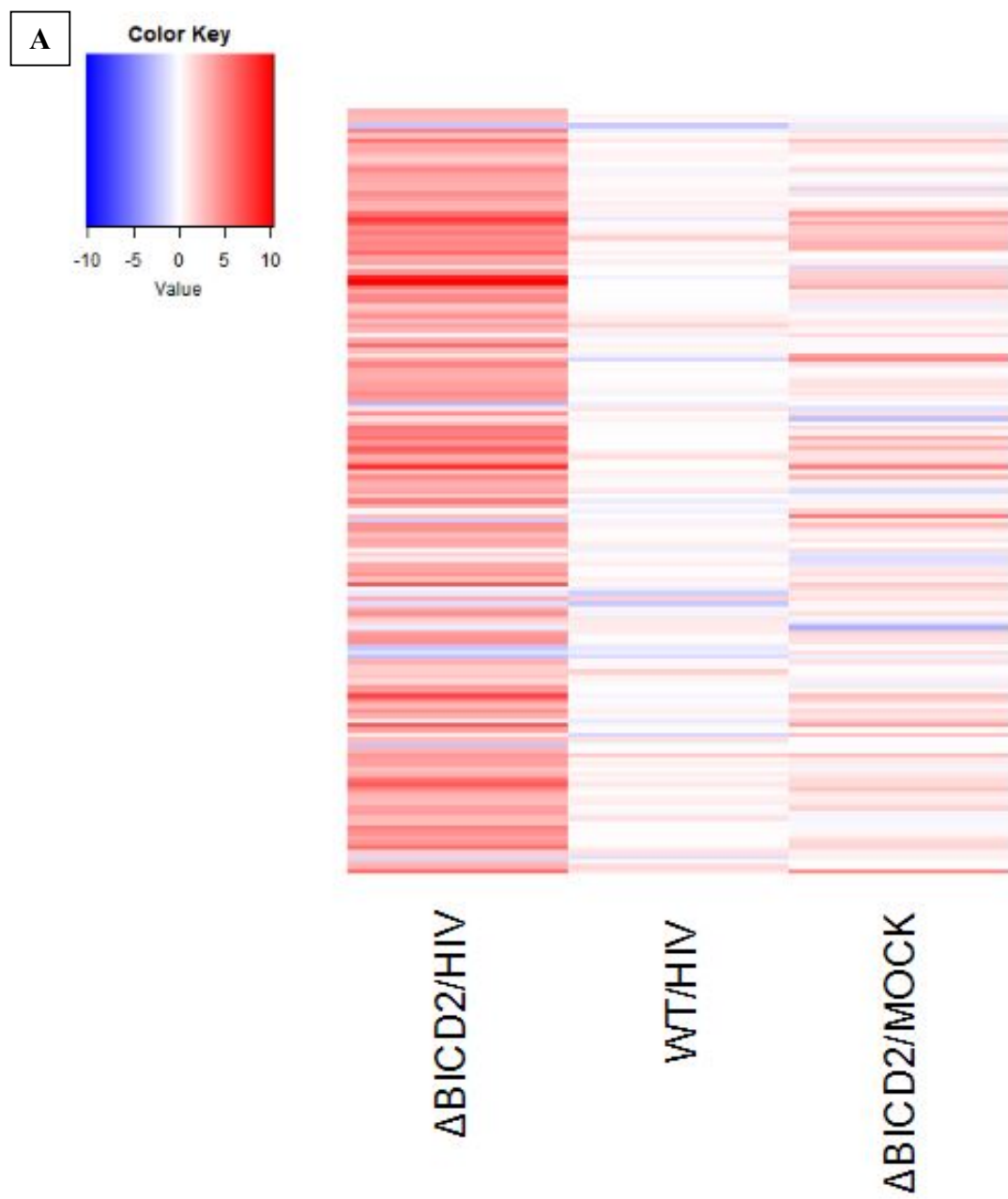


Figure S9. Additional Pathway Analysis (DAVID). In addition to the EnrichR pathway network shown in CHAPTER FOUR, we also generated pathway diagrams that allowed us to view where factors upregulated in our RNA-Seq analysis were present. A sample pathway generated, involved in general viral response (using Influenza A as an example), is shown below. Red asterisks denote differently expressed factors detected in the RNA-Seq analysis.



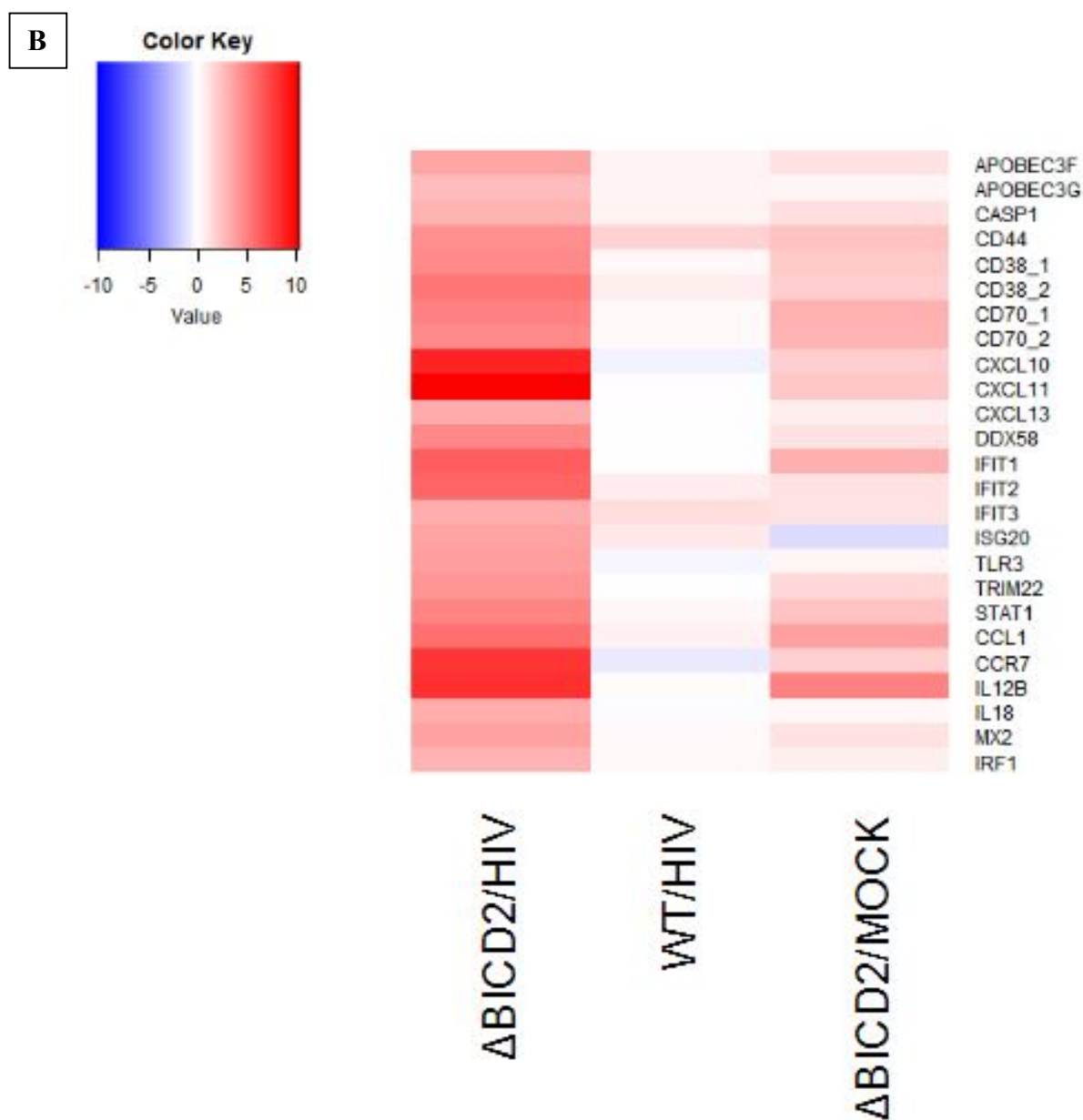


Figure S10. Additional Heatmaps (Log-Transformed Fold Change Values, Normalized to WT/MOCK). Heatmaps visualizing log-transformed fold changes of normalized gene counts relative to WT/MOCK for both A) all 157 differentially expressed genes and B) Selected antiviral genes of interest. Color keys are provided.

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VITA

The author, Omar Abdel-Rahim, was born in Normal, Illinois on January 21st, 1994 to Fatima and Majed Abdel-Rahim. He attended DePauw University in Greencastle, Indiana where he earned a Bachelor of Art in Biology, graduating magna cum laude.

He matriculated to Loyola University Chicago in July 2016, in the Infectious Disease & Immunology MS program. He joined the lab of Dr. Edward Campbell where he helped investigate the importance of cargo adaptor proteins in the context of HIV-1 infection and the host ISG response. After completion of his studies, Omar will continue his work at Loyola, in the IPBS PhD program.

