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The role of T cell specific factors and RNA Polymerase II pausing in HIV-1 replication in CD4+ T cells

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THE ROLE OF T CELL SPECIFIC FACTORS

AND RNA POLYMERASE II PAUSING

IN HIV-1 REPLICATION IN CD4$^+$ T CELLS

by

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DEDICATION

This thesis is dedicated to my grandfather, Franciszek Kaczmarek, who has always encouraged me to learn new things, and to my godmother, Jolanta Dziemianczyk Para, who has always supported me.

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THE ROLE OF T CELL SPECIFIC FACTORS

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ABSTRACT

In order to eradicate HIV-1 infection the virus needs to be specifically eliminated

from latently infected memory CD4$^+$ T cells. There does not seem to be a single

mechanism that promotes HIV-1 latency. RNA Polymerase II (RNAP II) pausing,

chromatin structure, tissue specific transcriptional repressors and transcriptional

interference have been implicated in regulating HIV-1 transcription. The transcription

factor B Lymphocyte-Induced Maturation Protein 1 (Blimp-1) is expressed in B and T

cells and upregulated in patients chronically infected with HIV-1. I hypothesized that

Blimp-1 is a T cell intrinsic factor that binds to HIV-1 LTR, inhibits HIV-1 transcription

and contributes to HIV-1 latency. Blimp-1 is expressed in primary peripheral blood CD4$^+$

T cells and is further induced by T cell activation. Importantly, Blimp-1 is highly

expressed in memory CD4$^+$ T cells compared to naïve CD4$^+$ T cells. Ectopic expression

of Blimp-1 in CD4$^+$ T cells represses HIV-1 transcription, whereas decreasing Blimp-1 in

memory CD4$^+$ populations activates HIV-1 transcription. Reduction of Blimp-1 in

infected primary T cells increases RNAP II processivity and histone H3 acetylation.

Blimp-1 binds downstream of the HIV-1 5'-LTR to the interferon-stimulated response

element (ISRE) in resting primary CD4$^+$ T cells and strongly represses Tat-dependent HIV-1 transcription. Upon T cell activation, Blimp-1 is released from the HIV-1 ISRE and this correlates with significant increase in HIV-1 transcription. These results demonstrate that Blimp-1 acts to limit HIV-1 transcription in memory CD4$^+$ T cells and promotes the establishment and maintenance of latency. I also examined whether neighboring host promoters could impact HIV-1 transcription. Using a set of inducible cell lines I observed that neighboring promoters have minimal impact on HIV-1 transcription and that enabling release of paused RNAP II by diminishing negative elongation factor (NELF) is sufficient to reactivate transcriptionally repressed HIV-1 provirus. The implications of my results in the different mechanisms regulating HIV-1 latency are discussed.

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LIST OF ABBREVIATIONS

Ab.. Antibody

AFF1 .... AF4/FMR2 Family Member 1

AFF4 .... AF4/FMR2 Family Member 4

AIDS .... Acquired Immunodeficiency Syndrome

AP-1 .... Activator Protein 1

APC.. Antigen Presenting Cell

APOBEC-3G......Apolipoprotein B mRNA-editing Enzyme Catalytic Polypeptide-like 3G

ASP .... Antisense Protein

asRNA.. Antisense RNA

BAF...BRG1-associated Factors

bNAb...Broadly Neutralizing Antibody

Brd4...Bromodomain-Containing Protein 4

BSA.. Bovine Serum Albumin

CA .... Capsid

CBF-1.. C-Promoter Binding Factor-1

CCR5...C-C Chemokine Receptor Type 5

CD4...Cluster of Differentiation 4

cDNA .... Complementary DNA

C/EBP ....CCAAT-enhancer-binding proteins

CpG...Cytodine and Guanine Separated by a Phosphate

CPNEI .... Copine I

Nef..Negative factor

NELF.. Negative Elongation Factor

NFAT .... Nuclear Factor of Activated T Cells

NFκB...Nuclear factor Kb

NP-40 .... Nonidet P-40

NRE... Negative Regulatory Element

Nuc-0..Nucleosome 0

Nuc-1..Nucleosome 1

PAF1 ....RNA Polymerase II-Associated Factor 1

PBS ....Phosphate-Buffered Saline

PCR.. Polymerase Chain Reaction

PD-1 .... Programmed cell death protein 1

PDZD8 .... PDZ domain containing 8

PHA.. Phytohemagglutinin

PIC .... Preintegration Complex

PM...Plasma Membrane

PMA.. Phorbol Myristate Acetate

PML ....Promyelocytic Leukemia Protein

PPM1A... Protein Phosphatase 1A

PR...Protease

PRD...Proline-rich Domain

PR Domain...PRDI-BF1-RIZ1 Homologous Domain

I. Introduction

1. Epidemiology of Human Immunodeficiency Virus

Infection with human immunodeficiency virus type 1 (HIV-1) leads to the development of Acquired Immunodeficiency Syndrome (AIDS), a disease whose hallmark is a decline in CD4$^+$ T cells and an increased susceptibility to opportunistic infections and cancer. In addition to directly impacting the number of CD4$^+$ T cells, HIV-1 infection leads to indirect immune exhaustion by activating neighboring or bystander cells and results in a general imbalance of all T cell populations, facilitating the immune dysregulation associated with AIDS (Chevalier and Weiss, 2013; Moreno-Fernandez et al., 2012; Paiardini and Muller-Trutwin, 2013). HIV-1 is a zoonotic disease that resulted from cross-species transmission of simian immunodeficiency virus (SIV) naturally infecting African non-human primates (Sharp and Hahn, 2011). HIV-1 is transmitted via bodily fluids and the life expectancy without treatment is 9 to 11 years (Programme and (UNAIDS), 2014). HIV-1 was first identified as a causative agent of AIDS in 1983 (Barre-Sinoussi et al., 1983; Gallo et al., 1983) but the first HIV-1 infection dates back to the 1920s (Faria et al., 2014). In 2013 the number of people living with HIV-1 worldwide reached an estimated 35 million (Programme and (UNAIDS), 2014) (**Fig. 1**). There were approximately 2.1 million new HIV-1 infections and 1.5 million AIDS-related deaths in 2013 (**Fig. 1**) (Programme and (UNAIDS), 2014). Although the disease has plateaued in the recent years due to treatment options and public health efforts, there is still no cure for HIV-1/AIDS.

The current combination therapy, called Highly Active Antiretroviral Therapy (HAART), was available to only 12.9 million people in 2013 (36.9% of infected people) (**Fig. 1**) (Programme and (UNAIDS), 2014). Drugs used in HAART target viral fusion, reverse transcription, integration and maturation of the virus. HAART does not inhibit viral transcription or release and because it targets viral proteins (with the exception of entry inhibitors) the virus mutates to escape the drugs action and develops resistance. In addition, HAART has many side effects and does not reverse the inflammation and premature aging associated with HIV-1 infection even when viral load is undetectable.
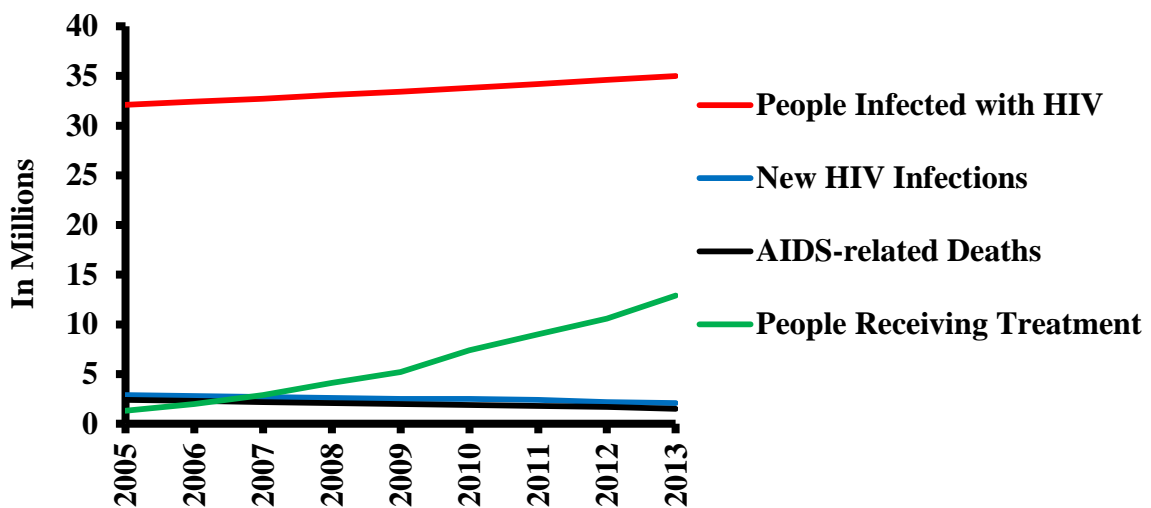
Figure 1. HIV-1 Epidemic in Numbers (based on UNSAIDS data).

2. Human Immunodeficiency Virus

Virion Structure

HIV-1 is a retrovirus belonging to the Lentivirus genus. It is a single-stranded, positive-sense, enveloped RNA virus. The hexameric p24 capsid (CA) protein forms a cone-shaped core which encloses two copies of viral RNA genome in complex with the p7 nucleocapsid (NC) protein, as well as the accessory proteins Tat and Rev. The capsid is surrounded by the p17 matrix (MA) protein which assembles into trimers. The outer surface of the HIV-1 membrane is a lipid bilayer derived from host cell during viral budding that is studded with envelope spikes composed of gp120 and gp41 trimers. The HIV-1 virion also contains three enzymes, reverse transcriptase, integrase and protease, enclosed inside the capsid, as well as accessory proteins Nef, Vpr and Vif (**Fig. 2**).
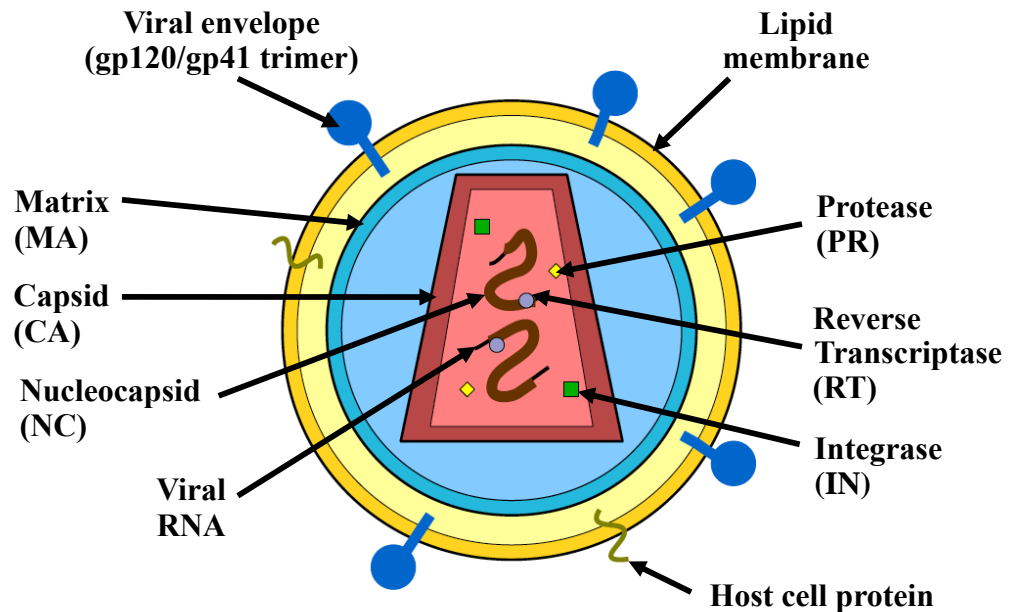
Figure 2. Structure of Human Immunodeficiency Virus.

The HIV-1 virion contains two copies of single-stranded, positive-sense RNA which interact closely with the nucleocapsid. The RNA together with three viral enzymes, protease, reverse transcriptase and integrase, is enclosed in viral capsid. The viral capsid is surrounded by matrix which in turn is surrounded by lipid bilayer derived from the host PM. The lipid membrane is embedded with host cell proteins and is studded with viral envelope spikes which are composed of trimers of gp120 and gp41.

HIV-1 Genome

The genome of integrated HIV-1 provirus is 9719 base pairs long and is flanked at each end by long terminal repeats (LTRs) (**Fig. 3**). The 5'-LTR serves as the viral promoter and transcriptional enhancer. The genes encoded by HIV-1 are as follow:

- ***gag*** – encodes the precursor polyprotein Pr55Gag which is cleaved by viral protease into MA, CA, NC, and p6, as well as two spacer peptides, SP1, and SP2. MA, CA and NC are structural proteins and the expression of Gag in the absence

of other viral proteins and RNA genome is sufficient for the production of non-infectious virus-like particles (VLPs). p6 is required for incorporation of Vpr into the HIV-1 virions and contains late domain motifs necessary for virus budding (Garrus et al., 2001).

- *pol* – encodes Pol polyprotein processed into HIV-1 protease, reverse transcriptase and integrase. HIV-1 protease cleaves viral polyproteins (Gag and Pol) and thus is required for viral maturation. HIV-1 reverse transcriptase is responsible for transcribing viral RNA into DNA, while HIV-1 integrase is required for integration of HIV-1 DNA into host genome (Sierra et al., 2005).

- *env* – encodes the viral envelope polyprotein gp160 which is spliced into gp120 and gp41. gp120 binds to CD4 receptor and CXCR4 or CCR5 co-receptors enabling insertion of gp41 into PM, which in turn leads to viral fusion with the target cell (Postler and Desrosiers, 2013). The cytoplasmic domain of gp41 also contains signaling motifs that can activate NFκB, which is important for HIV-1 transcription (Postler and Desrosiers, 2013).

- *tat* – encodes cell membrane-permeable Tat (trans-activator of transcription), which binds to trans-activation response (TAR) RNA stemloop and recruits p-TEFb to initiate HIV-1 transcripion (Watson and Edwards, 1999). Tat is cell membrane permeable (Watson and Edwards, 1999).

- *rev* – encodes Rev (Regulator of Expression of Virion Proteins), a protein required for viral mRNA splicing and export from the nucleus (Pollard and Malim, 1998).

- **nef** – encodes Nef (negative regulatory protein) which is important in modulating host cell signaling and reponses. The expression of Nef alone in animal models results in AIDS-like symptoms (Rahim et al., 2009).

- **vif** – encodes Vif (viral infectivity factor), a protein that increases viral infectivity by targeting APOBEC-3G, a cytidine deaminase (deanimates cytidine into uridine0, for proteasomal degradation (Goila-Gaur and Strebel, 2008).

- **vpr** – encodes Vpr (viral protein R) , a protein that induces cell cycle arrest in CD4$^+$ T cells and facilitates infection of macrophages by unknown mechanisms (Li et al., 2009).

vpu – encodes Vpu (viral protein U) important for viral budding by targeting tetherin for proteasomal degradation (Kueck and Neil, 2012).
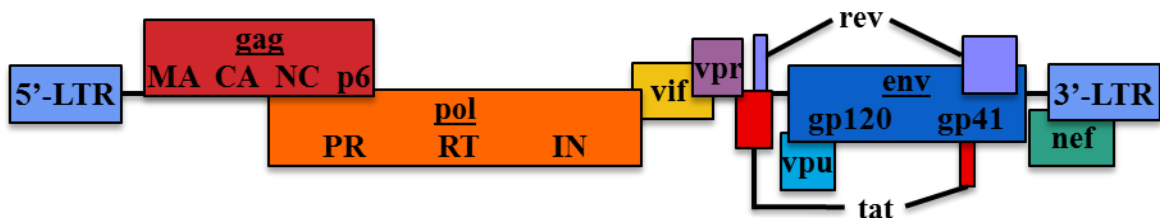
Figure 3. Organization of Human Immunodeficiency-1 Virus Genome.

HIV-1 genome has LTR at each of its ends that serve as viral promoter and enhancer. HIV--1 encodes gag, pol and env polyproteins, as well as accessory proteins: Tat, Vif, Vpr, Vpu, Rev and Nef.

3. Replication Cycle of HIV-1

HIV-1 replication cycle is composed of the following steps: virus binding, fusion, uncoating, reverse transcription, integration, transcription, translation, assembly and budding (**Fig. 4**). Upon the binding of the HIV-1 envelope to CD4 receptor and a chemokine co-receptor expressed at the surface of the target cell, the viral fusion peptide penetrates host plasma membrane (PM) and then the virion fuses with the PM or with endosomal compartment (Postler and Desrosiers, 2013). In the cytoplasm the viral core disassembles and viral RNA is reverse transcribed into double-stranded cDNA bythe viral reverse transcriptase. The uncoating of the virion might be blocked by TRIM5α (Yan and Chen, 2012). Reverse transcription is blocked by such host-restriction factors as APOBEC-3G (Yan and Chen, 2012), a cytidine deaminase that deaminates cytidine to uridine in the viral DNA during the process of reverse transcription, SAMHD1, which hydrolyzes dNTPs and degrades viral RNA (Yang and Greene, 2014) and TREX, an exonuclease digesting excessive HIV-1 DNA (Yan et al., 2010). Recent studies show that RNA polymerase II-associated factor 1 (PAF1) inhibits early steps of viral replication leading to decreased reverse transcription and proviral integration (Liu et al., 2011). The viral cDNA is imported to the nucleus by preintegration complex (PIC) and is integrated into the host genome by viral integrase. Lens epithelium-derived growth factor (LEDGF) binds to chromosomal DNA and HIV-1 integrase and favors HIV-1 integration into LEDGF-regulated, A-T-rich genes, while integrase interactor 1 (INI1) facilitates HIV-1 integration by remodeling nucleosomes (Ciuffi et al., 2005; Lesbats et al., 2011). The provirus is next transcribed by host transcriptional machinery into RNA. While the

multiply spliced viral RNAs encoding for Tat, Rev and Nef are processed by the cellular

splicing and nuclear export machinery, the unspliced viral RNA genome and singly-

spliced viral mRNAs (encoding Gag, Env, Vif, Vpr and Vpu) are transported to

cytoplasm, a process controlled by the viral protein Rev (Felber et al., 1989). The

envelope is synthesized on the rough endoplasmic reticulum (ER) and is transported

through the ER to Golgi and trans-Golgi network (TGN) (Saftig and Klumperman, 2009).

Gag is synthesized by membrane-unattached ribosomes (Perlman and Resh, 2006).

Newly synthesized Gag accumulates in the perinuclear region, and before reaching the

PM it passes through a late endosome-like compartment (Klein et al., 2007; Perlman and

Resh, 2006). Gag is responsible for targeting HIV-1 to lipid rafts in host plasma

membrane (PM) by binding to phosphatidylinositol-4,5-bisphosphate (PIP2) (Klein et al.,

2007). The MA domain targets Gag to the PM via the 14-carbon-long N-myristyl group

at the glycine 2 position, and the basic region at the amino-terminal end (Klein et al.,

2007). Gag binds to the genomic RNAs, and together with the envelope assembles into

HIV-1 virions, which then bud off the cell (Klein et al., 2007; Postler and Desrosiers,

2013). The budding of HIV-1 is blocked by such host factors as tetherin, ISG15, miRNA,

TIM-1, TIM-3 and TIM-4 (Chen et al., 2014; Li et al., 2014; Pincetic et al., 2010; Yan

and Chen, 2012). HIV-1 matures by cleavage of the Gag precursor, Pr55Gag, by viral

protease into MA, CA, NC, and p6, as well as two spacer peptides, SP1 and SP2.
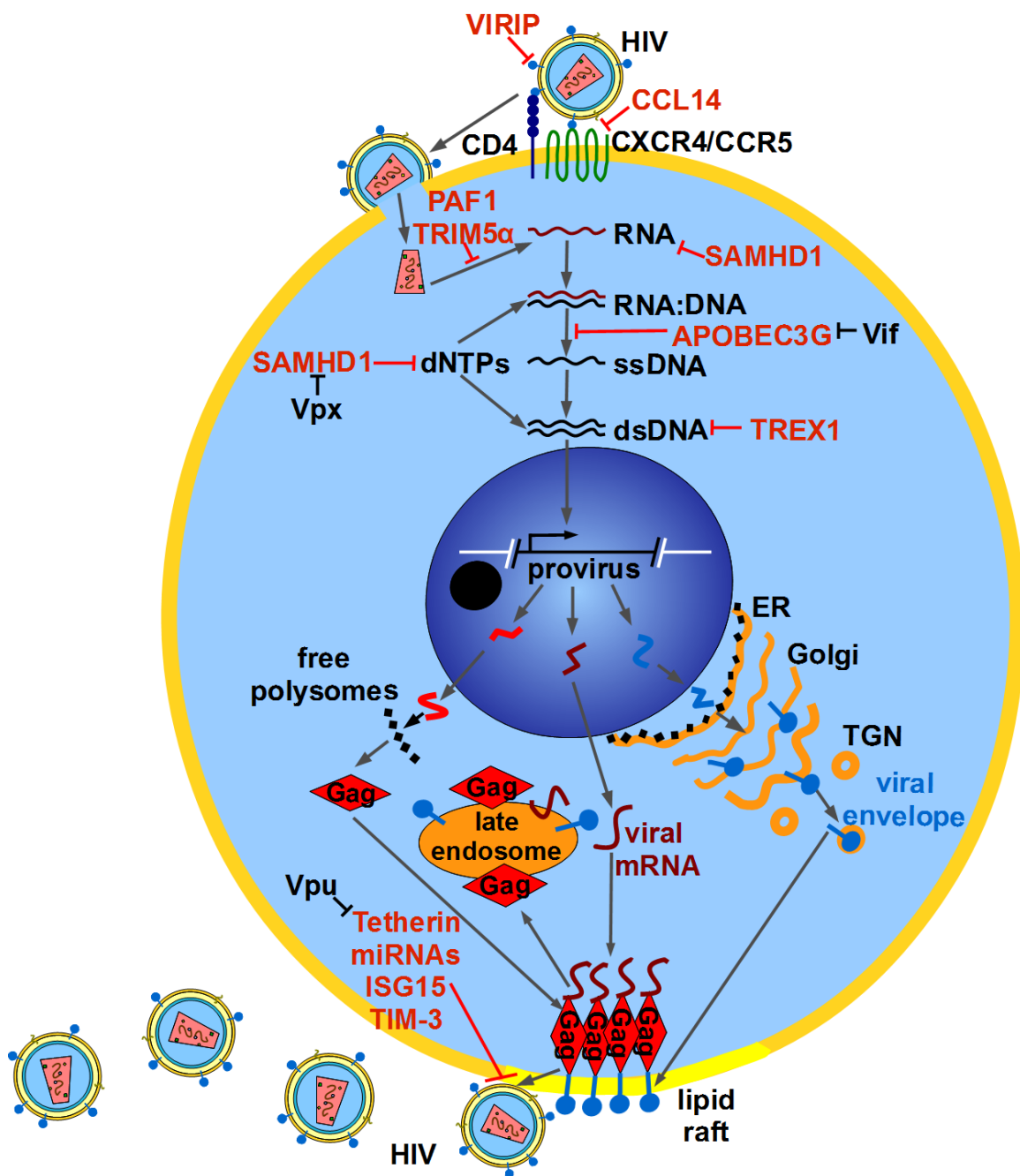
Figure 4. HIV-1 Replication Cycle.

The HIV-1 virion binds via gp120 to its receptor, CD4, and co-receptor, CCR5 or CXCR4. The binding is followed by fusion of the virion with the target cell which is mediated by gp41. Next the viral core undergoes uncoating and RNA genome is reversly transcribed into dsDNA., which is next imported into the nucleus. In the nucleus viral integrase mediates interation of HIV-1 into host genome. The provirus is transcribed, the viral mRNAs spliced and exported into the cytoplasm where they are translated by free polysomes or in ER. The viral proteins and mRNA are transported to the PM. The virus assembles and buds off from lipid rafts. The maturation of HIV-1 involves cleaving of Pr55Gag by viral protease. For a more detailed description of each step in the HIV-1 replication cycle please see the text.

4. HIV-1 Persistence During Therapy

HAART is a combination of several antiviral drugs that target viral fusion, reverse transcription, integration and maturation of HIV-1. A remaining challenge in efforts to cure HIV-1 infection is targeting cells harboring quiescent, yet replication-competent virus. HAART and host immune system do not affect latently-infected cells which are the source of viral rebound after cessation of antiretroviral treatments (Richman et al., 2009; Siliciano and Greene, 2011). Upon cessation of HAART, HIV-1 rapidly reemerges from latently infected cells to pretreatment viral loads (**Fig. 5**) (Richman et al., 2009; Siliciano and Greene, 2011). HIV-1 preferentially infects activated CD4^{+} T cells. HIV-1 infection either leads to productive HIV-1 replication and cell death or to the transition of effector cell into a memory CD4^{+} T cell and establishment of latency. Memory CD4^{+} T cells are considered the main reservoir of latent HIV-1. The latently infected cells persist for the life of an infected individual. In addition to memory CD4^{+} T cells, other latent reservoirs are thought to exist. Using dual-reporter virus

expressing one HIV-1 LTR-dependent marker to measure productive HIV-1 infection, and a second marker under a constitutive promoter independent of HIV-1 LTR it has been shown that HIV-1 establishes latency even in activated CD4$^+$ T cells (Calvanese et al., 2013). Strategies to target latent reservoir requires characterizing the cell populations that harbor latent HIV-1 and understanding the biochemical mechanisms that regulate provirus expression in these cells.

The size of a latently-infected viral reservoir is still a matter of debate (Stevenson, 2013). Originally, based on the ability to grow virus from blood samples in vitro (viral outgrowth assay), the size of latent reservoir was estimated to be 1 cell in a million. However, recent studies have suggested that the size of latent reservoir is at least 60-fold higher than previously thought (Ho et al., 2013). Plus, exactly what cells and tissues harbor latent infected cells has made it difficult to accurately measure the total size of the viral reservoir in vivo. It has been also estimated that the latent reservoir must be reduced by more that 10,000-fold to cure HIV-1 infection (Hill et al., 2014).
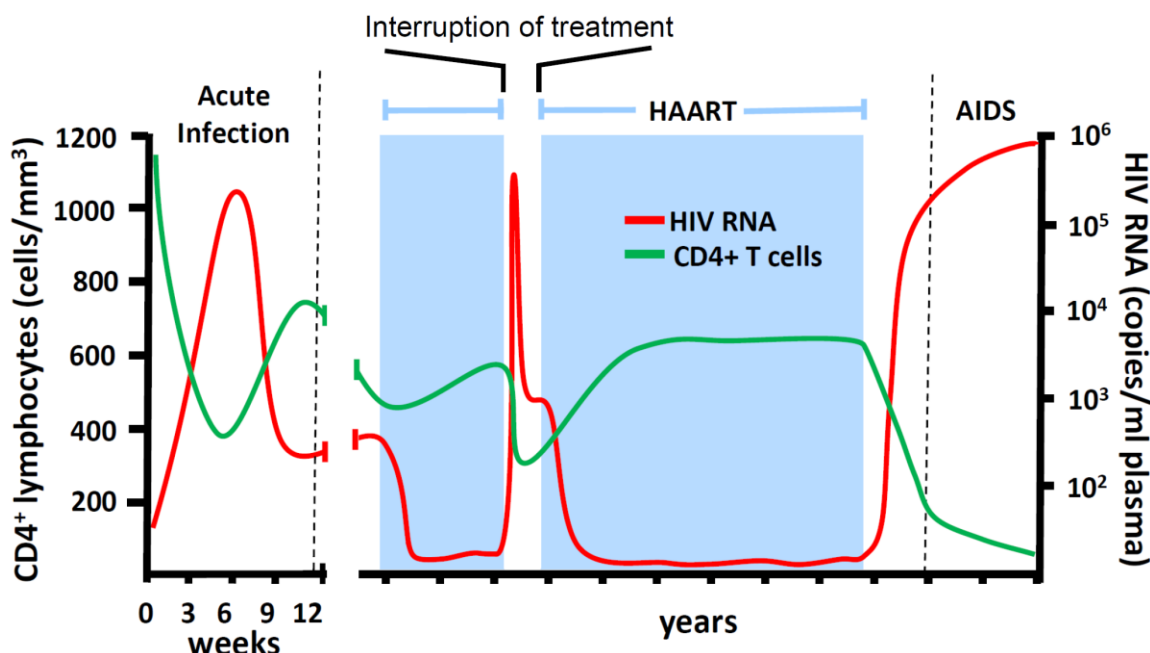
Figure 5. HIV-1 Clinical Progression. Modified from (Fauci et al., 1996)

During the acute infection the viral load rapidly increases while the number of CD4^{+} T cells falls drastically. Once the patient initiates HAART, which targets actively replicating virus, the viral load decreases to even undetectable levels, while the number of CD4^{+} T cells recovers, but not to the pre-treatment levels. However, treatment interruption results in a reemergence of virus from latently infected cells to high pre-treatment levels. The latently infected cells harbor replication-competent virus that is not actively transcribed until some signal triggers its reactivation. Without treatment the infection may result in AIDS which is characterized by high viral loads and CD4^{+} T cell numbers below 200 cells /mm^{3} which makes the patient susceptible to opportunistic infections and results in death.

The establishment of latent reservoir occurs within the first 3 days of SIV infection (Whitney et al., 2014). Studies using dual fluorescent reporter virus that allows detection of infected cells independent of transcription from HIV-1 LTR confirm that the majority of virus becomes latent shortly after infection and that it can be reactivated (Dahabieh et al., 2013). Thus, initiation of HAART as early as possible increases the chances of not only stopping viremia but also decreasing the possibility of viral rebound (Whitney et al., 2014). The "Mississippi Baby" who was perinatally infected with HIV-1

and who within 30 hours after birth was put on HAART for 18 months did not have a viral rebound for over 2 years after cessation of treatment (Ledford, 2014). In contrast, "Boston patients", whose viremia was actively suppressed by HAART prior to receiving bone marrow transplants to repopulate hematopoietic cells had viral rebound within a few months of HAART interruption (Henrich et al., 2014) suggesting that early treatment might diminish but not completely suppress virus reservoir establishment and is associated with positive prognosis.

5. Molecular Mechanisms of HIV-1 Latency

The mechanisms that establish HIV-1 latency remain incompletely defined and research has focused on general events that control gene expression including transcription initiation, elongation and epigenetic regulation of chromatin (Richman et al., 2009; Schiralli Lester and Henderson, 2012; Siliciano and Greene, 2011). HIV-1 latency is driven by transcriptional repression; however there does not seem to be a single mechanism that promotes HIV-1 latency. RNA Polymerase II (RNAP II) pausing, chromatin structure and recruitment of ubiquitously expressed transcription factors as well as non-coding RNAs have been implicated in the regulation of HIV-1 transcription. Additionally, T cell specific factors may be repressing HIV-1 transcription in memory $CD4^+$ T cells, the major reservoir of latent HIV-1. Transcriptional interference from neighboring host promoters is also thought to contribute to establishing and maintaining HIV-1 latency (**Fig. 6**).

Additionally, HIV-1 latency can be a stochastic process, where the virus enters latency and gets reactivated regardless of T cell activation level (Ho et al., 2013). The presence of Tat has been shown to inhibit the establishment of HIV-1 latency (Donahue et al., 2012). Superinfection with HIV-1 results in Tat-dependent reactivation of latent virus, suggesting that limiting amounts of Tat drive HIV-1 latency (Donahue et al., 2013). Strategies targeting signals which regulate recruitment of transcription factors, chromatin remodeling complexes or regulators of RNAP II have been devised to activate and purge HIV-1 latent reservoirs (Deeks et al., 2012; Lafeuillade, 2012; Margolis and Hazuda, 2013; Richman et al., 2009; Siliciano and Greene, 2011; Tyagi and Bukrinsky, 2012).
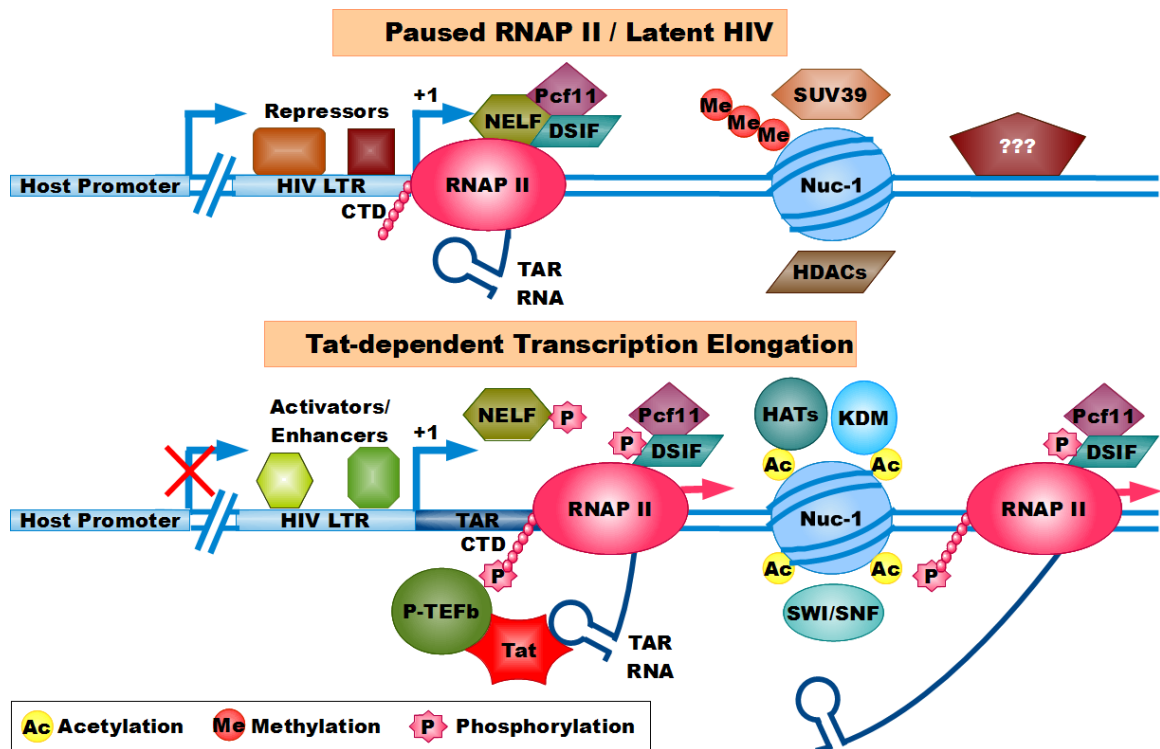
Figure 6. Model for HIV-1 Transcriptional Regulation.

During initiation of HIV-1 transcription RNAP II is recruited to HIV--1 LTR and transcribes nascent TAR RNA. However, transcriptional repressors bind to HIV--1 LTR and NELF forms a complex with RNAP II, DSIF and Pcf11 resulting in paused RNAP II at HIV--1 LTR. Additionally, methyltransferases and HDACs condense chromatin making it inaccessible to the transcriptional machinery. All these events are cooperative and combinatorial. Upon cell stimulation, transcriptional activators and enhancers are recruited to HIV--1 LTR, Tat binds to TAR RNA and recruits P-TEFb which activates RNAP II by phosphorylating its CTD. P-TEFb also phosphorylates NELF and DSIF. Phosphorylated NELF dissociates from RNAP II, while phosphorylation of DSIF changes it from transcriptional repressor to transcriptional activator. Histone modifying factors remodel and remove methyl groups from Nuc-1 making the chromatin more accessible to the transcriptional machinery. DSIF and Pcf11, which is required for transcript termination, travel down the provirus with RNAP II. For a more detailed description of HIV-1 transcription please see the text.

5.1 Transcription Factors

HIV-1 LTR controls provirus transcription by functioning as a promoter and enhancer recruiting host transcription factors and co-activators necessary to initiate transcription. HIV-1 LTR has three binding sites for the specificity protein 1 (Sp1) and two binding sites for the nuclear factor κB (NFκB). Binding of Sp1 and NFκB to HIV-1 LTR, as well as their physical interaction is required for robust HIV-1 transcription (Perkins et al., 1993). In addition to Sp1, NFκB acts in synergy with the activator protein 1 (AP1), a heterodimer of c-Fos and c-Jun, to induce HIV-1 transcription (Yang et al., 1999). HIV-1 LTR also contains three binding sites for CCAAT-enhancer-binding proteins (C/EBP) (Tesmer et al., 1993), which although not required, enhance basal and active HIV-1 transcription in macrophages (Henderson and Calame, 1997; Henderson et

al., 1995). Upon cell activation, the transcription factor II human (TFIIH) gets recruited to HIV-1 LTR where it phosphorylates CTD of RNAP II enabling HIV-1 reactivation (Kim et al., 2006). T cell stimulation also activates RBF2, a complex composed of USF-1, USF-2 and TFII-I to enhance HIV-1 transcription (Chen et al., 2005). Ets-1 stably binds to HIV-1 LTR 1 and physically interact with NFκB, NFAT and USF-1 to induce HIV-1 transcription (Bassuk et al., 1997; Gegonne et al., 1993; Seth et al., 1993; Sieweke et al., 1998). A subset of T cell transcription factors have been suggested to rapidly reactivate latent HIV-1 (Kaczmarek et al., 2013). NFAT was considered critical for overcoming latency and induction of HIV-1 transcription in different T cell subsets (Dahabieh et al., 2011; Malcolm et al., 2008), although recent studies have questioned its role in the reactivation of latent HIV-1 (Bosque and Planelles, 2009; Kinoshita et al., 1998; Pessler and Cron, 2004; Robichaud et al., 2002).

HIV-1 LTR also can be bound by transcriptional repressors and contains negative regulatory element (NRE) between -420 and -154 of HIV-1 LTR which mediates inhibition of HIV-1 transcription (Rosen et al., 1985; Siekevitz et al., 1987). p50 homodimers, a subunit of NFκB, bind to HIV-1 LTR and recruit histone deacetylase 1 (HDAC1), which in turn decreases histone acetylation and recruitment of RNAP II repressing proviral transcription (Williams et al., 2006). p50 can also form heterodimers with E2F1 protein which repress NFκB-induced HIV-1 transcription (Kundu et al., 1997). Yin Yang 1 (YY1) has been shown to recruit HDAC1 to a positioned Nucleosome-1 (Nuc-1) adjacent to the HIV-1 transcriptional start site, to decrease histone H4 acetylation at Nuc-1, and to co-operate with LSF to represses HIV-1 (He and Margolis, 2002;

Margolis et al., 1994; Romerio et al., 1997). However, recent studies showed that decreasing YY1 re-activates latent HIV-1 without affecting the recruitment of HDACs or the levels of acetylated histone H3 (Barton and Margolis, 2013). C-promoter binding factor-1 (CBF-1), a heterochromatin marker, represses HIV-1 transcription by inhibiting recruitment of RNAP II and acetylation of Nuc-1 (Tyagi and Karn, 2007). The T cell commitment factor B-cell lymphoma 11b (Bcl-11b, also known as CTIP-2) (Liu et al., 2010) binds HIV-1 LTR and limits transcription by binding Tat and redirecting it to heterochromatic regions (Le Douce et al., 2012; Marban et al., 2007; Rohr et al., 2003).

5.2 RNA Polymerase II Promoter Proximal Pausing

RNAP II pausing is defined by stalling of the RNAP II at a promoter and is a a critical checkpoint in controlling gene transcription (Gaertner et al., 2012; Rahl et al., 2010). RNAP II pausing is characterized by the accumulation of short initiated mRNA transcripts but lack of transcriptional elongation. RNAP II pausing may be beneficial for the transcription of a gene, as deletion of eleven-nineteen lysine-rich leukemia (ELL), which facilitates RNAP II pause site entry, results in loss of P-TEFb recruitment to pre-initation complex and disrupts elongation (Byun et al., 2012). Negative elongation factor (NELF) and DRB sensitivity-inducing factor (DSIF) induce promoter-proximal pausing of RNAP II (Wada et al., 1998a; Yamaguchi et al., 1999). DSIF is a heterodimer composed of hSpt4 and hSpt5 that interacts with RNAP II and nascent RNA. NELF is

composed of four subunits (NELF-A, NELF-B, NELF-C/D and NELF-E) and binds to pre-formed DSIF-RNAP II complexes (Narita et al., 2003; Yamaguchi et al., 2002). P-TEFb, a heterodimer of Cdk9 and cyclin T1, phosphorylates C-terminal domain (CTD) of RNAP II activating it and releasing the pause induced by NELF and DSIF (Fujinaga et al., 2004; Peterlin and Price, 2006; Wada et al., 1998b; Yamaguchi et al., 1999). P-TEFb also phosphorylates NELF-E causing its release and hSpt5 subunit of DSIF, switching DSIF to a stimulator of RNAP II processivity (Fujinaga et al., 2004; Ivanov et al., 2000; Wada et al., 1998a; Yamada et al., 2006). P-TEFb can be brought to poised genes by bromodomain-containing protein 4 (Brd4), which recruits it from the repressive 7SK/HEXIM1 complex composed of 7SK small nuclear RNA (snRNA), HEXIM, LARP7 and MEPCE (Jang et al., 2005; Krueger et al., 2008; Yang et al., 2005). CDK7 is a subunit of TFIIH that recruits DSIF to promoters inducing RNAP II pausing, and that is also required for P-TEFb-mediated phosphorylation enabling transcriptional elongation (Larochelle et al., 2012). In addition, Gdown-1 inhibits transcriptional initiation by TFIIF (Cheng et al., 2012). TRIM28 has been recently implicated in regulating RNAP II pausing at mammalian genes (Bunch et al., 2014). Nonphosphorylated TRIM28 at S824 stabilizes paused RNAP II, while S824-phosphorylated TRIM28 is required for release of paused RNAP II (Bunch et al., 2014). c-Myc is another factor required for the release of paused RNAP II (Rahl et al., 2010). While these cellular factors have been shown to be necessary for general transcription from host promoters, roles in HIV-1 LTR-mediated transcription have still not been confirmed for all.

In latently-infected cells, transcription of HIV-1 provirus is inhibited at the step of transcription elongation. NELF binds at HIV-1 LTR to RNAP II/DSIF complex inducing RNAP II promoter proximal pausing (Zhang et al., 2007). NELF also recruits Pcf11 and NCoR1-GPS2-HDAC3 complex to HIV-1 LTR which results in premature termination of transcription and condensed chromatin, respectively (Natarajan et al., 2013). Recently, the microprocessor complex composed of RNase III Drosha and the dsRNA-binding protein Dgcr8 has been shown not only to cleave the 5' stem loop RNA structure, TAR, but also to recruit Setx, Xrn2, and Rrp6 leading to RNAP II stalling at HIV-1 LTR (Wagschal et al., 2012). Setx and Xrn2 are transcription termination factors mediating premature termination of transcription, while Rpr6 processes cleaved TAR RNAs to repress HIV-1 transcription (Wagschal et al., 2012). To overcome RNAP II promoter proximal pausing, HIV-1 encodes a transcriptional activator, Tat, which via cyclin-dependent kinase substrate 1 (NUCKS1)-dependent mechanism binds TAR RNA (Kim et al., 2014) and gets activated by the lysine methyltransferase Set7/9-KMT7 (Pagans et al., 2010). Cyclin T1-associated Tat recruits AF4/FMR2 family member 1 (AFF1)/P-TEFb complexes (Lu et al., 2014a) to HIV-1 LTR that are normally sequestered away by HEXIM1–7SK snRNA regulatory complex (du Chene et al., 2007; Garber et al., 1998; Wei et al., 1998). P-TEFb alleviates transcriptional repression by phosphorylating components of the paused RNAP II complex including the CTD of RNAPII facilitating productive transcription elongation (Isel and Karn, 1999). Protein phosphatase 1A (PPM1A) is present at high levels in resting CD4$^+$ T cells and inhibits P-TEFb activation by preventing CKD9 T-loop phosphorylation, which in turn leads to repression of HIV-1

transcription (Budhiraja et al., 2012). In addition, Brd4 competes with Tat for binding to P-TEFb (Bisgrove et al., 2007). The splicing-associated c-Ski-interacting protein, SKIP, binds to Tat/P-TEFb complexes, and together with c-Myc and Menin transactivates Tat, as well as promotes transcriptional elongation of HIV-1 (Bres et al., 2005; Bres et al., 2009). The Super Elongation Complex (SEC) composed of P-TEFb, Eleven-Nineteen Lysine-Rich Leukemia (ELL2), AFF1, AF4/FMR2 Family Member 4 (AFF4), eleven-nineteen-leukemia (ENL), and ALL1-fused gene from chromosome 9 protein (AF9) binds to RNAP II and promotes efficient elongation (He et al., 2011).

5.3 Chromatin Structure

Regardless of the integration site the 5' end of HIV-1 provirus is associated with Nucleosome-0 (Nuc-0) and Nuc-1 (Verdin et al., 1993). Nuc-0 is positioned upstream of 5'-HIV-1 LTR, while Nuc-1 is located directly downstream of the HIV-1 LTR at approximately 100 nt after the transcription start site (TSS) and is better studied in regulation of HIV-1 transcription than Nuc-0 (Verdin et al., 1993). Histones can be post-translationally modified by acetylation, methylation, phosphorylation and ubiquitination. Factors associated with the HIV-1 LTR recruit histone acetyltransferases (HATs) and KDM demethylases that regulate chromatin organization of the integrated provirus (Schiralli Lester and Henderson, 2012). HATs such as p300/CBP and P/CAF, are recruited by Tat to acetylate not only histones but also NF-κB (Deng et al., 2001; Furia et

al., 2002). However, transcriptional repressors are also recruited to HIV-1 LTR, such as methyltransferases including SUV39H1 (du Chene et al., 2007), G9a (Imai et al., 2010), GLP (Ding et al., 2013) and enhancer of Zeste 2 (EZH2) (Friedman et al., 2011), as well as HDACs (Keedy et al., 2009), which methylate and deacetylate histones, respectively, within positioned nucleosomes favoring condensation of chromatin and making the proviral LTR less accessible for efficient transcription. HDAC2 and HDAC3 have been shown to be present in the nuclei of latent cells and to repress HIV-1 transcription (Keedy et al., 2009). In microglial cells Bcl-11b recruits HDAC1, HDAC2, and SUV39H1 to HIV-1 LTR resulting in deacetylation and methylation of Nuc-1 (Marban et al., 2007). Furthermore, Bcl-11b facilitates binding of heterochromatin protein 1 (HP1) (Marban et al., 2007). In addition, CpG methylation of the 5'-HIV-1 LTR by methyl-CpG binding domain protein 2 (MBD2) is not required for the establishment of HIV-1 latency, but has been associated with increased resistance to reactivation (Blazkova et al., 2009; Kauder et al., 2009). BRG1-associated factors (BAF), a member of switching-defective-sucrose non-fermenting (SWI/SNF) family, represses HIV-1 transcription by re-positioning Nuc-1closer to the HIV-1 LTR (Rafati et al., 2011). PBAF, another member of (SWI/SNF family), gets recruited by Tat and enables remodeling of Nuc-1 and transcriptional elongation (Easley et al., 2010; Rafati et al., 2011). USF-1 co-operates with Sp1, NF-kB and LEF-1 to remove histone NH(2) tails from DNA (Angelov et al., 2000). Integration of HIV-1 close to nuclear bodies containing promyelocytic leukemia protein (PML) is associated with increased H3K9me2-metylation mediated by the methyltransferase G9a and thus increased latency (Lusic et al., 2013). Overall, these multiple posttranslational

changes of histones contribute to coordinated mechanism that can positively and negatively influence the epigenetic state of HIV-1.

5.4 Influence of Neighboring Promoters

Three-fourths of HIV-1 provirus preferentially integrates into introns of actively transcribed host genes (Ding et al., 2013; Lenasi et al., 2008; Lewinski et al., 2005; Shan et al., 2011; Sherrill-Mix et al., 2013). Transcriptional interference is thought to occur when HIV-1 integrates next to a gene with stronger promoter. HIV-1 can integrate in three orientations relative to the host gene: parallel or tandem, convergent and divergent orientation (**Fig. 7**). Transcriptional interference has been shown to suppress gene expression, especially when the genes are in a convergent or parallel orientation (Eszterhas et al., 2002). In the parallel orientation scenario, RNAP II from the upstream gene reads through HIV-1 LTR displacing its transcriptional machinery (**Fig. 7**) (Lenasi et al., 2008). The inhibition of transcription from the upstream promoter can induce HIV-1 transcription in the parallel orientation (Lenasi et al., 2008). In the convergent orientation the two transcriptional machineries from two promoters are thought to collide leading to transcriptional interruption of both genes (**Fig. 7**). In the divergent orientation scenario the two promoters may be competing for limited quantities of transcriptional activators and RNAP II (**Fig. 7**). The integration site of provirus has been shown to not only affect the transcriptional activity of provirus (Jordan et al., 2001), but also the

persistence and expansion of HIV-1-infected cells (Maldarelli et al., 2014). For example, integration of provirus into introns of BACH2 and MKL2 in the same transcriptional orientation correlates with clonal expansion and persistence of infected cells (Ikeda et al., 2007; Maldarelli et al., 2014). Early studies showed that transcriptional interference is strongest in the convergent orientation, while it has the weakest influence when promoters are in a divergent orientation (Eszterhas et al., 2002). The integration site played the strongest role on transcriptional activity in the parallel orientation (Eszterhas et al., 2002).
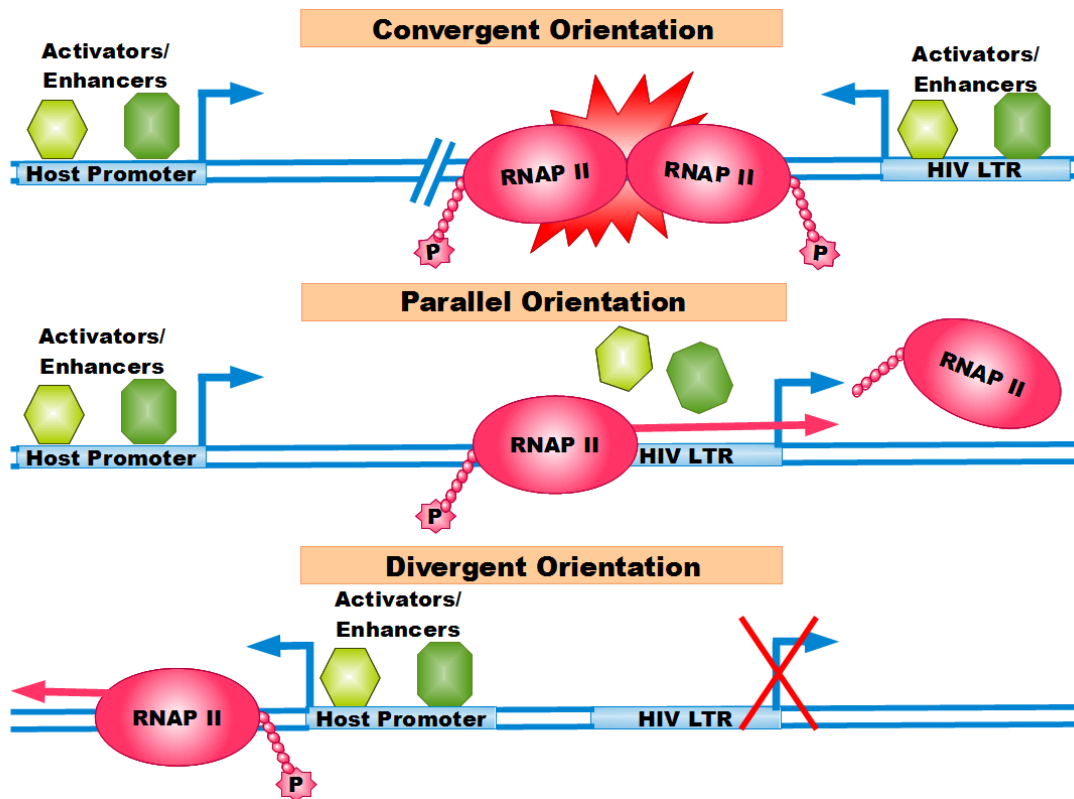
Figure 7. Potential Role of Transcriptional Interference in HIV-1 Transcription

HIV-1 can integrate in three different orientations relative to the host gene: convergent, parallel and divergent. In the convergent orientation the RNAP IIs from the two promoters collide leading to termination of transcription. In the parallel orientation the RNAP II from the upstream host gene reads through HIV--1 LTR leading to occlusion and dissociation of transcription factors from the viral promoter. In divergent orientation the two promoters are competing for limiting amounts of transcriptional activators and RNAP II.

Additionally, numerous antisense RNA (asRNA) transcripts that may originate from transcriptional interference have been identified which might also regulate HIV-1 transcription and replication (Landry et al., 2007; Michael et al., 1994). In addition, HIV-1 LTR has been also implicated in the generation of asRNAs (Bentley et al., 2004; Peeters et al., 1996), which have been shown to halt viral transcription (Kobayashi-Ishihara et al., 2012; Tagieva and Vaquero, 1997). A recent study detected antisense protein (ASP) which is encoded by a region in the minus strand env gene, and which regulates HIV-1 replication by inducing autophagy (Torresilla et al., 2013).

5.5 T Cell Transcription Factors Implicated in Regulation of HIV-1 Transcription

CD4$^+$ effector T cell subsets possess diverse specialized functions. CD4$^+$ T helper cells (T$_H$1, T$_H$2, T$_H$17) are responsible for the production of cytokines stimulating specific immune responses, follicular B helper T cells (T$_{FH}$) support B cell activation, regulatory T cells (T$_{reg}$) suppress immune responses elicited by CD4$^+$, CD8$^+$ T cells and

B cells, while memory T cells are important for the recall of immune response (Murphy and Stockinger, 2010; van Leeuwen et al., 2009; Zhu et al., 2010). During infection antigen presenting cells (APC) display antigenic peptides in the context of MHC II to naïve $CD4^+$ T cells (T_H0), promoting their clonal expansion and polarization into effector T cells. A subset of activated $CD4^+$ cells will generate memory T cells responsible for rapid recall of the adaptive immune response upon re-exposure. The differentiation of $CD4^+$ T cells is driven in part by avidity of T cell receptor (TCR) engagement, strength of signaling, co-stimulatory signals and tissue microenvironments which include cytokine milieu and differential interactions with APC (**Fig. 8**) (Zhu et al., 2010). Additionally, $CD4^+$ T cell development is controlled by a constellation of transcription factors that activate and repress batteries of genes that influence proliferation, differentiation and lineage commitment (Murphy and Stockinger, 2010; van Leeuwen et al., 2009; Yamane and Paul, 2013).
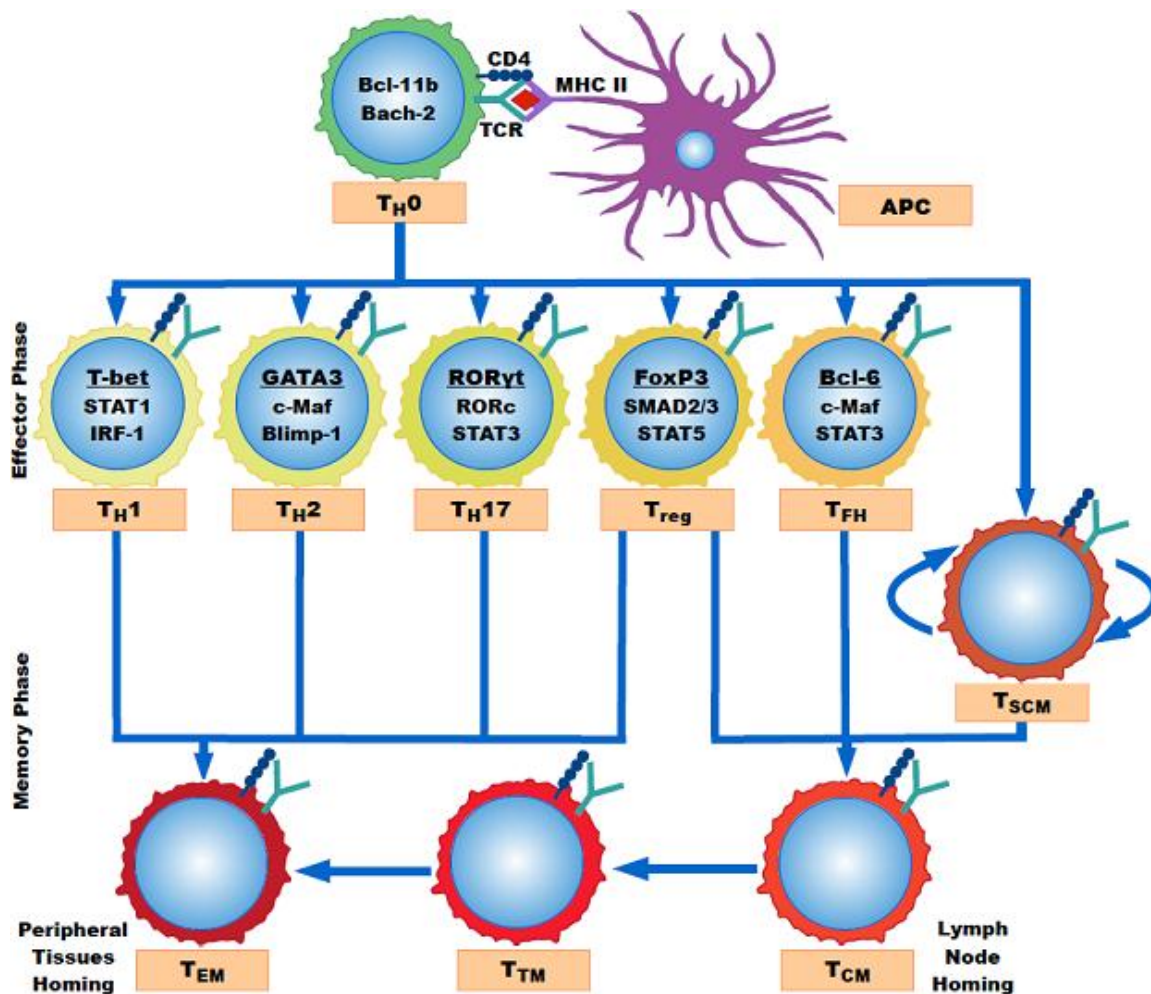
Figure 8. Model of Sequential CD4$^+$ T Cell Differentiation. Modified from (Kaczmarek et al., 2013)

Upon activation by antigen presenting cell (APC), naïve T cell (T$_H$0) undergoes differentiation into effector or memory populations. The different effector populations have capacity to mature into effector memory T cells (T$_{EM}$). Stem cell memory T cells (T$_{SCM}$), follicular helper T cells (T$_{FH}$) and regulatory T cells (T$_{reg}$) mature into central memory T cells (T$_{CM}$), which then can become transitional memory T cells (T$_{TM}$) and further convert into T$_{EM.}$

Although all CD4$^+$ T cells are susceptible to HIV-1 infection due to their expression of CD4 and chemokine receptors, CXCR4 and CCR5, the ability of different T cell populations to support HIV-1 replication varies (Chevalier and Weiss, 2013;

Moreno-Fernandez et al., 2012; Paiardini and Muller-Trutwin, 2013), possibly reflecting differential expression of T cell-specific transcription factors that regulate HIV-1 expression. It is possible that these T cell factors, by promoting HIV-1 transcription, influence the dissemination of virus at different stages of AIDS or, by repressing proviral transcription contribute to the establishment of latently infected T cells. By targeting $CD4^+$ effector T cells, HIV-1 has a dramatic impact on the depletion, expansion and function of the different polarized T cell subsets. The maturation of T cell lineages is in part driven by intrinsic transcription factors which potentially influence how efficiently HIV-1 replicates (**Table 1**). Quiescent memory $CD4^+$ T cells have been implicated as the primary HIV-1 reservoir because they are susceptible to HIV-1 infection, are long-lived and, with their ability to self-renew, potentially maintain pools of latently infected cells. Whether there are T cell specific factors that predispose memory cells to latent HIV-1 infection has not been demonstrated.

The polarization of T_H1 verses T_H2 cells is mediated by two primary transcription factors, T-bet and GATA-3, respectively, whereas RORγt facilitates the differentiation of T_H17 cells (Amsen et al., 2009; Muranski and Restifo, 2013; Zhu et al., 2010). T_H1 cells have been reported to be limited in their ability to support HIV-1 replication, although there is no evidence that T-bet, the T_H1 master transcription factor, directly influences HIV-1 transcription (Gosselin et al., 2010). T-bet antagonizes GATA-3 function, which is considered the key regulator of T_H2 differentiation (Amsen et al., 2009; Kanhere et al., 2012; Zhu et al., 2010). GATA-3 binds to several sites in the HIV-1 LTR and induces HIV-1 transcription (Galio et al., 1997; Yang and Engel, 1993). Therefore, T-bet

potentially limits HIV-1 transcription by targeting GATA-3. In addition to GATA-3, c-Maf promotes T_H2 differentiation (Ho et al., 1998) and, in regards to HIV-1 transcription, binds HIV-1 LTR and cooperates with NF-κB and NFAT to enhance HIV-1 transcription (Zhang et al., 2012). The ability of GATA-3 and c-Maf to activate HIV-1 transcription is consistent with findings that T_H2 cells support HIV-1 replication (Gosselin et al., 2010; Zhang et al., 2012). T_H17 cells also support robust HIV-1 replication (Rodriguez-Garcia et al., 2014), although RORγt and RORc, factors which are abundant in T_H17 cells (Gosselin et al., 2010), have not been shown to directly impact HIV-1 transcription. Thus, induction of downstream T cell signaling pathways that culminate in activation of NF-κB, NFAT and STATs probably strongly influence HIV-1 transcription in T_H17 cells.

There has been recent interest in characterizing the role of T_{reg} cells in HIV-1 (Chevalier and Weiss, 2013; Moreno-Fernandez et al., 2012). In general, changes in T_{reg} numbers and function have been documented in patients and include higher T_{reg} frequencies in untreated AIDS patients and diminished ability of these cells to suppress immune activation in HAART treated patients (Chevalier and Weiss, 2013; Moreno-Fernandez et al., 2012). Whether these changes are a direct result of HIV-1 infection or reflect more general immune dysfunction requires further investigation. T_{reg} development is in part driven by the transcription factor FoxP3 (Zhu et al., 2010). The data as to whether FoxP3 directly impacts HIV-1 transcription are conflicting. Ectopically expressed FoxP3 in primary CD4^{+} T cells inhibits activation and recruitment of transcriptional activators NF-κB, CREB and NFAT2 to the LTR (Grant et al., 2006; Selliah et al., 2008). FoxP3 may also facilitate HIV-1 transcription by inhibiting HDAC1

(Holmes et al., 2011; Holmes et al., 2007). However, overexpression of FoxP3 in different T cell subsets may result in different transcriptional outcomes. For example, forced expression of FoxP3 polarized naïve CD4$^+$ T cells towards a T$_{reg}$ phenotype and enhanced HIV-1 replication, but had no impact on T memory cell phenotypes or HIV-1 transcription (Oswald-Richter et al., 2004). These conflicting results may reflect intrinsic differences of T cell subsets or challenges associated with overexpressing factors, and underscore the need to further study HIV-1 transcription in primary T$_{reg}$ cells.

A unique feature of the adaptive immune response is the generation of memory. For CD4$^+$ T cells two distinct memory populations have been well characterized based on the expression of surface markers, homing capacity and function upon reactivation; T central memory (T$_{CM}$) and T effector memory (T$_{EM}$) cells (Pepper and Jenkins, 2011; Sallusto et al., 2004; van Leeuwen et al., 2009). Recently, stem cell memory T cells (T$_{SCM}$) have been described in humans (Gattinoni et al., 2011). These self-renewing memory CD4$^+$ cells are reported to generate T memory and effector populations. The array of factors, including cytokines, signaling events and transcription factors, that influence the proliferation and generation of T$_{SCM}$, T$_{CM}$ and T$_{EM}$ have not been fully elucidated. Critical transcription factors that regulate the generation and survival of T$_{CM}$ include Schnurri-2, STAT3, STAT5a TBR2, FOXO3a, Bmi1 and LKLF (Catalfamo et al., 2008; Hirschhorn-Cymerman et al., 2012; Juffroy et al., 2010; Kimura et al., 2007; Kuo et al., 1997; van Grevenynghe et al., 2008; Yamashita et al., 2008). Furthermore, Bach-2, IRF-1 and p27 (Kip1) have been suggested to suppress T$_{EM}$ differentiation (Jatzek et al., 2012; McElligott et al., 1997; Tsukumo et al., 2013b). Relevant to HIV-1,

memory cells, especially T_{CM} and T_{SCM}, have been implicated as primary reservoirs harboring latent provirus because they are susceptible to HIV-1 infection, long-lived and, with their ability to self-renew, may maintain and/or renew the pool of cells harboring latent provirus (Bosque et al., 2011; Buzon et al., 2014). This homeostatic proliferation of infected T_{CM} and T_{SCM} in the absence of T cell activation and robust HIV-1 transcription presents a major barrier to eradicating persistent HIV-1 infection and underscores the need to characterize the tissue distribution and factors that regulate these different T cell memory subsets (Henrich and Gandhi, 2013). The predisposition for HIV-1 to establish latency in T_{CM} may reflect the expression levels of transcription factors; T_{CM} express T-bet (Marshall et al., 2011) and have lower levels of Cyclin T1 and phosphorylated CDK9, the two subunits of P-TEFb required for transcription elongation (Budhiraja et al., 2013), whereas, T_{EM} have increased expression of factors shown to activate HIV-1 transcription including GATA-3, c-Maf and RORγt. (Dutta et al., 2013; Wang et al., 2010) The discrepancy of what key check points limit HIV-1 transcription in latently infected cells may reflect heterogeneity of memory cells, difference in cell culture conditions and isolation of cell subsets. The factors that repress transcription in T memory cells have not been defined but most likely include epigenetic factors such as HDACs or methyltransferases (Richman et al., 2009; Siliciano and Greene, 2011).

Table 1. Factors Influencing CD4$^+$ T Cells Differentiation and HIV-1 Transcription.

Transcription Factor	T Cell Subset Promoted	T Cell Subset Inhibited	Effect on HIV-1 Transcription	Reference
Bcl-6	T_{FH}, T_{CM}	T_H2	Inhibition, Activation (?)	(Baron et al., 1997; Ichii et al., 2007; Pepper et al., 2011; Sawant et al., 2013; Yu et al., 2009)
Bcl-11b (CTIP-2)	All		Inhibition	(Le Douce et al., 2012; Liu et al., 2010; Marban et al., 2007; Rohr et al., 2003)
Blimp-1		T_H1, T_H17, T_{FH}	Repression, Activation	(Cimmino et al., 2008; Johnston et al., 2012; Lin et al., 2014)
BRG1	T_H1, T_H2		Activation & Inhibition	(Agbottah et al., 2006; Henderson et al., 2004; Rafati et al., 2011; Wurster and Pazin, 2008; Zhang and Boothby, 2006)
c-Maf	T_H2, T_{FH}, T_{EM}	T_H1	Activation	(Ho et al., 1998; Kroenke et al., 2012; Sato et al., 2011;

				Zhang et al., 2012)
c-Myb	T_H2, T_{EM}		Activation	(Dasgupta et al., 1990; Kozuka et al., 2011; Nakata et al., 2010)
CIITA	T_H2 (?)		Activation & Inhibition (?)	(Okamoto et al., 2000; Otten et al., 2003; Saifuddin et al., 2000; Sung and Simon, 2004)
CREB	T_H1, T_{reg}		Activation	(Rabbi et al., 1997a; Rabbi et al., 1997b; Ruan et al., 2009; Yao et al., 2013)
CTCF	T_H1, T_H2		Inhibition	(Li et al., 2008; Ribeiro de Almeida et al., 2009; Sekimata et al., 2009)
Ets-1	T_H1, T_{reg}	T_H17	Activation	(Gegonne et al., 1993; Grenningloh et al., 2005; Moisan et al., 2007; Mouly et al., 2010)
FoxP3	T_{reg}		Activation & Inhibition (?)	(Chen et al., 2003; Grant et al., 2006; Holmes et al., 2011; Holmes et al., 2007; Selliah et al., 2006)

GATA3	T_H2, T_{EM}	T_H1	Activation	(Yang and Engel, 1993; Zheng and Flavell, 1997)
IRF-1	T_H1	T_{EM}	Activation	(Lohoff et al., 1997; Sgarbanti et al., 2002; Taki et al., 1997)
IRF-8		T_H17	Inhibition	(Ouyang et al., 2011; Sgarbanti et al., 2002)
JunB	T_H2, T_{reg}		Activation	(Blonska et al., 2012; Roebuck et al., 1996; Son et al., 2011)
JunD		T_H1, T_H2	Activation	(Meixner et al., 2004; Roebuck et al., 1996)
Lef1	T_{reg}		Activation	(Fu et al., 2012; Sheridan et al., 1995)
Menin	T_H17		Activation	(Bres et al., 2009; Watanabe et al., 2014)
NFAT	T_H1, T_{reg}	T_H2	Activation	(Cron et al., 2000; Ranger et al., 1998; Tone et al., 2008; Wu et al., 2006)
PU.1	T_H9, memory	T_H2, T_{reg}	Activation	(Chang et al., 2009; Chang et al., 2010; Hadjur et al.,

	T_H9			2009; Lodie et al., 1998)
SATB1	T_H2, T_{reg}		Inhibition	(Fu et al., 2012; Kumar et al., 2005; Notani et al., 2010)
SIRT1		T_{reg}	Activation & Inhibition	(Pagans et al., 2005; van Loosdregt et al., 2010; Zhang and Wu, 2009)
SMAD3	T_H2, T_{reg}, $T_{EM,}$ T_H9	T_H1, T_H17	Activation	(Blokzijl et al., 2002; Coyle-Rink et al., 2002; Elyaman et al., 2012; Giroux et al., 2011; Hu et al., 2012; Xiao et al., 2008; Zhang et al., 2013)
SMAD4	T_H1, T_{reg}		Inhibition	(Coyle-Rink et al., 2002; Huss et al., 2011; Yang et al., 2008b)
STAT1	T_H1, T_{reg}, T_{FH}		Activation & Inhibition	(Chang et al., 2002; Choi et al., 2013; Ouaked et al., 2009; Owaki et al., 2005)
STAT3	T_{FH}, T_H17, T_H1, T_H2, T_{CM}	T_{reg}	Unknown	(Ma et al., 2012; Owaki et al., 2008; Sawant et al., 2013; Siegel et al., 2011a;

				Sundaravaradan et al., 2010; Wei et al., 2008)
STAT5	T_H2, T_{reg}, T_{CM}	T_H1, T_{FH}, T_H17	Activation & Inhibition	(Burchill et al., 2007; Crotti et al., 2007; Della Chiara et al., 2011; Johnston et al., 2012; Laurence et al., 2007; Riou et al., 2007; Selliah et al., 2006; Takatori et al., 2005; Zhu et al., 2003)
T-bet	T_H1	T_{reg}, T_H2	Unknown	(Lazarevic et al., 2011; Lugo-Villarino et al., 2003; Mullen et al., 2001)
TRAF-2		T_H2	Activation	(Horie et al., 2007; Lieberson et al., 2001; Tsitsikov et al., 1997)

5.6 B Lymphocyte-Induced Maturation Protein 1

The *Prdm1* gene located on chromosome 6q21 encodes B lymphocyte-induced maturation protein-1 (Blimp-1), a 789 amino acid-long, weighting 87,990 Da, nuclear transcription factor (Martins and Calame, 2008). Blimp-1 is comprised of N-terminal acidic domain, PRDI-BF1-RIZ1 homologous (PR) domain, proline-rich domain (PRD), five Kruppel-like (C2H2) zinc-fingers and another acidic domain at C-terminus (**Fig. 9**). The PRD interacts with Grucho family proteins (Ren et al., 1999), the lysine demethylase LSD1 (Su et al., 2009) and HDAC2 (Yu et al., 2000). Full length Blimp-1 is called Blimp-1α, while Blimp-1 lacking PR domain is called Blimp-1β. Even though Blimp-1β binds the same genes as full length Blimp-1, it has attenuated ability to repress them (Gyory et al., 2003). The C-terminal C2H2 zinc-fingers contain a nuclear localization signal and mediate binding to DNA (Martins and Calame, 2008), as well as recruitments of the SET domain histone methyltransferase G9a (Gyory et al., 2004) and the arginine methyltransferase Prmt5 (Ancelin et al., 2006). The N-terminal PR domain and two acidic domains of Blimp-1 have been also shown to be critical in repression of gene transcription and can act independently of each other (Yu et al., 2000).
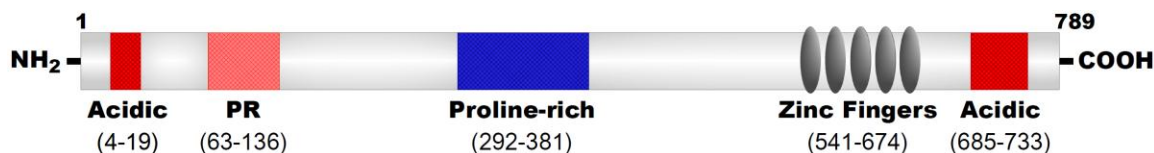
Figure 9. Blimp-1 Structure. Modified from (Martins and Calame, 2008)

Blimp-1 proteins is composed of two acidic regions, one PR domain, one proline-rich domain and five zinc-fingers. The zinc-fingers mediate the binding of Blimp-1 to DNA. The proline-rich region has been shown to interact with methyltransferases and histone deacetylases and to recruit them to Blimp-1 target-genes. The acidic and PR domains have also been shown to be involved in Blimp-1-mediated transcription.

Blimp-1 is critical for the differentiation of mature B cells into long-lived, Ig-secreting plasma cells and has been recently demonstrated to be expressed in dendritic cells, macrophages, keratinocytes and T cells (Chan et al., 2009; Chang et al., 2000; Chiang et al., 2013; Kim et al., 2011; Lin et al., 2002; Lin et al., 1997; Magnusdottir et al., 2007; Shaffer et al., 2002; Shapiro-Shelef et al., 2003; Smith et al., 2011; Turner et al., 1994). In T cells Blimp-1 regulates the activation and generation of CD4 and CD8 T cell effector populations (Hua et al., 2013; Kallies et al., 2006; Kallies et al., 2009; Martins et al., 2006) (**Fig. 10**). Blimp-1 inhibits both T cell proliferation and differentiation into T_{FH} (Crotty et al., 2010). Murine $CD4^+$ T cells express high Blimp-1 levels in effector and memory subsets and minimal Blimp-1 expression in T_H0 cells (Savitsky et al., 2007) (**Table 2**). In addition to its role in the differentiation of immune cells, Blimp-1 plays a crucial role in limb, pharynx and heart morphogenesis (Bikoff et al., 2009).
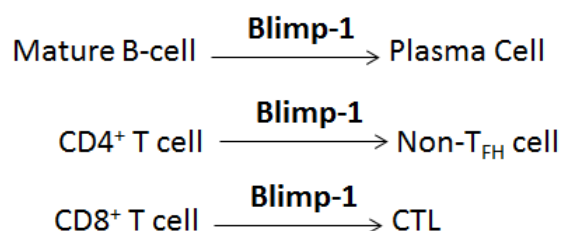
Figure 10. Role of Blimp-1 in Immune Cell Differentiation. Modified from (Crotty et al., 2010)

Blimp-1 is important regulator of differentiation and maturation of immune cells. Overexpression of Blimp-1 in B cells causes them to differentiation into long-lived, Ig-secreting plasma cells. Blimp-1 is expressed at different levels in CD4$^+$ T cell subsets and represses the differentiation of T$_{FH}$. In CT8$^+$ T cells the expression of Blimp-1 causes them to differentiate into cytotoxic T cells.

Table 2. Expression of Blimp-1 in Murine CD4$^+$ T Cell Subsets.

Phase	T Cell Subset	Blimp-1 Levels
Naïve	T$_H$0	+
Effector	T$_H$1, T$_H$2, T$_H$17, T$_{reg}$	+++
	T$_{FH}$	+
Memory	T$_{CM}$, T$_{TM}$, T$_{EM}$	++

Blimp-1 represses the transcription of several regulatory factors including Bcl-6, T-bet, IL-2, IL-6, TNFα, IFN-γ, IFN-β and type-III IFNs while enhancing the expression of IL-10 and Mcl-1 (Chan et al., 2009; Cimmino et al., 2008; Iwasaki et al., 2013; Keller and Maniatis, 1991; Lin et al., 2007; Martins et al., 2008; Smith et al., 2010; Swider et

al., 2014). Blimp-1-deficient DCs secret significantly higher amounts of IL-6 and CCL2, and favor the differentiation of T_{FH} (Chan et al., 2009; Kim et al., 2011). Blimp-1 also plays an important role in innate immunity by activating granzyme B, murine p202, a cytosolic DNA-sensor that inhibits AIM2 inflammasome formation, and repressing AIM2, and NLRP-12 (Gong and Malek, 2007; Lord et al., 2009; Panchanathan et al., 2012; Yin et al., 2013) (**Fig. 11**).
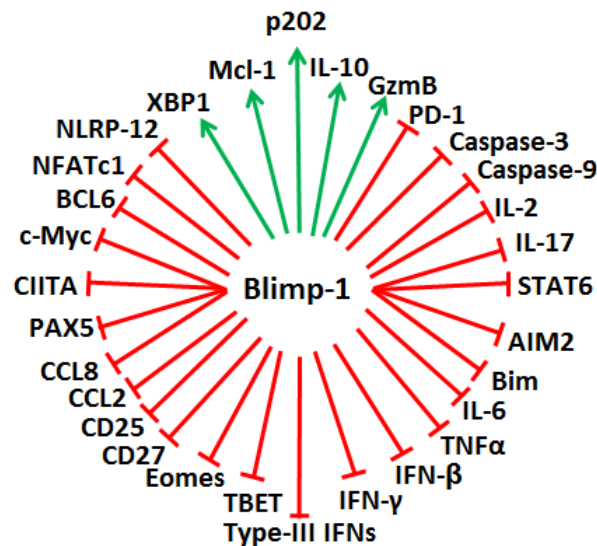
Figure 11. Blimp-1 is a Global Transcriptional Regulator.

Blimp-1 represses a constellation of genes in different cell types. However, Blimp-1 has been also shown to induce transcription of some genes.

Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Sendai virus and LPS induce Blimp-1 expression in bone marrow–derived macrophages (Severa et al., 2014). Blimp-1 is regulated by several mechanisms in different cell types. It is induced by IL-2, IL-21, OX40, STAT3, STAT5, IRF5, IFN-α, PPARγ, RXRα and TLR9 (Boettler

et al., 2013; Garcia-Bates et al., 2009; Genestier et al., 2007; Hodge et al., 2012; Lien et al., 2010; Nurieva et al., 2012; Panchanathan et al., 2012; Parlato et al., 2013) and inhibited by RIG-I (Sathe et al., 2014) and Bach2, a transcription factor suppressing CD4$^+$ T cell differentiation (Tsukumo et al., 2013a) (**Fig. 12**).
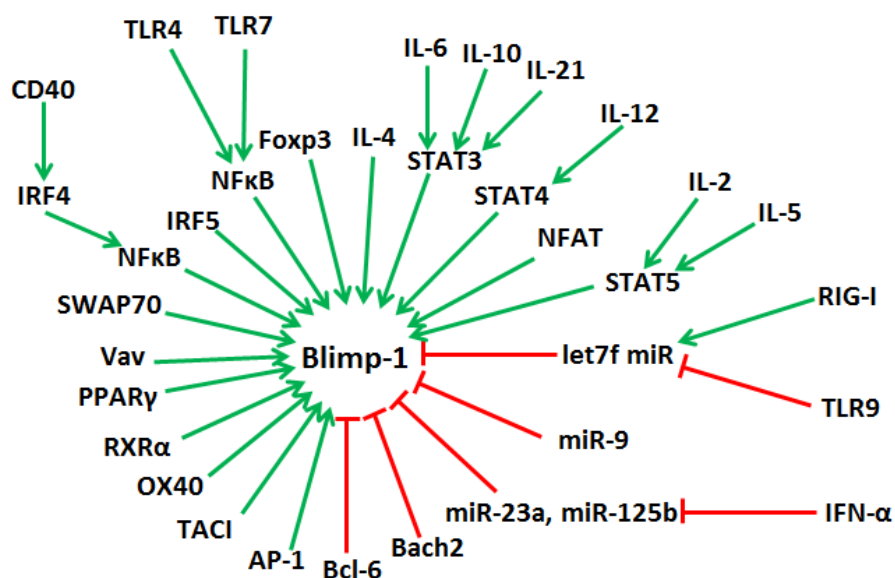
Figure 12. Regulation of Blimp-1 Expression.
Blimp-1 is induced via various mechanisms in different immune cells.

In the context of HIV-1, Blimp-1 expression is increased in chronically infected patients but remains unaltered in long term non-progressors and correlates with enhanced expression of negative regulators of T cell activation including PD-1, LAG3 and CTLA-4, and with T cell exhaustion and apoptosis (Che et al., 2012; de Masson et al., 2014; Seddiki et al., 2013; Shankar et al., 2011). Interestingly, HIV-1 LTR contains binding

sites for both Bcl-6 and Blimp-1, suggesting that these factors directly regulate HIV-1 transcription (Baron et al., 1997; Kaczmarek et al., 2013).

T_{FH} cells support productive HIV-1 infection and are reported to expand during the course of HIV-1 infection (Lindqvist et al., 2012; Perreau et al., 2013). Bcl-6 is the master transcription factor for the generation of T_{FH} cells (Yu et al., 2009). In particular, Bcl6 and Blimp-1 have an antagonistic relationship (Crotty et al., 2010; Martins and Calame, 2008). Unlike Bcl-6, Blimp-1 is highly expressed in T_H2 cells compared to T_H1 cells and represses T_H1 differentiation by repressing interferon, T-bet and Bcl-6 expression (Cimmino et al., 2008).

6. Hypothesis

Memory $CD4^+$ T cells are the main cell reservoir harboring replication-competent, yet transcriptionally quiescent HIV-1. Though HIV-1 latency is a rare event, primarily because the majority of HIV-1 proviruses are preferentially integrated in actively transcribed host genes, establishment of this replication competent but transcriptionally quiescent provirus does occur and is a significant barrier to a functional cure of HIV-1. Blimp-1 is a DNA-binding transcription factor that recruits multiple chromatin-modifying complexes, such as HDACs and methyltransferases, to targeted promoters. Blimp-1 is a key maturation factor of T cells and is upregulated in chronically HIV-1-infected patients. Together, these findings lead us to hypothesize that Blimp-1

binds to the HIV-1 LTR, inhibits HIV-1 transcription and contributes to HIV-1 latency

(**Fig. 13**). A second hypothesis that is explored in this dissertation is that host promoters

proximal to the HIV-1 LTR in the integrated provirus are active but have minimal impact

on HIV-1 transcription.
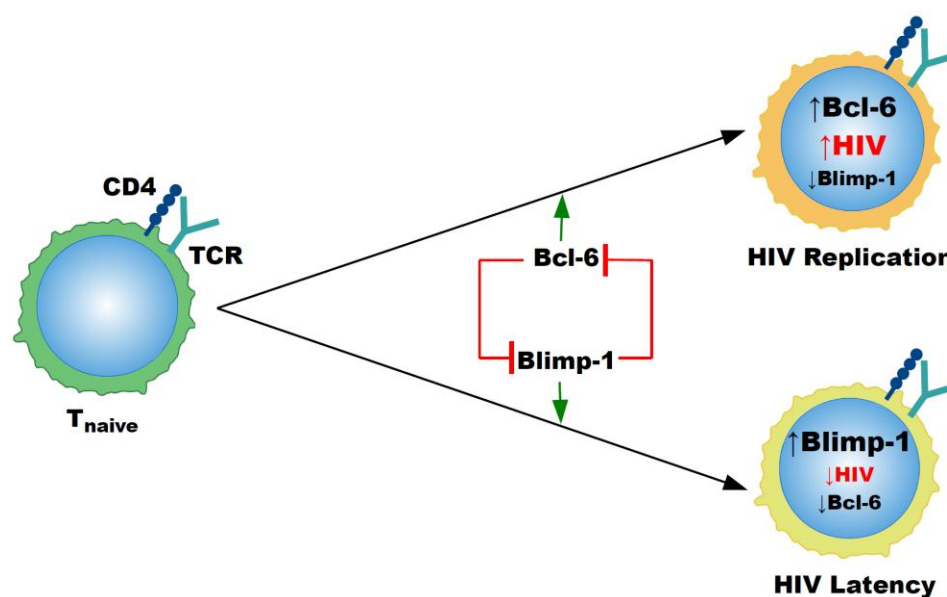
Figure 13. Model for Potential Role of Blimp-1 in HIV-1 Transcription.

Blimp-1 and Bcl-6 have potential binding sites in HIV--1 LTR. I hypothesize that Blimp-1, an antagonist of Bcl-6, contributes to paused RNAP II and thus to the establishment of HIV-1 latency. See text for details.

II. Materials and Methods

Cell Culture. Discarded deidentified tissues from otolaryngology surgeries performed at Boston Medical Center were mechanically separated and cultured on plastic plates for 2-3 d to eliminate adherent cells. Cells in suspension were positively selected for CD4$^+$ using the Dynabeads CD4 Positive Isolation Kit (Invitrogen). Peripheral blood mononuclear cells were isolated from whole blood by centrifuging through Histopaque gradient (Sigma-Aldrich). CD4$^+$ T cells were positively selected using the Dynabeads CD4 Positive Isolation Kit. Jurkat clone E6-1 was originally purchased from American Type Culture Collection (ATCC, Manassas, VA). Primary CD4$^+$ T cells and Jurkat cells were propagated in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin (P/S), and 0.2 M L-glutamine. Human embryonic kidney 293T cells (HEK293T) were purchased from ATCC and cultured in Dulbecco's modified Eagle's medium containing 10% FBS and P/S. Inducible Jurkat cell lines were a kind gift from Dr. Olaf Kutch and were previously described (Duverger et al., 2009). Cells were incubated in a 37°C humidified incubator with 5% CO$_2$. Cells were either left untreated, or activated with 0.1 μg/ml anti-human CD3 (BD Biosciences) and 1.0 μg/ml anti-human CD28 (BD Biosciences) for 30 min. 5 μg/ml of goat anti-mouse antibody (Sigma) was added to crosslink the receptors. T cells were harvested 24 h post-stimulation.

Polychromatic flow cytometry. CD4$^+$ T cells were isolated from whole blood by negative selection using RosetteSep^{TM} Human CD4$^+$ T Cell Enrichment Cocktail (STEMCELL Technologies). To sort T cell subsets cells were stained with CD3-

PacificBlue (BD Biosciences), CCR7-PE-Cy7 (BioLegend), CD45RA-PE-Cy5.5

(Invitrogen), CD27-PE (BD Biosciences), CD4-APC (BioLegend). T cell subsets were

separated with FACSAria.

Transfections, Virus Generation and Infections. HIV-1 LTR/GLS-luciferase reporter

constructs with an intact or mutated (G<u>AAA</u>GCG<u>AAA</u>G mutated to G<u>CCC</u>GCG<u>CCC</u>G)

ISRE or NL4-3ΔTat-luciferase, Blimp-1, Tat, RSV LTR-LUC (Yamamoto et al., 1980),

FIP-LUC(Meiering et al., 2001) and DHFR-LUC (Gummuluru and Emerman, 1999)

expression constructs were transiently transfected into HEK293T cells via calcium

phosphate transfection as described previously (Natarajan et al., 2013). The RSV-LUC,

FIP-LUC and DHFR-LUC were generously provided by Dr. S. Gummuluru, Boston

University School of Medicine. Luciferase assays were performed 48 h post-transfection

using Luciferase Assay System (Promega).

Lentiviral vectors pNL4-3-Luc(+)Env(−)Nef(−) (Henderson et al., 1995) (obtained from

NIH AIDS Research and Reference Reagent Program), Blimp-1 shRNA (Dharmacon),

FUGW Blimp-1 (kindly provided by Dr. Kathryn Calame, Columbia University, New

York), NELF shRNA (Dharmacon) were packaged by cotransfecting Tat, RSV-Rev,

Gag/Pol and VSV-G into HEK293T cells using calcium phosphate as previously

described (Natarajan et al., 2013). HIV-1 titers were determined using a p24 ELISA

(PerkinElmer). Viruses were collected 48 h post-transfection and filtered through a

Puradisc 25 Syringe Filter with 0.45-μm Polyethersulfone membrane (Whatman). Jurkat

cells were infected by culturing with supernatants containing HIV-1-LUC or lentiviral

constructs for 12–16 h. CD4^{+} T cells were activated with 2μg/mL PHA and 10ng/mL

PMA for 16 h, rested for 12 h and infected by culturing with HIV-1-containing

supernatant or spinoculated with HIV-1-LUC supernatant plus 1 µg/ml polybrene for 1.5

h at 1,200 ×g without prior activation described by O'Doherty et al (O'Doherty et al.,

2000).

Quantitative RT-PCR. RNA was prepared by resuspending cells in TRIzol (Life

Technologies), and cDNA was generated using SuperScript II Reverse Transcriptase

(Invitrogen) and random primers (Promega). GoTaq qPCR Master Mix (Promega) was

used for quantitative real-time PCR reaction. Blimp-1 transcripts (+2074 to +2372) were

amplified using 5'-CAGCTCGCCCACCTGCAGAA-3' and 5'-

GCCGCAGCGCAGTTCCCTTT-3' primers. Initiated HIV-1 transcripts (+1 to +40)

were amplified using 5′- GGGTCTCTCTGGTTAGA-3′ and 5′-

AGAGCTCCCAGGCTCA-3′ primers and elongated HIV-1 transcripts (+5396 to +5531)

were amplified using 5′-GACTAGAGCCCTGGAAGCA-3′ and 5′-

GCTTCTTCCTGCCATAGGAG-3′ primers as described previously (Natarajan et al.,

2013). β-actin mRNA was amplified using a QuantiTect primer assay (Qiagen). PCR was

carried out for 45 cycles, and the relative expression was calculated using the $\Delta\Delta Ct$

method (Livak and Schmittgen, 2001), normalizing specific amplification of the

transcripts of interest to the β-actin control for each specific sample. The product detected

in the sh-Control was a calibrator, and the transcript levels in samples were calculated as

fold changes in comparison to sh-Control.

Immunoblot Analysis and Antibodies. Whole-cell lysates were prepared by washing

cells with cold PBS and lysing them with buffer containing 10 mM Tris-Cl (pH 7.4), 150

mM NaCl, 1.0 mM EDTA (pH 8.0), 2.0 mM sodium vanadate, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 1% Triton X-100, 1.0 mM phenylmethylsulfonyl fluoride, and protease inhibitor mixture III (Calbiochem). Protein was measured using the BSA assay (Pierce). Samples were heated for 5 min at 100 °C before loading onto a 10% SDS-PAGE gel. Proteins were transferred to a polyvinylidene difluoride membrane (Millipore) by electroblotting. Antibodies used were as follows: anti-Blimp-1 serum (kindly provided by Dr. Kathryn Calame, Columbia University, New York), anti-β-actin (Sigma-Aldrich), anti-Tat (4138; NIH AIDS Research and Reference Reagent Program), anti-Sp1 (Upstate), anti-IRF-1 (Santa Cruz Biotechnology), anti-IRF-8 (Santa Cruz Biotechnology), anti-RNAP II (Santa Cruz Biotechnology), anti-AcH3 (Upstate Biotechnology) and rabbit IgG (Upstate Biotechnology).

ChIP-qPCR. Chromatin immunoprecpitations were performed as previously described (Natarajan et al., 2013). Quantitative real-time PCR analysis was carried out using SYBR green reagents and the primers 5′-GACTTTCCGCTGGGGACTTTC-3′ and 5′-CTAACCAGAGAGACCCAGTAC-3′, which amplify the −102 to +16 region of HIV-1 LTR, 5'-CTGGGAGCTCTCTGGCTAACTA-3'and 5'-TTACCAGAGTCACACAACAGACG-3', which amplify the +30 to +134 region of HIV-1, 5′-TCCCTCAGACCCTTTTAGTCAG-3′ and 5′-GTCGAGAGAGCTCCTCTGGTTT-3′, which amplify the +142 to +237 region of HIV-1, and 5'-ACAGTACTGGATGTGGGTGATG-3'and 5'-AATCCCTGGTGTCTCATTGTTT-3', which amplify the +2415 to +2522 region of provirus.

Statistical analysis. Statistical analysis was carried out using Student t test. A two-tailed distribution was performed on paired samples. Values of <0.01 were considered significant.

III. Blimp-1 Represses HIV-1 Transcription in Memory CD4^{+} T Cells

1. Introduction

Blimp-1 is a key regulator of T cell differentiation and reciprocal inhibitor of Bcl-6. Bcl-6 supports differentiation of T_{FH}, a cell subset that supports robust HIV-1 replication (Lindqvist et al., 2012; Perreau et al., 2013). In CD4^{+} T cells Blimp-1 represses transcription of IL-2 gene (Martins et al., 2008) whose promoter share common cis-elements with HIV-1 LTR. Recent studies have shown that Blimp-1 is expressed at high levels in CD4^{+} T cells from HIV-1-infected patients and that it is induced in T cells by stimulation with HIV-1-pulsed DCs (Che et al., 2012; de Masson et al., 2014; Seddiki et al., 2013; Shankar et al., 2011). Therefore, it is important to decipher the role that Blimp-1 plays in HIV-1 infection

In this chapter I show that Blimp-1 binds downstream of HIV-1 LTR to a region called ISRE and represses Tat-dependent and Tat-independent HIV-1 transcription. Blimp-1 favors closed chromatin structure at Nuc-1 by inhibiting its acetylation. Blimp-1 contributes to the accumulation of short HIV-1 mRNA transcripts and decreases RNAP II

processivity. I also show that Blimp-1 contributes to the development and maintenance of

HIV-1 latency in memory CD4$^+$ T cells, the main reservoir of latent HIV-1.

2. Results

2.1. Blimp-1 is Expressed in Primary Human CD4$^+$ Cells Including Memory CD4$^+$ T Cells.

Although Blimp-1 expression has been characterized in murine T cells I wanted to

confirm that it shared a similar expression pattern in human primary CD4$^+$ T cells. CD4$^+$

T cells were isolated from tonsils, a rich source of follicular helper T cells (T$_{FH}$), and

from peripheral blood. Blimp-1 mRNA was detected by qRT-PCR and protein with

immunoblots. Consistent with previous reports (Johnston et al., 2009) tonsillar CD4$^+$ T

cells expressed low levels of Blimp-1 compared to the CD4$^+$ T cells isolated from whole

blood (**Fig. 14A**). Activation with anti-CD3 and anti-CD28 antibodies resulted in 3-fold

increase in Blimp-1 mRNA and protein levels (**Fig. 14B and C**).

I also examined Blimp-1 expression in different memory CD4$^+$ T cell populations

obtained from peripheral blood. Flow cytometry based on CD45RA, CD27 and CCR7

expression was used to enrich for naïve T cells (T$_N$; CD4$^+$CD3$^+$CD45RA$^+$), central

memory T cells (T$_{CM}$; CD4$^+$CD3$^+$CD45RA$^-$CCR7$^+$CD27$^+$), transitional memory T cells

(T$_{TM}$; CD4$^+$CD3$^+$CD45RA$^-$CCR7$^-$CD27$^+$) and effector memory T cells (T$_{EM}$;

CD4$^+$CD3$^+$CD45RA$^-$CCR7$^-$CD27$^-$) (**Fig. 14D**). Although T$_{CM}$ have been implicated as

the primary cell type that is latently infected, recent reports suggest that T_{EM} and T_{TM} contribute to the latent HIV-1 reservoir (Bacchus et al., 2013; Chomont et al., 2009; Lassen et al., 2012). Blimp-1 mRNA was measured in memory cells by qRT-PCR and protein levels were determined by immunoblots. Blimp-1 expression was 10-fold higher in long-lived T_{CM} compared to naïve T cells, whereas, even higher levels of Blimp-1 was observed in T_{TM} and T_{EM} (**Fig. 14E, F**). Overall, Blimp-1 is present in multiple T cell populations but is expressed at significantly higher levels in CD4$^+$ T memory cells found within the latent reservoir.
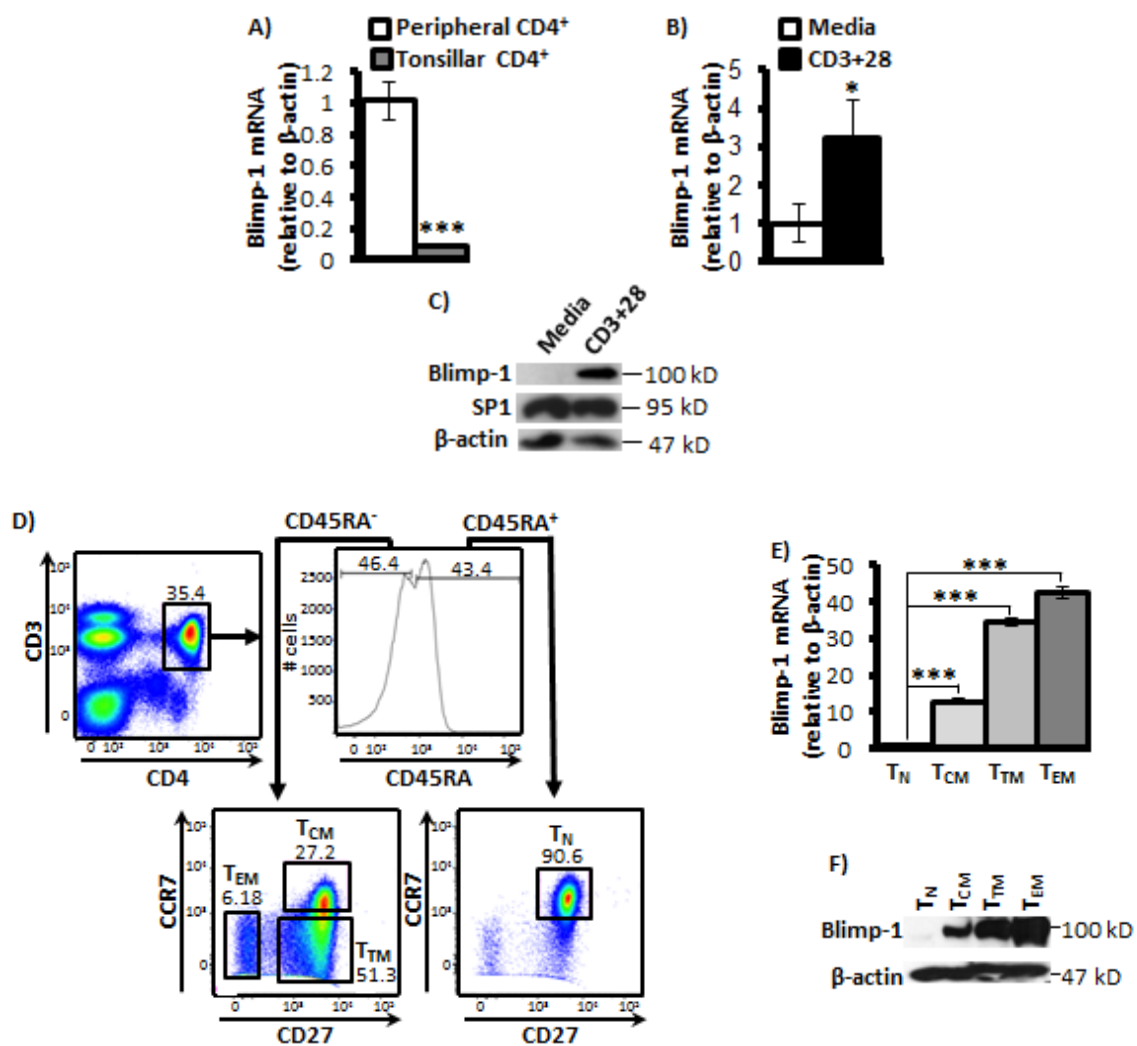
Figure 14. Blimp-1 is Expressed in CD4$^+$ Memory T Cells.

A) CD4$^+$ cells were isolated from peripheral blood and tonsils and Blimp-1 was measured by qRT-PCR. B) Blimp-1 levels were measured by qRT-PCR in unstinmulated or CD3 + CD28 stimulated primary peripheral CD4$^+$ T cells. β-actin was used as a reference gene to normalize values. C) Immnoblots of lysates from resting and CD3 + CD28 activated primary peripheral CD4$^+$ T cells for Blimp-1, Sp1 and β-actin. Sp1, a transcription factor, and β-acting served as loading controls. D) Sorting strategy and staining profiles for T cell subsets. T cells were stained with Pacific Blue–labeled antibody to CD3, APC–labeled antibody to CD4, PE-Cy7–labeled antibody to CCR7, PE-Cy5.5-labeled antibody to CD45RA and PE-labeled antibody to CD27. T cell subsets were separated by FACSAria into naïve T cells (T$_N$; CD4$^+$CD3$^+$CD45RA$^+$), central memory T cells (T$_{CM}$; CD4$^+$CD3$^+$CD45RA$^-$CCR7$^+$CD27$^+$), transitional memory T cells (T$_{TM}$; CD4$^+$CD3$^+$CD45RA$^-$CCR7$^-$CD27$^+$) and effector memory T cells (T$_{EM}$; CD4$^+$CD3$^+$CD45RA$^-$CCR7$^-$CD27$^-$). E) and F) Expression of Blimp-1 in sorted memory CD4$^+$ T cell populations measured by E) qRT-PCR using β-actin as a reference gene and by F) immunoblots. These experiments represent CD4$^+$ cells obtained from at least three healthy individuals. Bars show average values ±SD, n=3. *p < 0.05 and ***p < 0.001 (Student's t test).

2.2. Blimp-1 Binds to the ISRE Element Downstream of the 5' HIV-1 LTR in Primary CD4$^+$ Cells

Based on the transcriptional repressor function of Blimp-1 and my data showing its high expression in CD4$^+$ memory T cells I hypothesized that Blimp-1 limits HIV-1 expression in these cells. To determine if Blimp-1 regulates HIV-1 LTR activity I performed co-transfection experiments with Blimp-1 and an HIV-1 LTR reporter. Blimp-1 inhibited HIV-1 LTR-driven luciferase reporter gene (HIV-1 LTR-LUC) by 67% when overexpressed in HEK293T cells (**Fig. 15A**). Overexpression of the HIV-1 transcriptional activator, Tat, was unable to rescue HIV-1 LTR-mediated transcription in the presence of Blimp-1 (**Fig. 15A**). In addition, Blimp-1 inhibited an HIV-1 clone that lacked Tat (ΔTat-

HIV-1-LUC) by only 30% but repressed transcription by greater than 70% when Tat was added back by co-transfection (**Fig. 15B**). Blimp-1 is not acting as a general repressor in these experiments since it failed to repress expression driven by Rous sarcoma virus LTR (RSV LTR) (Yamamoto et al., 1980) and foamy virus internal promoter (FIP) (Meiering et al., 2001) and even activated the dihydrofolate reductase (DHFR) (Gummuluru and Emerman, 1999) promoter (**Fig. 15C**). These data indicate that Blimp-1 inhibits Tat-dependent HIV-1 transcription.
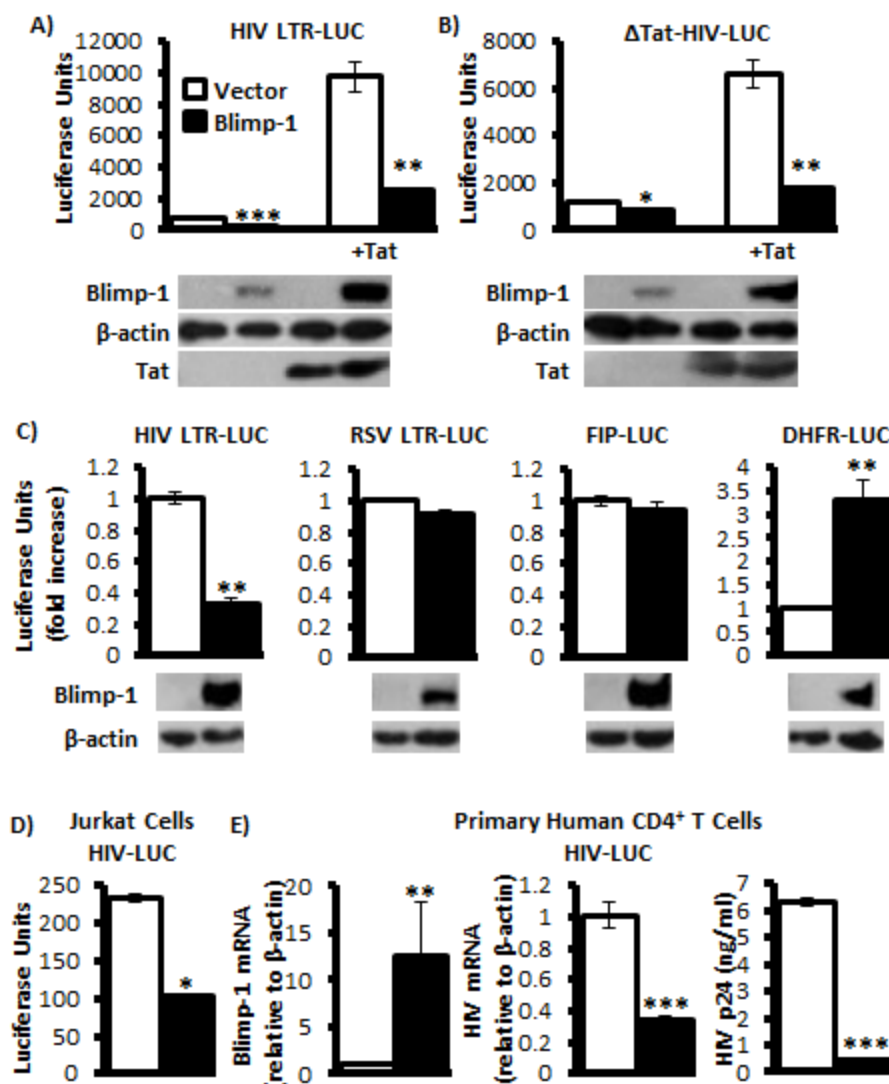
Figure 15. Blimp-1 Represses Tat-dependent HIV-1 Transcription.

A) and B) HEK293T cells were transfected with viral clones HIV--1 LTR-LUC containing the TAR element (A) or ΔTat-HIV-1-LUC (B) and vector control or Blimp-1 in the absence or presence of Tat. Luciferase assays and western blot analyses were performed 48 h post-transfection. (C) HEK293T cells were transfected with HIV--1 LTR-LUC, RSV LTR-LUC, FIP-LUC, DHFR-LUC and vector control or Blimp-1. Luciferase assays and western blot analyses were performed 48 h post-transfection. (D) Jurkat T cells stably transduced with Blimp-1 lentivirus were infected with HIV-1. 48 h post-infection cells were lysed and luciferase activity was measured (E) 16 h post-HIV-1 infection primary CD4$^+$ cells were transduced with empty lentiviral vector or with vector expressing Blimp-1. 72 h post-transduction expression of Blimp-1 and HIV-1 were measured by qRT-PCR. HIV-1 released into supernatants was assessed by measuring p24 by an ELISA. These experiments were performed in triplicates and the data represent at least three independent experiments. Experiments with primary cells were performed with cells from at least three different people. White bars indicate vector control; black bars indicate Blimp-1 lentiviral vector. Bars show average values ±SD, n=3. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ (Student's t test).

I explored whether Blimp-1 regulates HIV-1 expression in CD4$^+$ T cells. I established a Jurkat T cell line that stably overexpressed Blimp-1 and infected it with a single cycle env-minus HIV-1-luciferase virus (HIV-1-LUC). HIV-1 transcription, as measured by the luciferase assay, was decreased by 55% in cells overexpressing Blimp-1 (**Fig. 15D**). In addition, primary human CD4$^+$ T cells were infected with a HIV-1-LUC virus (Henderson et al., 1995) and Blimp-1 was overexpressed using a lentiviral vector. Transduction of cells with Blimp-1 lentivirus resulted in over 13-fold increase in Blimp-1 mRNA (**Fig. 15E**). Cells overexpressing Blimp-1 had 65% decrease in HIV-1 transcription measured by HIV-1 mRNA levels (**Fig. 15E**) and over 90% inhibition of HIV-1 replication measured by HIV-1 p24 ELISA (**Fig. 15E**). The data from Jurkat T cells and primary human CD4$^+$ T cells confirmed that Blimp-1 is a repressor of HIV-1 transcription.

HIV-1 provirus has four putative Blimp-1 binding sites, GAAAG, with two sites upstream of the transcription start site that overlap the NF-κB sites (-104 to − 80 bp) and a second set of sites located downstream of the transcriptional start site in an interferon sensitive response element (ISRE; +200 to +218 bp) (Liang et al., 1997) (**Fig. 16A**). Both regions have been reported to modulate HIV-1 transcription (Liang et al., 1997; Nabel and Baltimore, 1987). Mutating the HIV-1-ISRE ameliorated Blimp-1-mediated repression of HIV-1 following co-transfection into HEK293T cells (**Fig. 16B**). Furthermore, chromatin immunoprecipitations (ChIPs) demonstrate that Blimp-1 directly binds HIV-1 provirus. Chromatin was prepared from $CD4^+$ T cells infected with HIV-1 and protein-DNA complexes were enriched with Blimp-1-specific antibody. ChIPs show modest Blimp-1 binding at the -104/-80 bp site and 9-fold higher Blimp-1 binding at the +200/+218 bp ISRE site (**Fig. 16C**). Following T cell activation, Blimp-1 binding was not detected at the HIV-1 ISRE element despite increases in Blimp-1 mRNA and protein (**Fig. 16C, Fig. 14B and C**). Blimp-1 did not exclude binding of interferon regulatory factors (IRFs) IRF-1 and IRF-8 to the HIV-1 ISRE (**Fig. 16D**). These results suggest that Blimp-1 represses HIV-1 transcription by directly binding the proviral ISRE element.

A)

-102F   +16R

p50 p65

HIV LTR   TAR   nuc-1   ISRE

+142F   +237R

-104   -80   +200   +218

GGGACTTTCCGCTGGGGACTTTCA   TTGAAAGCGAAAGGGAAAC

mISRE: TTGCCCGCGCCCGGGAAAC

B)

Luciferase Units (fold increase)

□ Vector
■ Blimp-1

WT HIV LTR   Tat   mISRE HIV LTR   Tat

Blimp-1

β-actin

Tat

C)

Percent Input

□ Media
■ CD3+28

Ctrl IgG   Blimp-1   Ctrl IgG   Blimp-1

Primer Set -102 F/+16 R   +142 F/+237 R

D)

Percent Input

□ Media
■ CD3+28

Ctrl IgG   Blimp-1   IRF-1   IRF-8
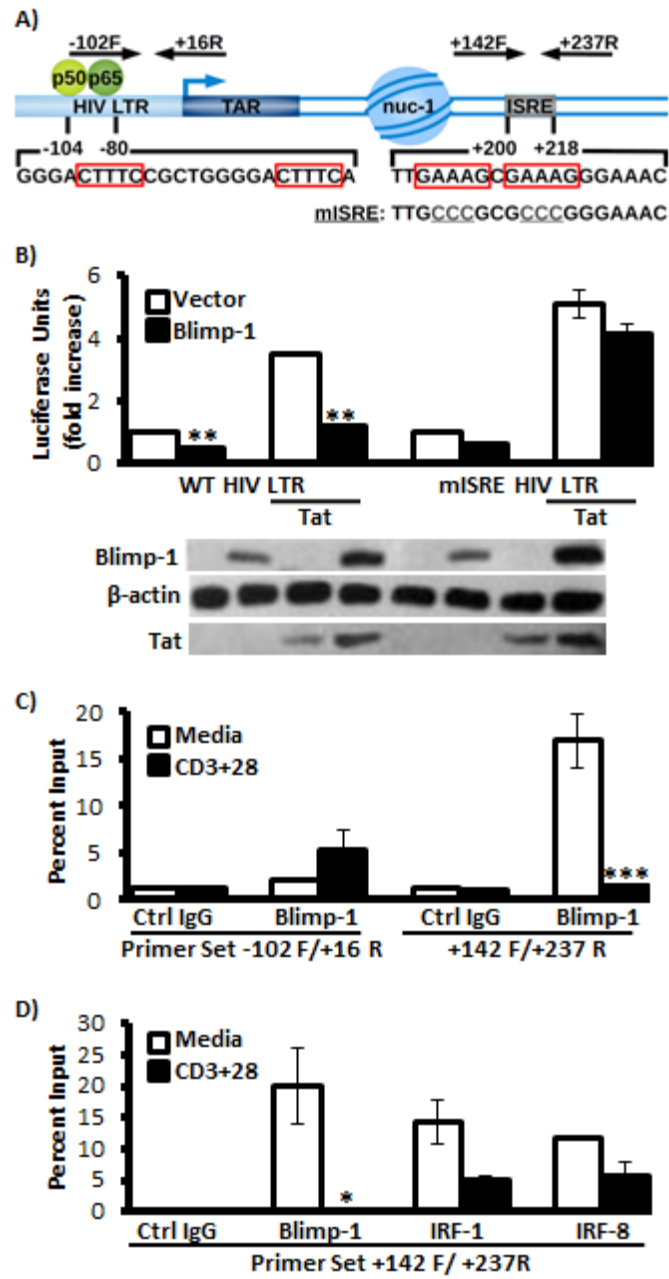
Primer Set +142 F/ +237R

Figure 16. Blimp-1 Binds HIV-1 Provirus.

(A) The location of HIV--1 LTR, four putative Blimp-1-binding sites and ISRE mutations in provirus. (B) HEK293T cells were transfected with HIV--1 LTR-LUC or mISRE-HIV--1 LTR-LUC and control vector or Blimp-1 in the absence or presence of Tat. Luciferase assays and western blot analyses were performed 48 h post-transfection. (C, D) 96 h post-HIV-1 infection primary human CD4$^+$ cells were activated with anti-CD3 and anti-CD28 antibodies for 24 h and ChIPs were performed using anti-Blimp-1, anti-IRF-1, anti-IRF-8 or anti-rabbit antibody. Binding was detected with -102F/+16R and +142F/+237R HIV-1 primer sets. These data are performed in triplicate and represent at three independent experiments. Bars show average values ±SD, n=3. *p < 0.05, **p < 0.01 and ***p < 0.001 (Student's t test).

The above gain of function experiments demonstrated that Blimp-1 represses HIV-1 expression. To determine if Blimp-1 limits HIV-1 expression in the context of primary cells I reduced Blimp-1 expression in primary CD4$^+$ T cells using shRNA. I infected CD4$^+$ T cells with HIV-1-LUC, transduced the infected cells with sh-Blimp-1 lentivirus and monitored HIV-1 expression. The efficacy of Blimp-1 knock-down was confirmed 96 h post-transduction by qRT-PCR (**Fig. 17A**) and immunoblots (**Fig. 17B**). Diminishing Blimp-1 increased basal HIV-1 transcription and replication as measured by qRT-PCR (**Fig. 17C**), luciferase assay (**Fig. 17D**) and p24 ELISA (**Fig. 17E**) indicating that Blimp-1 limits HIV-1 transcription in primary CD4$^+$ T cells. Activating T cells through CD3+CD28 following Blimp-1 knockdown did not further induce HIV-1 transcription and actually decreased HIV-1 transcription by 80% suggesting that Blimp-1 was required for optimal induction of HIV-1 transcription (**Fig. 17C**). These data indicate that Blimp-1 acts as both a repressor and transcriptional activator in the context of HIV-1-infected primary T cells. However, the ability of Blimp-1 to activate HIV-1

transcription is independent of Blimp-1 binding to provirus since ChIPs show it is displaced following T cell activation (**Fig. 17C**).

To gain insight into how Blimp-1 limits HIV-1 transcription I examined with ChIPs the distribution of RNA Polymerase II (RNAP II) on the HIV-1 genome. In cells expressing Blimp-1 there was an accumulation of RNAP II at the transcriptional start site and modest amounts of RNAP II downstream suggesting that Blimp-1 leads to RNAP II promoter proximal pausing (**Fig. 17F**). Diminishing Blimp-1 reduced RNAP II at the promoter and increased RNAP II downstream in the provirus by 3-fold consistent with RNAP II release and greater processivity (**Fig. 17F**). I also used qRT-PCR to measure initiated versus elongated transcripts. In control cells there was an accumulation of initiated short HIV-1 mRNA but low expression of full length mRNA (**Fig. 17G**) as would be expected with RNAP II pausing (Zhang et al., 2007). Decreasing Blimp-1 with shRNA altered the ratio of initiated to elongated HIV-1 mRNA so that the ratio was approximately one indicating processive transcriptional elongation (Fig 17G). Furthermore, knocking down Blimp-1 led to an increase in histone H3-acetylation (AcH3) at the positioned nucleosome (nuc-1) (**Fig. 17H**). These data suggest that Blimp-1 targets multiple steps of transcription regulation to limit HIV-1 transcriptional elongation.
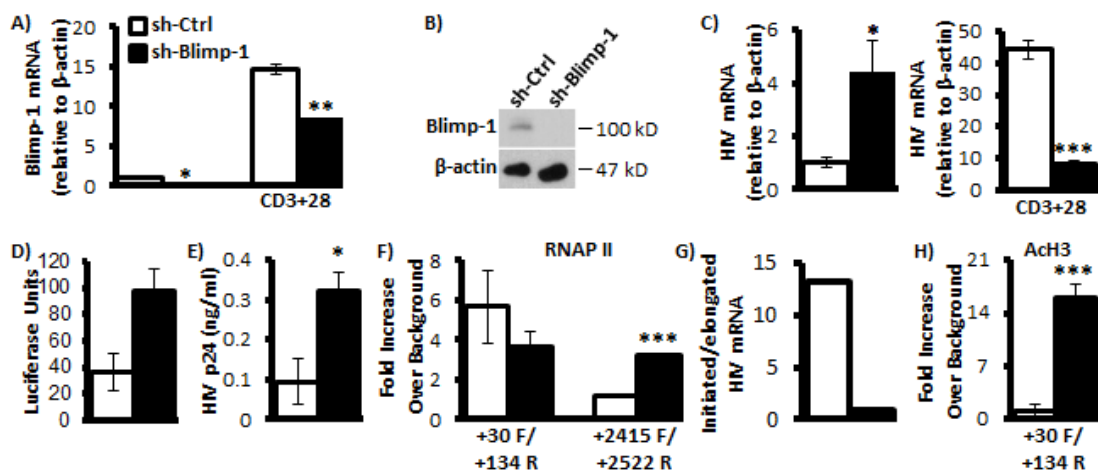
Figure 17. Blimp-1 Represses HIV-1 Transcription in Resting CD4⁺ T Cells.

HIV-1-infected primary CD4⁺ cells were transduced with sh-Ctrl and sh-Blimp-1. 72 h post-knockdown cells were activated with anti-CD3 and anti-CD28 antibodies for 24 h. Expression of Blimp-1 was measure by A) qRT-PCR and B) immunoblots. HIV-1 expression was assayed by qRT-PCR using primers for (C) elongated HIV-1 mRNA and (G) initiated short transcripts, D) luciferase assay and E) HIV-1 p24 ELISA. ChIP analysis used anti-rabbit and anti-RNAP II (F) oranti-AcH3 (H) antibody and +30F/+134R and +2415F/+2522R HIV-1 primer sets. These experiments were performed in triplicate and the data are representative of at least three independent experiments. White bars indicate sh-control; black bars indicate sh-Blimp-1. Bars show average values ±SD, n=3. *p < 0.05, **p < 0.01 and ***p < 0.001 (Student's t test).

Blimp-1 was most highly expressed in memory CD4⁺ T cells which do not support efficient HIV-1 transcription. To examine whether Blimp-1 was limiting HIV-1 transcription in these cells I infected isolated memory CD4⁺ T cells with HIV-1 by spinoculation (O'Doherty et al., 2000) which has been shown to facilitate infection of cells without using mediators that activate T cells and then decreased Blimp-1 with sh-Blimp-1 lentivirus. Spinoculation enhances viral binding which increases infection of cells without activating stimuli and results in higher integration rates than infection of cells in the absence of spinoculation. Decreasing Blimp-1 levels in all three CD4⁺ T cell

memory populations resulted in significant increases, 2 to 12-fold, in HIV-1 transcription
(**Fig. 18B**). The levels of induction correlated with the efficiency of Blimp-1 knockdowns
observed with more modest induction in T_{CM} and T_{EM} cells in which Blimp-1
knockdowns were inefficient compared to robust induction in T_{TM} where Blimp-1
expression was decreased by greater than 80% (**Fig. 18A**). These findings support a
model in which Blimp-1 expression correlates with limited basal HIV-1 transcription in T
memory cells.
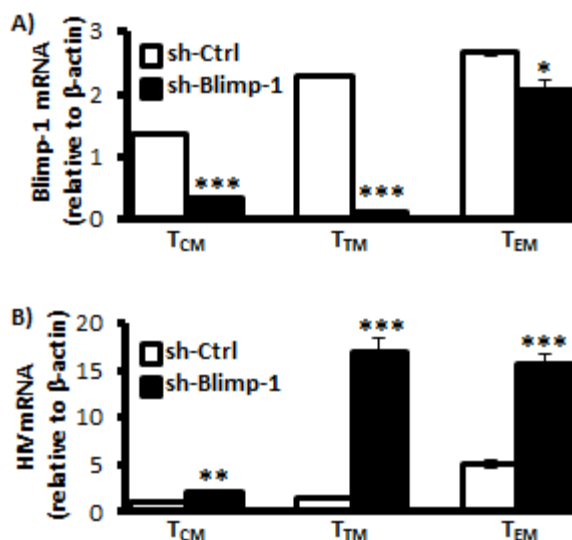
Figure 18. Blimp-1 Represses Basal HIV-1 Transcription in Primary Memory CD4$^+$ T Cells.

T_{CM}, T_{TM} and T_{EM} sorted as described above were infected with NL4-3 HIV-1 by spinoculation. 16 h post infection cells were transduced with sh-Ctrl and sh-Blimp-1. 72 h post-knockdown mRNA was collected. Expression of (A) Blimp-1 and (B) HIV-1 was measured by qRT-PCR using β-actin as a reference gene. These experiments were performed in triplicate and are representative of three separate infections from T cells obtained from three patients. Bars show average values ±SD, n=3. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ (Student's t test).

2.3. T Cell Activation and Treatment with G9a and HDACs Inhibitors Do Not Abolish Blimp-1-Mediated HIV-1 Repression in Jurkat Cells

Posttranslational modifications of histones have been shown to regulate transcription of HIV-1. Trichostatin A (TSA), an inhibitor of class I and II HDACs, has been shown to increase HIV-1 transcription by favoring open chromatin structure (Van Lint et al., 1996). BIX01294, an inhibitor of the methyltransferase G9a, was also demonstrated to increase HIV-1 transcription and to reactivate latent HIV-1 (Imai et al., 2010). To gain insight into the mechanism by which Blimp-1 halts HIV-1 transcription I have treated HIV-1-infected Jurkat cells stably overexpressing Blimp-1 with TSA and BIX01294 and activated them with anti-CD3+28 Abs in the presence of the inhibitors. TSA induced HIV-1 transcription, however Blimp-1 was still able to repress it (**Fig. 19**). Treatment with BIX01294 did not induce HIV-1 transcription and Blimp-1 was able to repress HIV-1 transcription in not activated cells at the highest concentration (**Fig. 19**). In contrast to primary CD4$^+$ cells, the CD3+28 activation of Jurkat cell lines did not abolish Blimp-1 mediated repression of HIV-1 transcription highlighting the differences between the two cell types (**Fig. 19**). These results suggest that Blimp-1-mediated repression of HIV-1 transcription does not depend on HDACs and G9a. However, these experiments lack the appropriate controls and should be repeated in primary CD4$^+$ T cells in which Blimp-1 affects HIV-1 transcription in different manner than in Jurkat cell lines upon T cell activation.
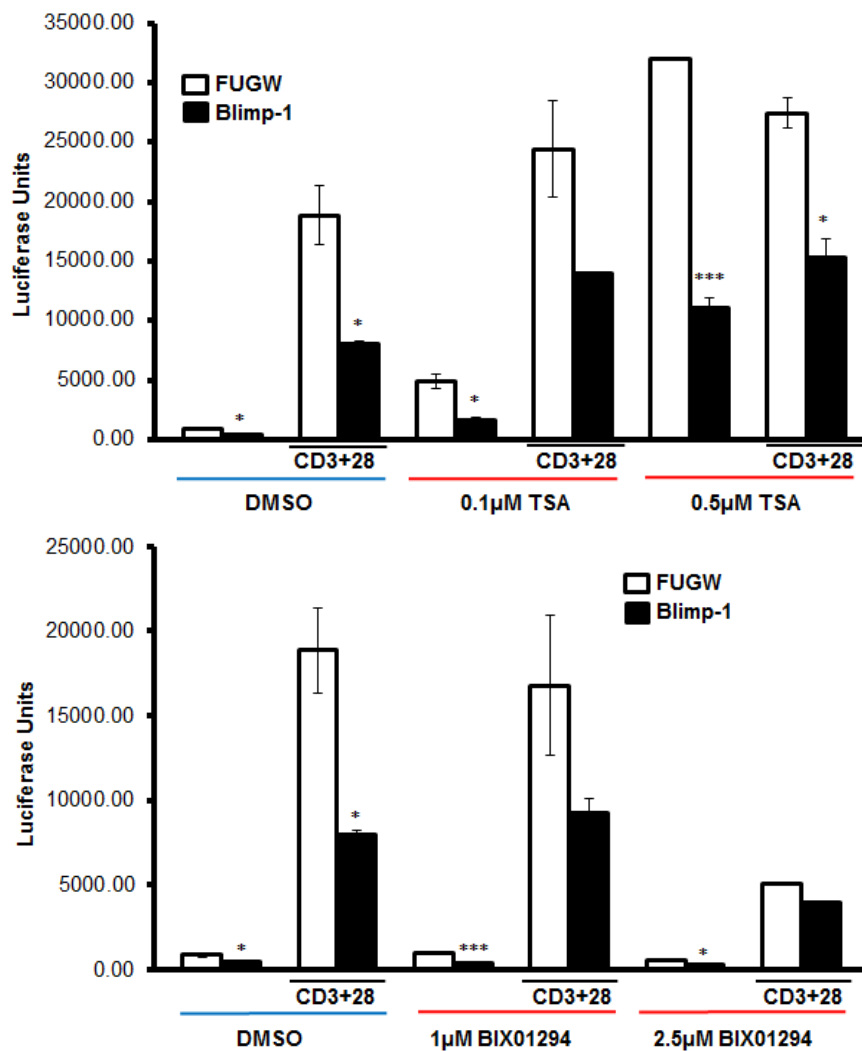
Figure 19. G9a and HDACs Inhibitors do not Inhibit Blimp-1-mediate Repression of HIV-1 Transcription.

Jurkat T cells stably over-expressing Blimp-1 were infected with HIV-1 LUC. 12 h post infection cells were pre-treated with different concentrations of TSA, BIX01294 or DMSO for 1 h. Cells were activated with anti-CD3+28 antibodies in the presence of inhibitors for 16 h. Cells were lysed and luciferase activity was measured. Bars show average values ±SD, n=3. *p < 0.05 and ***p < 0.001 (Student's t test).

3. Conclusions and Discussion

Memory $CD4^+$ T cells are a major reservoir of latent HIV-1 and their longevity and homeostatic proliferation prevents virus clearance and supports HIV-1 persistence. Although several mechanisms potentially inhibit HIV-1 transcription, T cell specific transcription factors that intrinsically program the maturation of $CD4^+$ memory T cells could contribute to the propensity of HIV-1 to become repressed in these cells. I present evidence that the lymphoid differentiation factor Blimp-1 limits HIV-1 transcription in $CD4^+$ memory T cell subsets.

Blimp-1 is expressed in a range of immune cells, such as B cells, macrophages, dendritic cells and T cells. I confirmed that Blimp-1 is differentially expressed in $CD4^+$ T cells, with low levels in T_N and $CD4^+$ tonsillar cells but elevated expression in cells that have been shown to contribute to the latent reservoir, T_{CM}, T_{TM} and T_{EM}. I also showed that Blimp-1 is induced upon T cell activation. Blimp-1 in part influences T cell maturation and function by regulating the expression of key lineage restricting transcription factors including PAX5, STAT6, Bcl-6 and T-bet (Cimmino et al., 2008; Lin et al., 2002; Shaffer et al., 2002). Furthermore, Blimp-1 is required for robust CD4 and CD8 T cells antiviral responses against influenza and lymphocytic choriomeningitis virus (LCMV) and mediates murine gamma herpesvirus latency in splenocytes (Hua et al., 2013; Kallies et al., 2009; Rutishauser et al., 2009; Shin et al., 2009; Siegel et al., 2010). Blimp-1 is associated with increased expression of inhibitory receptors and decreased polyfunctionality of exhausted $CD4^+$ T cells during LCMV infection

(Crawford et al., 2014). In T_H1 Blimp-1induces the expression of IL-10, a cytokine that suppresses anti-viral T cell responses, while in CD8$^+$ T cells Blimp-1-mediated repression of CD25 and CD27 decreases their survival and differentiation into memory cells during LCMV infection (Parish et al., 2014; Shin et al., 2013). During *Toxoplasma gondii* infection Blimp-1 suppresses inflammation which increases survival rate (Neumann et al., 2014). In chronically infected HIV-1 patients Blimp-1 is elevated in CD4$^+$ T cells and correlates with an increase in T cell exhaustion markers (de Masson et al., 2014; Seddiki et al., 2013). This increase in Blimp-1 may be a direct result of infection since HIV-1-pulsed dendritic cells enhance Blimp-1 expression (Che et al., 2012; Shankar et al., 2011). My results demonstrate that Blimp-1 limits HIV-1 transcription in T memory cells consistent with its function as a transcriptional repressor in different T cell subsets. Blimp-1 is expressed in macrophages and dendritic cells and it may limit HIV expression and promote latency in them although this possibility has not been explored.

Blimp-1 binds a GAAAG consensus sequence which is also found in ISREs present in Blimp-1 regulated genes CIITA, IDO1 and IFN-λ1 (Barnes et al., 2009; Piskurich et al., 2000; Siegel et al., 2011b). Although HIV-1 provirus contains four putative Blimp-1-binding sequences (Kaczmarek et al., 2013), I detected Blimp-1 binding only at the HIV-1 ISRE element. This cis-element has been implicated in regulating HIV-1 transcription by recruiting IRF family proteins IRF-1 and IRF-8 (Sgarbanti et al., 2002). Blimp-1 binds this element in resting cells and is displaced following T cell activation. It is possible that Blimp-1 antagonizes or competes with the transcriptional activator IRF-1, although, I do not observe significant changes in IRF-1 or IRF-8 binding in the absence

or presence of Blimp-1 or in response to T cell activation. There is also a possibility that T cell activation results in post-translational modifications of Blimp-1, such as sumoylation, phosphorylation or ubiquitination.

Blimp-1 mediates repression of genes by recruiting epigenetic factors such as methyltransferases G9a, Prmt5 and LDS1, Groucho-proteins and histone deacetylases to promoters (Ancelin et al., 2006; Gyory et al., 2004; Ren et al., 1999; Su et al., 2009; Yu et al., 2000). LSD-1 and G9a limit HIV-1 transcription and are candidates for the repression observed in CD4^{+} memory T cells (Imai et al., 2010; Le Douce et al., 2012). I observed that decreasing Blimp-1 increases acetylation at nuc-1 and releases RNAP II pausing. Blimp-1 limiting transcription elongation is also consistent with the observation that Tat cannot rescue HIV-1 expression in the presence of Blimp-1 and suggests that it is targeting a step prior to transcription elongation and recruitment of P-TEFb which is mediated by Tat.

Blimp-1 can act as a transcriptional activator and is necessary for the induction of IL-10 and XBP-1 (Cretney et al., 2011; Iwasaki et al., 2013; Lin et al., 2002). I also observe that Blimp-1 can act as a repressor and trans-activator in co-transfections with select promoters and in primary CD4^{+} T cells. Intriguingly, Blimp-1 is required for efficient induction of HIV-1 transcription upon T cell activation. However, Blimp-1 does not occupy either set of binding sites following T cell activation despite increased Blimp-1 expression following CD3 + CD28 activation suggesting that Blimp-1 is not directly inducing HIV-1 transcription. The mechanism by which Blimp-1 induces HIV-1 transcription following activation is not clear and may reflect Blimp-1 interacting with

other transcription factors, or its ability to influence the expression of other host genes,

cytokines and restriction factors that regulate HIV-1 transcription. Another transcription

factor that functions in a similar manner is DSIF. Unphosphorylated DSIF functions as a

transcriptional repressor promoting RNAP II pausing at promoters (Yamaguchi et al.,

2002). Phosphorylation of DSIF by P-TEFb changes it to a transcriptional activator

(Peterlin and Price, 2006).

I propose a model in which Blimp-1 is highly expressed in memory CD4$^+$ T cells

which do not support robust HIV-1 replication. In resting cells Blimp-1 binds the HIV-1

ISRE and represses HIV-1 transcription elongation whereas upon T cell activation

Blimp-1 is released from HIV-1 provirus derepressing proviral transcription (**Fig. 20**). I

show that Blimp-1 is a transcriptional repressor of HIV-1 and its expression in memory

CD4$^+$ T cells makes them prone to HIV-1 latency. Understanding how Blimp-1 is

regulated and the transcriptional processes it coordinates to silence HIV-1 expression will

provide insights into the establishment and maintenance of the HIV-1 reservoir.
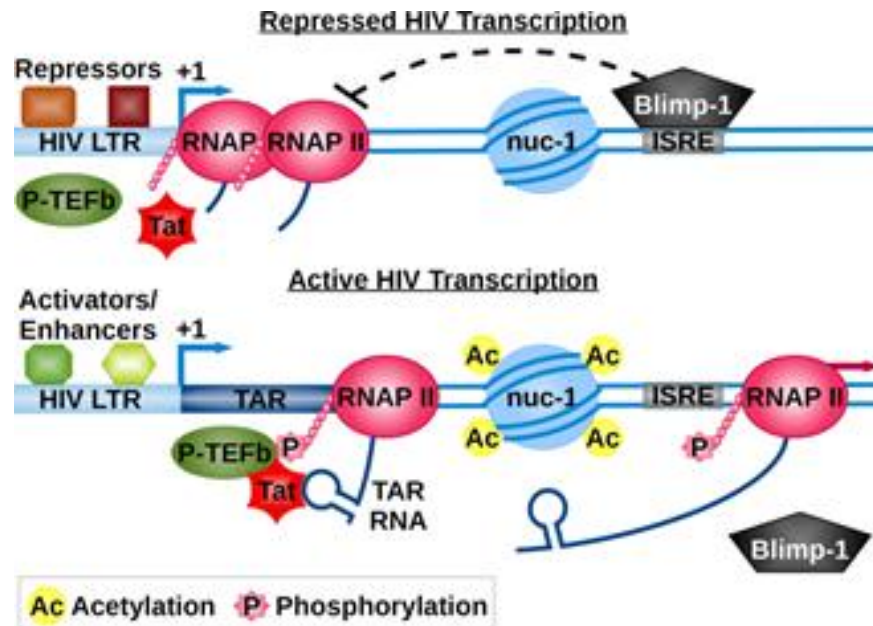
Figure 20. Model for the Role of Blimp-1 in HIV-1 Transcription.

Blimp-1 is highly expressed in memory CD4$^+$ T cells, binds the HIV-1 ISRE and inhibits Tat-dependent HIV-1 transcription. Following T cell activation Blimp-1 is released from HIV-1 provirus which correlates with increased RNAP II processivity, histone H3 acetylation and enhanced HIV-1 transcription.

IV. Neighboring Promoters and Their Influence on HIV-1 Transcription

1. Introduction

HIV-1 latency is regulated by binding of factors to HIV-1 LTR, chromatin structure, intrinsic T cell factors, as well as NELF-mediated stalling of RNAP II at the viral promoter. Transcription from host neighboring genes in which HIV-1 is integrated has been suggested to influence HIV-1 transcription and establishment of latency. However, although HIV-1 has been shown to integrate into transcriptionally active host genes, proviral latency is established. Therefore, I hypothesized that active host genes have minimal impact on HIV-1 transcription. To investigate the role of proximal host promoters on HIV-1 transcription I have used five different cell lines with inducible expression of HIV-1; one with parallel promoters, three with convergent promoters and one with provirus integrated between two host genes (**Fig. 21**). My results demonstrate that these cells are differentially responsive to reactivation induced by T cell activation. Four out of five inducible cell lines were minimally responsive to treatment with HDAC inhibitor indicating that chromatin remodeling agents are not a general mechanism of repression. Additionally, I show that transcription of host genes does not exclude transcription from a proximally integrated HIV-1 LTR, and that HIV-1 integration may lead to an increase in transcription of the neighboring host gene. The majority of the cells had accumulation of short HIV-1 mRNA transcripts and bound RNAP II and NELF at the repressed HIV-1 LTR. Decreasing NELF levels led to reactivation of provirus suggesting

that NELF-mediated RNAP II pausing is a general mechanism of proviral repression in these cells.

2. Results

2.1. Characterization of Inducible Cell Lines

The inducible Jurkat cell lines, CA5, BA1, 11B10, EF7 and CG3 were a kind gift from Dr. Olaf Kutsch and the mapping of integration and orientation of HIV-1 provirus in these cells was performed by Dr. Frank Wolschendorf. The inducible cell lines were generated by infecting Jurkat T cells with HIV-1-GFP, activating GFP-non-expressors with PMA+PHA and establishing clonal cell lines from cells in which PMA+PHA treatment induced GFP expression (Duverger et al., 2009).CA5 cells have provirus integrated in the exon of RNA binding motif protein 12 (RBM12) in a parallel orientation (**Fig. 21**). RBM12 has been implicated in meibomian cell carcinoma (Kumar et al., 2007). RBM12 shares promoter and 5'exons with copine I (CPNE1) (Yang et al., 2008a). The function of RBM12 remains unidentified, while CPNE1 plays role in cell-cycle and proliferation (Skawran et al., 2008). BA1 cells have HIV-1 integrated in the intron of PDZ domain containing 8 (PDZD8) in a convergent orientation (**Fig. 21**). PDZD8 is a cytoskeleton-regulating protein that binds HIV-1 Gag and stabilizes HIV-1 capsid contributing to enhanced reverse transcription (Guth and Sodroski, 2014; Henning et al.,

2010). PDZD8 is also a suppressor of Herpes Simplex Virus type-1 (HSV-1) replication (Henning et al., 2011). 11B10 cell line has HIV-1 inserted in the intron of HELZ in a convergent orientation (**Fig. 21**). HELZ is a zinc-finger containing RNA-helicase important for global translational initiation (Hasgall et al., 2011). HELZ interacts with RNAP II and histone methyltransferases, Smyd2 and Smyd3 (Diehl et al., 2010; Hamamoto et al., 2004). In EF7 cells HIV-1 is integrated in the intron of WHSC1 in a convergent orientation (**Fig. 21**). Wolf–Hirschhorn syndrome candidate 1 (WHSC1), also known as Multiple Myeloma SET domain (MMSET), is a histone H3 lysine 36 (H3K36) trimethyltransferase which together with the histone chaperone HIRA is implicated in incorporation of histone H3.3 into actively transcribed genes and which is required for IFN-induced transcription (Sarai et al., 2013). WHSC1 also interacts with Brd4 and P-TEFb facilitating transcriptional elongation (Sarai et al., 2013). In CG3 cell line provirus is integrated between two genes, tigger transposable element derived 5 (TIGD5) and PYCRL, with a parallel orientation to TIGD5 and a convergent orientation to PYCRL (**Fig. 21**). TIGD5 is a member of the tigger subfamily of the pogo superfamily of DNA-mediated transposons. PYCRL is cytosolic pyrroline-5-carboxylate reductase that catalyzes the reduction of ornithine to proline (De Ingeniis et al., 2012).
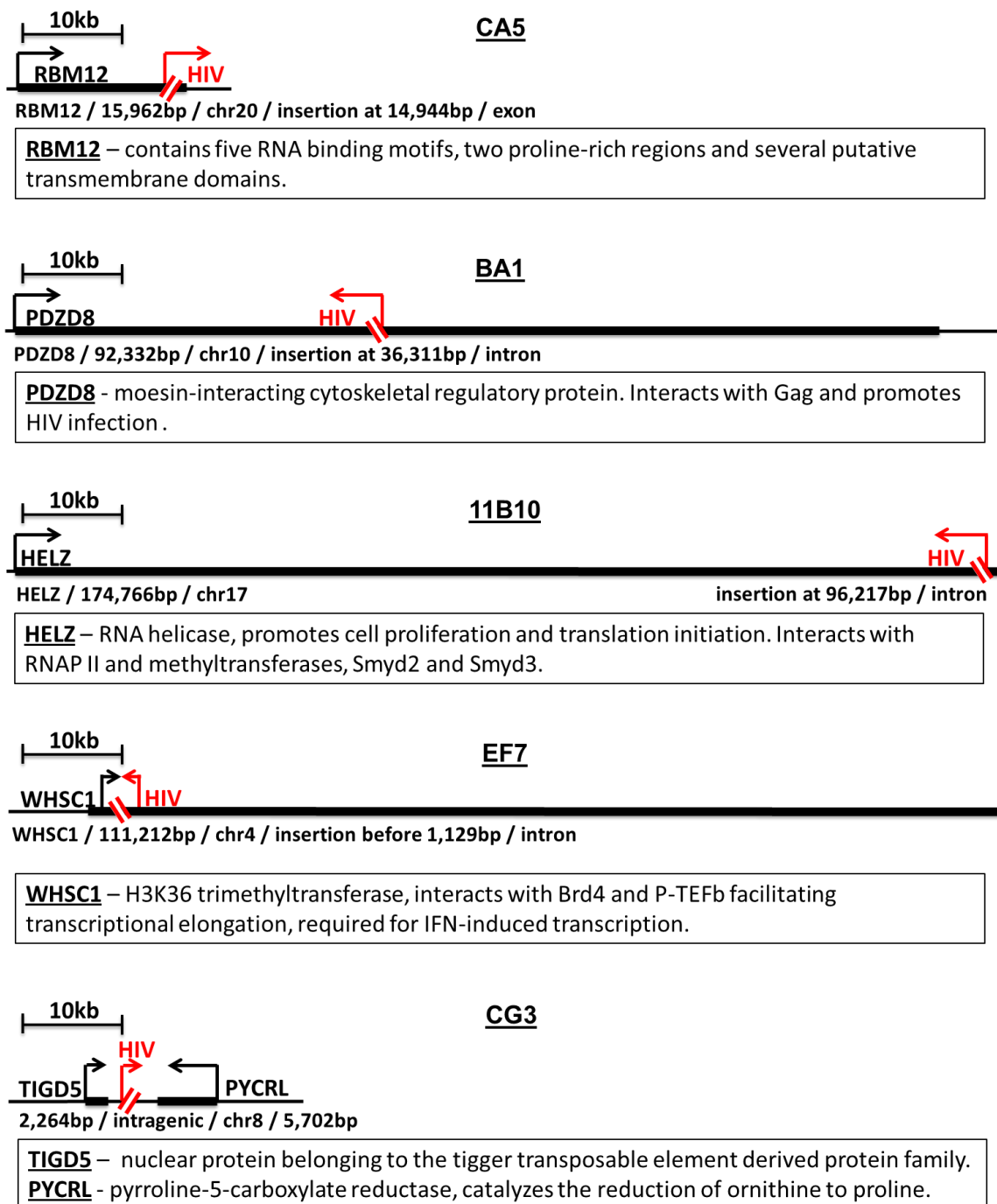
Figure 21. Proviral Integration Sites in CA5, BA1, 11B10, EF7 and CG3.

Schematic of HIV-1 provirus integration sites relative to neighboring host promoter for latently infected Jurkat cells (obtained from the Kutsh lab UAB).

2.2. Repressed HIV-1 is Differentially Inducible

Signals that activate T cells, such as signaling downstream of the CD3 and CD28 receptors as well as treatment with Phorbol 12-myristate 13-acetate (PMA), a phorbol ester and phytohemagglutinin (PHA), a plant lectin present in red kidney beans, lead to induction of HIV-1 transcription and effective replication (Peterlin and Price, 2006). Activation with PHA requires intact CD3/TCR and increases intracellular free Ca^{2++} (Weiss and Imboden, 1987) whereas PMA binds to and activates PKC (Manger et al., 1987). To see if cells harboring repressed proviruses with divergent integration sites are responding in the same manner to T cell activation stimuli, CA5, BA1, 11B10, EF7 and CG3 cell lines were treated with PMA+PHA. Results indicate that provirus induction levels in response to PMA+PHA treatment range from 90-30% among T cell lines with the CA5 cells being the most inducible and the CG3 cells (harboring intragenic HIV-1 provirus) being the least inducible (**Fig. 22 A**).

HDACs have been shown to contribute to the repression of HIV-1 transcription by removing acetyl groups from histones, making the chromatin more condensed (Keedy et al., 2009). To explore the contribution of chromatin structure in limiting HIV-1 expression I have treated cells with Trichostatin A (TSA), an inhibitor of the class I and II HDACs, which has been shown to be a potent inducer of HIV-1 transcription. The treatment with TSA was not as effective as treatment with PMA+ PHA at reactivating latent provirus (**Fig. 22 B**). TSA induced most HIV-1 expression in 11B10 and EF7 cells in which HIV-1 provirus and the host genes have convergent orientation (**Fig. 22 B**).

However, BA1 cells, which have both the host promoter and the HIV-1 LTR in the same orientation were minimally responsive to the TSA treatment (**Fig. 22 B**), implying that chromatin structure does not contribute to HIV-1 latency in all cell lines and that the promoter orientation does not impose a specific mode of transcriptional regulation of HIV-1 provirus. My data indicate that class I and II HDACs are not general regulators of HIV-1 transcription in these cell lines. This is consistent with the hypothesis that neighboring host genes being active and having open chromatin structure can influence transcription from the proximal HIV-1 LTR.
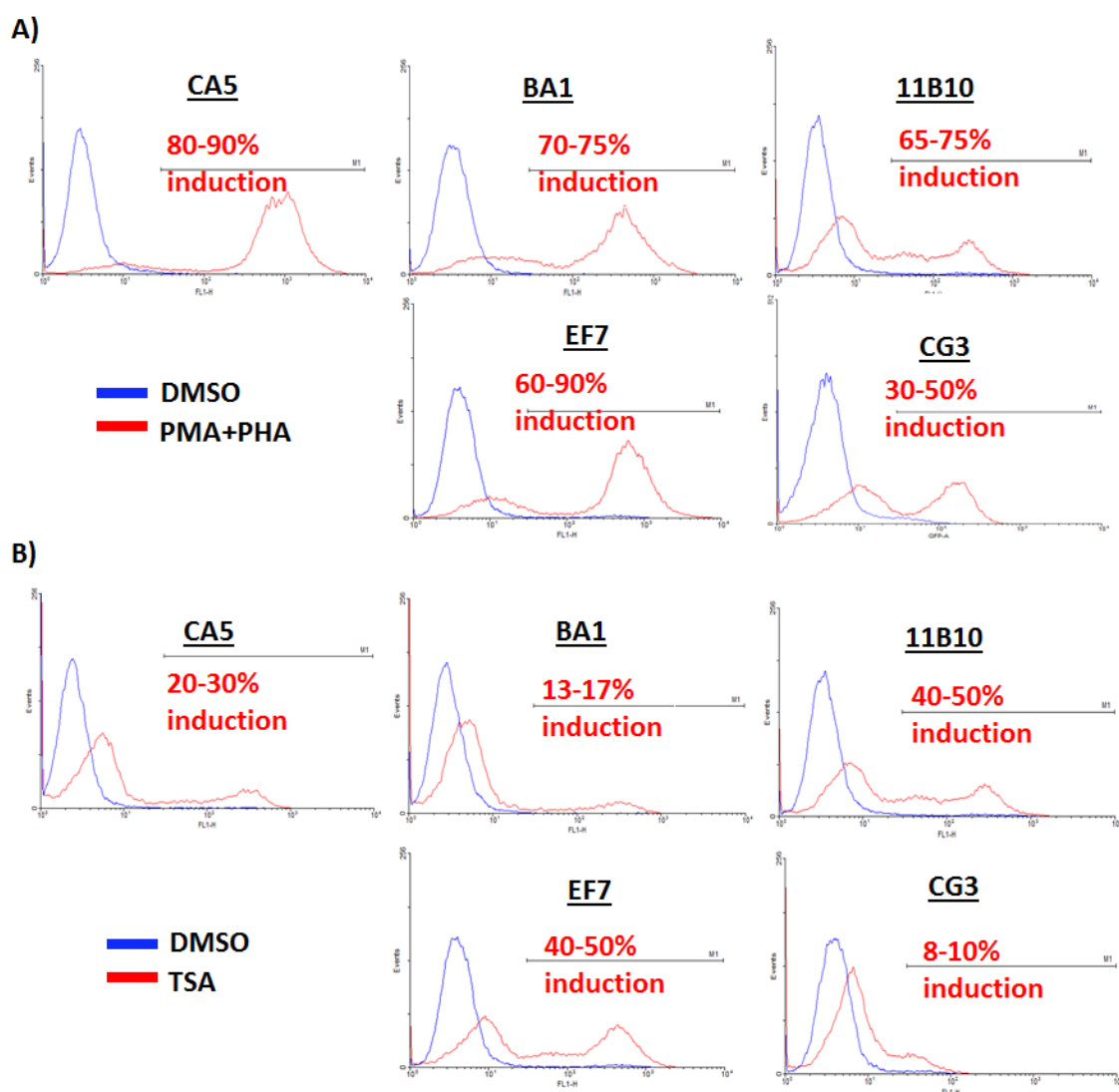
Figure 22. Provirus Integration Site Affects Reactivation Rate of Latent HIV-1.

Cells were treated with 2μg/mL PHA and 10ng/mL PMA or 0.5μM TSA for 24 h. GFP expression was measured by flow cytometry. Data represent fold induction over background HIV-1-GFP expression.

2.3. Correlation between the Expression of Host Genes and HIV-1 Induction

Because HIV-1 preferentially integrates into active host genes one may expect that HIV-1 transcription would interfere with transcription of the host gene (Lenasi et al., 2008). Thus, the reactivation of HIV-1 would be at the expense of the host, leading to a decreased expression of the endogenous gene. However, treatment with PMA+PHA resulted in decreased host gene expression only in 11B10 and CG3 (PYCRL gene) (**Fig. 23**). The m-RNA levels of the majority of neighboring host genes increased (BA1, EF7, CG3 (TIGD5 gene)) or remained constant (CA5 cells) upon T cell activation with PMA+PHA (**Fig. 23**). In addition, the integration of HIV-1 led to an increase in host gene expression in BA1, 11B10, EF7 and CG3 (TIGD5 gene). It is possible that host gene activity is crucial for the reactivation of provirus. These data indicated that host gene expression does not necessarily exclude HIV-1 expression.
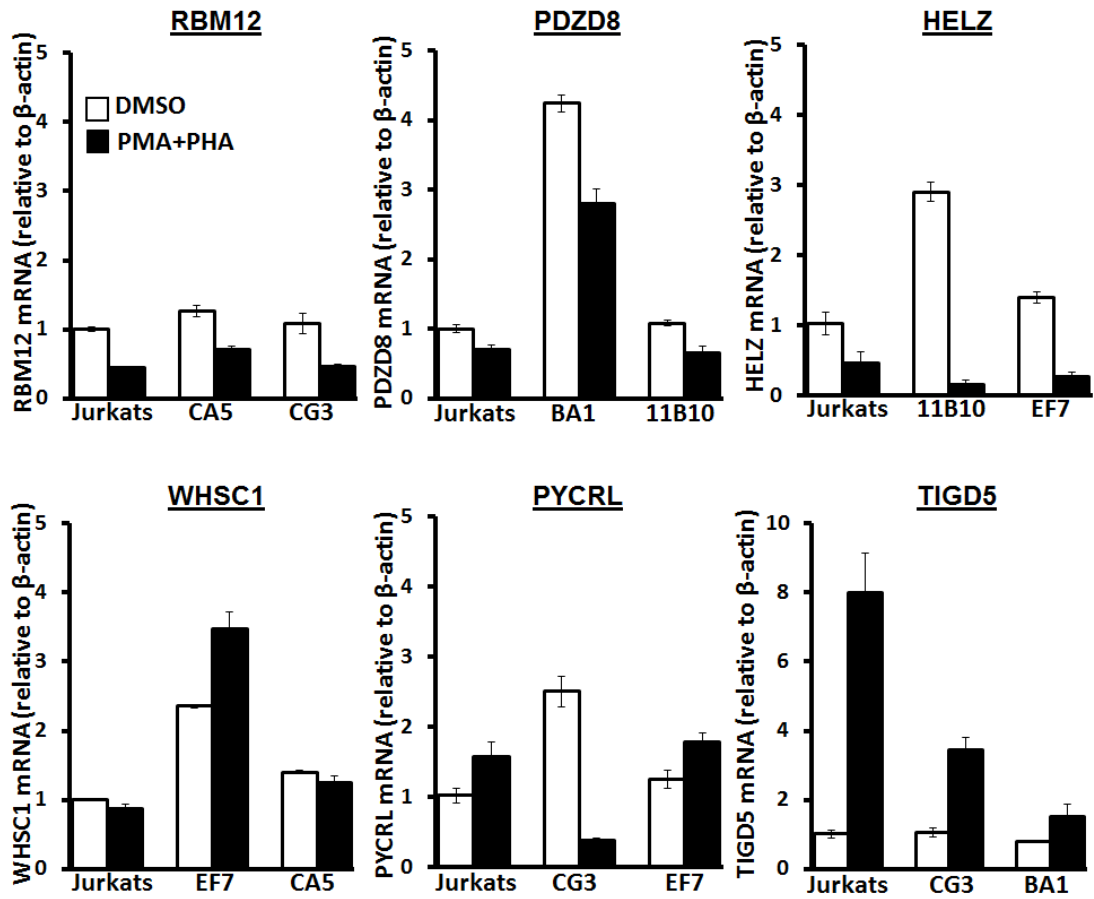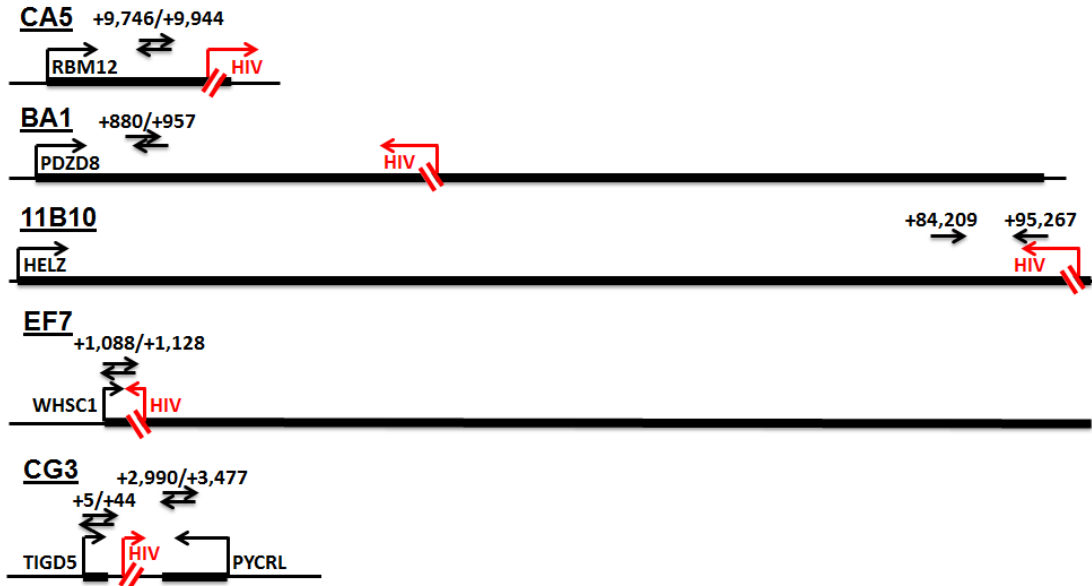
Figure 23. Activation of Latent Cells Results in Altered Host Gene Expression.

Uninfected Jurkat T cells as well as CA5, BA1, 11B10, EF7 and CG3 cells were treated with 2μg/mL PHA and 10ng/mL PMA for 24 h. Expression of the neighboring host gene mRNA was measured by qRT-PCR and normalized to β-actin.

2.4. RNAP II Pausing as Common Mechanism of HIV-1 Latency

To determine if HIV-1 transcription in the inducible Jurkat cell line is inhibited at the step of transcriptional initiation or elongation I looked at the accumulation of short HIV-1 mRNA transcripts. Using qRT-PCR I have calculated the ratio of initiated to elongated HIV-1 mRNAs (**Fig. 24**). The ratio of initiated to elongated HIV-1 mRNA transcripts decreased upon PMA+PHA treatment in CA5, BA1 and 11B10 cells, indicating productive RNAP II transcription. CG3 cells also had accumulated short HIV-1 transcripts, but to a much smaller degree (**Fig. 24**). However, EF7 cells did not contain accumulated initiated HIV-1 mRNAs (**Fig. 24**). The accumulation of short HIV-1 mRNAs is consistent with the paused RNAP II at HIV-1 LTR which decreases upon T cell activation. Effective HIV-1 transcription is initiated and elongated HIV-1 mRNA is produced shifting the ration of initiated to elongated transcripts to "1".
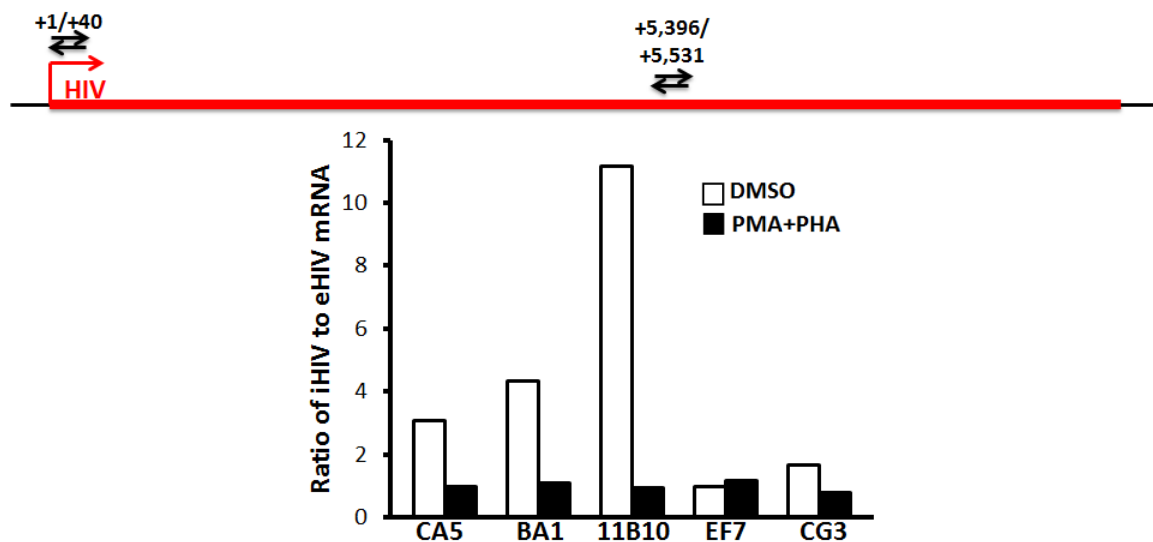
Figure 24. RNAP II Pausing Occurs in Latent Cell Lines.

Latently HIV-1-infected Jurkat cell lines were treated with 2μg/mL PHA and 10ng/mL PMA for 24 h. Expression of initiated and elongated HIV-1 was measured by qRT-PCR. These data are performed in triplicate and represent at three independent experiments.

RNAP II pausing is characterized by accumulation of RNAP II and NELF at the transcriptionally repressed promoter. The RNAP II promoter proximal pausing induced by NELF was confirmed in ChIP experiments looking at the binding of RNAP II and NELF to HIV-1 LTR. Using a ChIP assay, I detected high binding of RNAP II at HIV-1 LTR in uninduced cells and this binding decreased upon activation with PMA+PHA representing release of a paused RNAP II (**Fig. 25**). Upon T cell activation, the levels of NELF, an inducer of RNAP II pausing at HIV-1 LTR, also decreased at the viral promoter (**Fig. 25**). These data suggest that HIV-1 induction correlates with a decrease in RNAP II pausing at HIV-1 LTR.
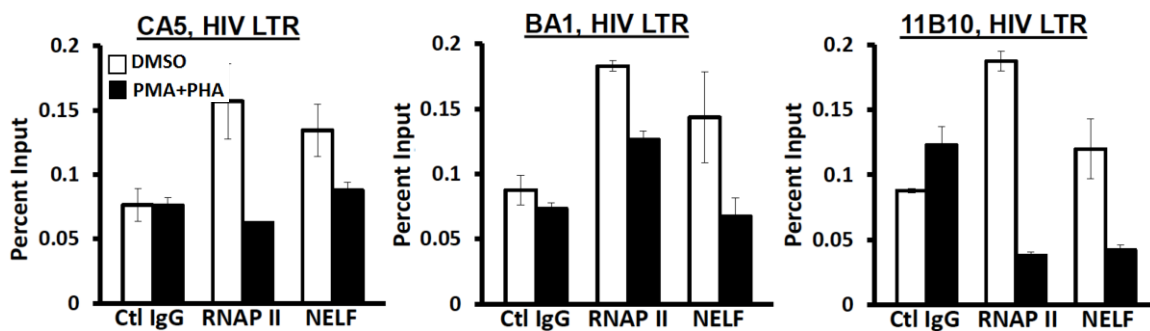
Figure 25. T Cell Activation Results in Altered Recruitment of RNAP II.

Cells were treated with 2μg/mL PHA and 10ng/mL PMA for 24 h. ChIP assays were performed to examine RNAP II and NELF binding to HIV--1 LTR.

Our laboratory identified the negative elongation factor (NELF) as a key player in mediating RNAP II promoter proximal pausing and repressing HIV-1 transcription 93 (Natarajan et al., 2013). Data obtained by Dr. Malini Natarajan and Dr. Gillian Schiralli Lester indicate that knockdown of NELF in these inducible Jurkat cell lines reactivates provirus, although to varying degree (**Fig. 26**). Thus, HIV-1 provirus in the inducible cell lines is repressed by NELF-induced RNAP II pausing, a widespread mechanism of gene regulation (Gaertner et al., 2012; Rahl et al., 2010), which I propose as a general mechanism of HIV-1 repression and latency.
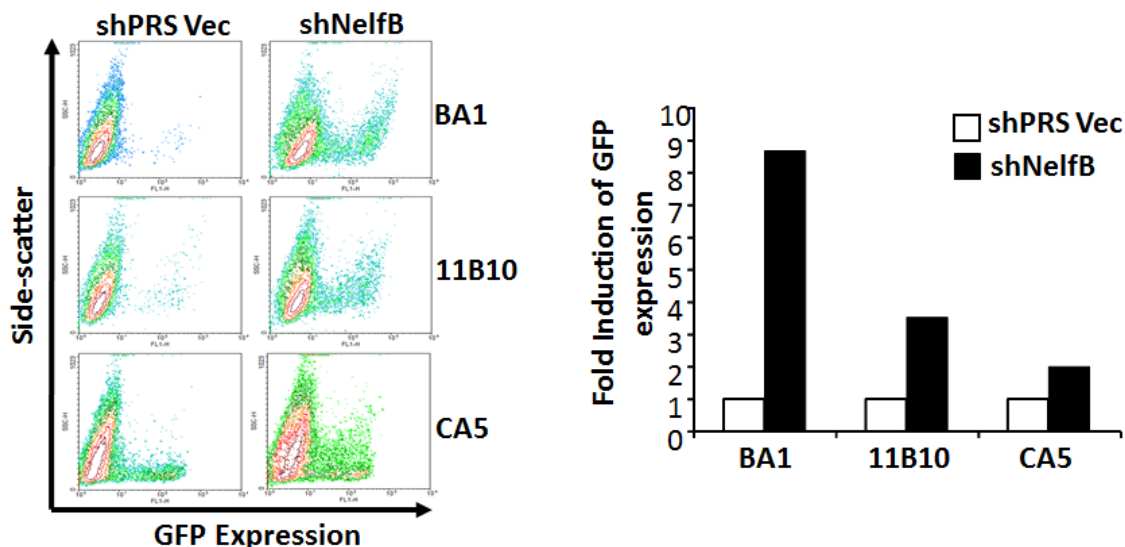
Figure 26. NELF Limits HIV-1 Transcription in Inducible Cells.

Cell lines were transduced with shPRS Vector or shNelf-B specific lentivirus, 72 h post-transduction GFP expression was measured by flow cytometry. The fold induction of GFP expression over the shPRS Vector sample is graphed for the representative experiment.

3. Conclusions and Discussion

I explored the effect of neighboring host promoters on HIV-1 expression in different inducible Jurkat cell lines. Previous reports showed that transcription from the proximal host promoters may lead to repression of HIV-1 transcription. However, my data demonstrate that genes in the vicinity of the HIV-1 provirus and chromatin structure have minimal impact on the maintenance of HIV-1 latency in the inducible Jurkat cells. The fact that CG3 cells, which harbor intergenic provirus, are the least inducible suggests that HIV-1 integration into transcriptionally active genes helps reactivate HIV-1. Thus

integration of the HIV-1 provirus into transcriptionally active genes instead of non-coding DNA may actually help in its reactivation by assisting with promoter clearance of repressive factors or open chromatin. In fact, HIV-1 integration increased expression of the neighboring host gene by an unknown mechanism. It is possible that HIV-1 induces transcription of the host gene by disrupting repressive chromatin structure, obstructing binding of transcriptional repressors to the host gene or recruiting them away from the host neighboring promoter to HIV-1 LTR. The high transcriptional activity of host neighboring promoter was decreased upon HIV-1 induction only in 11B10 cells, implying that in this particular cell line high transcriptional activity of the host gene may lead to repression of HIV-1 LTR. It remains to be determined if the decrease in the HELZ gene expression in 11B10 is a result of interruption of transcriptional initiation or elongation of this gene.

Recently RNAP II pausing has been shown to be one of the mechanisms of transcriptional interference (Palmer et al., 2009). My data show that all the inducible cell lines have high levels of RNAP II at HIV-1 LTR, as well as accumulation of short HIV-1 mRNA transcripts, implying that proviruses are inhibited at the step of transcriptional elongation rather than initiation. The data showing HIV-1 induction upon NELF knock-down are supportive of RNAP II promoter proximal.as the primary mechanism of HIV-1 transcriptional repression.

Upon cell activation, BA1, EF7 and 11B10 have differential expression of the neighboring host gene despite having HIV-1 integrated in convergent orientation. HIV-1 induction decreased HELZ levels, did not affect PDZD8 expression, while it induced

WHSC1 levels. These differences may be possibly explained by the different distances of HIV-1 LTR from the proximal host gene promoter as well as the host gene promoter strength. In 11B10 cells, provirus is integrated 96,217 bp of HELZ compared to 36,311 bp of PDZD8 in BA1 cells and 1,129 bp of WHSC1 in EF7 cells. These data suggest that the inhibition of neighboring host gene expression correlates positively with the distance between the host gene and viral promoter. One can also assume that the promoter of HELZ is weaker, while the promoters of WHSC1 and PDZD8 have the same or greater strength than HIV-1 LTR.

My data demonstrate that T cell activation, inhibition of HDACs, as well as NELF-mediated RNAP II pausing play a differential role in maintaining the repression of provirus in inducible Jurkat cell lines. My data also suggests that although HIV-1 insertion and induction differentially affect neighboring host genes, the expression of a host gene does not necessarily prevent the reactivation of the HIV-1 provirus. I propose a model in which HIV-1 transcription is repressed by a combination of mechanisms rather than by a single one. In this model NELF causes RNAP II pausing at HIV-1 LTR characterized by accumulation of short viral transcripts, while the closed chromatin structure at Nuc-1 may additionally prevent RNAP II from processing downstream provirus after its release from the pause. T cell activation leads to recruitment of transcriptional activators to HIV-1 LTR as well as the displacement of transcriptional repressors, such as Blimp-1, from provirus allowing efficient HIV-1 transcription.

V. Conclusions

The inability to target and eliminate latently infected memory CD4$^+$ T cells remains a barrier to eradicating HIV-1 infection. In an effort to cure HIV-1 infection, strategies to purge transcriptionally repressed HIV-1 provirus from latent reservoirs have been employed to complement current antiretroviral therapies (Margolis and Hazuda, 2013). Recent therapeutic approaches have focused on overcoming the repressive effects of chromatin, which has been implicated as a key regulator of HIV-1 transcription (Richman et al., 2009; Siliciano and Greene, 2011). For example, clinical trials have used HDAC inhibitors valproic acid (Archin et al., 2010; Routy et al., 2012) and vorinostat (Archin et al., 2012), which, despite modest ability to induce HIV-1 transcription in peripheral blood of HAART patients, did not decrease the HIV-1 reservoir. The limited success of these initial trials probably reflect the complexity of the latent reservoir in regards to the cells that are included in this compartment as well as the multiple mechanisms that establish and maintain latency (Richman et al., 2009; Schiralli Lester and Henderson, 2012; Siliciano and Greene, 2011). An additional confounder is that many of the factors that limit HIV-1 transcription are general transcriptional regulators which are necessary for normal gene expression. Targeting RNAP II, P-TEFb, and chromatin remodeling factors will likely be toxic, lack specificity, and have an impact on global gene expression.

I wanted to gain insight into the mechanisms that might contribute to HIV-1 latency in the context of T cells. My work explored two possible mechanisms of HIV-1

latency: tissue specific factors and host neighboring promoters. I show that Blimp-1 is an important transcriptional repressor of HIV-1 in memory CD4$^+$ T cells and that neighboring genes have minimal impact on HIV-1 transcription. I also demonstrate that RNAP II pausing appears to be necessary for establishment of proviral latency in both scenarios.

Blimp-1-mediated repression of HIV-1 transcription in memory CD4$^+$ T cells

CD4$^+$ T cell subsets differentially support HIV-1 replication. For example, quiescent CD4$^+$ memory T cells are susceptible to HIV-1 infection but do not support robust HIV-1 transcription and have been implicated as the primary reservoir of latent HIV-1. T cell transcription factors that regulate maturation potentially limit HIV-1 transcription and mediate the establishment and maintenance of HIV-1 latency. Attempts to purge HIV-1 from the latent reservoir by targeting general biochemical pathways have had modest success; however, events regulated by T cell specific factors may provide a more cell specific targeting strategy that would minimize potential off-target gene activation. I suggest that T cell restricted transcription factors strongly influence HIV-1 proviral transcription and that these factors may provide specific targets for eliminating latent HIV-1. I report that Blimp-1, a critical regulator of B and T cell differentiation, is highly expressed in memory CD4$^+$ T cells compared to naïve CD4$^+$ T cells and is induced following T cell activation. Furthermore, I show that Blimp-1 binds sequences downstream of HIV-1 LTR, called HIV-1 ISRE, and is displaced following T cell activation. Blimp-1 inhibits Tat-dependent HIV-1 transcription. Reduction of Blimp-1 in

infected primary T cells, including CD4$^+$ memory T cells, increases RNAP II processivity, histone H3 acetylation and baseline HIV-1 transcription. Treatment with protease inhibitors (Pacenti et al., 2006) as well as HIV-1 Tat have been shown to increase Blimp-1 expression in T cells (Sforza et al., 2014), thus promoting HIV-1 latency. Therefore, the transcriptional repressor, Blimp-1, is an intrinsic factor that predisposes CD4$^+$ memory T cells to latent HIV-1 infection. I predict that modulating the expression of Blimp-1 or targeting its cofactors would reactivate and potentially purge latent virus in specific T cell subpopulations without global T cell activation. However, further research is required to understand the Blimp-1-mediated mechanisms of repression of HIV-1 transcription in different T cell subsets and to assess which Blimp-1-interacting factors are the best candidates for the development of novel treatment strategies. NELF-induced RNAP II promoter proximal pausing is a common mechanism of repressing proviral expression. Further studies are required to elucidate whether RNAP II pausing at HIV-1 LTR can be disturbed by modulating intrinsic T cell factors.

Blimp-1 inhibits Tat-dependent and Tat-independent HIV-1 transcription. Based on the latter finding, I would speculate that that Blimp-1 represses the generation of TAR RNA, thus inhibiting the recruitment of Tat and P-TEFb to HIV-1 LTR. Blimp-1 has been shown to inhibit transcription of c-Myc, a factor implicated in the release of paused RNAP II (Lin et al., 1997; Rahl et al., 2010). It remains to be determined if targeting of c-Myc by Blimp-1 is another mechanism via which Blimp-1 inhibits HIV-1 transcriptional elongation and generation of TAR RNA stem loop. Blimp-1 is a transcription factor that regulates its target genes by recruiting histone-modifying complexes, such as

methyltransferases and HDACs. Decreasing Blimp-1 levels increased acetylation of histone H3 at Nuc-1 positioned next to HIV-1 LTR. It remains to be seen if Blimp-1 recruits histone-modifying enzymes to Nuc-1. Preliminary data shows that treatment with TSA, an inhibitor of HDACs I and II, and BIX02194, a G9a inhibitor, did not abolish Blimp-1 mediated repression of HIV-1 transcription. However, the experiments should contain proper controls verifying that the inhibitors are effective, such as ChIPs looking at the acetylation and methylation of Nuc-1. It will be critical to fully characterize the mechanism by which Blimp-1 leads to increase in HIV-1 transcription upon T cell activation. It may be informative in future studies to look if Blimp-1 recruits LDS1 (Su et al., 2009), a histone lysine demethylase, which has been shown to repress HIV-1 transcription by inducing trimethylation of H3K4 and H3K9 (Le Douce et al., 2012) as well as to activate Tat (Sakane et al., 2011). The interaction of Blimp-1 with LSD1 could provide a mechanism for Blimp-1-mediated repression of HIV-1 in resting T cells where Blimp-1 would recruit LSD1 to methylate histones, and for Blimp-1 mediated induction of HIV-1 transcription upon T cell activation where Blimp-1 may recruit LSD1 to Tat to activate it.

I also speculate that Blimp-1 mediated enhancement of HIV-1 transcription upon T cell activation may be a result of Blimp-1 inhibiting another transcriptional repressor of HIV-1, since upon T cell activation Blimp-1 is no longer bound to provirus. Further studies to investigate the induction of HIV-1 transcription by Blimp-1 in activated T cells could also investigate the interaction of Blimp-1 with the viral transcriptional activator Tat or P-TEFb complex. It remains to be determined why Blimp-1 dissociates from the

HIV-1 ISRE upon T cell activation. I show that Blimp-1 co-occupies the HIV-1 ISRE with IRF-1 and IRF-8 and that T cell activation leads to a decrease in the binding to this site of all of these factors. Thus IRF-1 and IRF-8 do not replace Blimp-1 at the ISRE in the HIV-1 LTR upon activation. Future studies investigating the post-translational modification of Blimp-1 may provide useful information as to why Blimp-1 is released from the HIV-1 ISRE upon T cell activation. Future experiments should look at the change in Blimp-1 phosphorylation, ubiquitination and SUMOylation upon T cell activation and identify the protein responsible for these modifications. Co-culture of HIV-1-pulsed DC with T cells induces the expression of Blimp-1, as well as markers of exhaustion, including PD-1, Tim-3, LAG-3 and CTLA-4 (Shankar et al., 2011). However, Blimp-1 has been recently shown to repress the expression of PD-1and NFATc1, its activator, in $CD8^+$ T cells (Lu et al., 2014b). It is also possible that by inhibiting NFAT, a transcriptional activator of HIV-1, Blimp-1 contributes to repression of HIV-1 transcription. It remains to be determined if Blimp-1 directly regulates the expression of other exhaustion molecules in HIV-1 infection. Bach-2 is a repressor of Blimp-1 and T cell differentiation (Tsukumo et al., 2013a) that has been shown to be enriched for integrated provirus site over time (Ikeda et al., 2007). It is possible that the disruption of BACH2 gene by provirus is in part responsible for the induction of Blimp-1 levels in HIV-1-infected patients.

Role of neighboring host genes in HIV-1 transcription

I also examined the influence of host neighboring promoters on HIV-1 transcription to identify common mechanisms controlling HIV-1 latency. About 40% of human transcripts overlap with each other (Birney et al., 2007) suggesting that neighboring promoters affect each other's expression. HIV-1 integrates into transcriptionally active host genes and its transcription is carried out and controlled by the host transcriptional machinery. I have shown that inducible cell lines are differentially responsive to T cell activation and treatment with HDACs inhibitor. In addition, integration of provirus in the convergent orientation resulted in the induction of the host gene, while HIV-1 transcription did not correlate with the neighboring host gene expression levels. Furthermore, my data indicates that expression of the host gene allows HIV-1 transcriptional activation. My research has demonstrated that the majority of repressed provirus integrated into host genes in the parallel and convergent orientation has NELF and stalled RNAP II at the LTR. Decreasing NELF was able to induce HIV-1 transcription. My data supports a model in which HIV-1 latency is established and maintained by multiple mechanisms, including RNAP II promoter proximal pausing, condensed chromatin structure, T cell activation and intrinsic T cell transcription factors but is not dependent on proximity or orientation to the neighboring host promoters.

My data suggests that neighboring promoters have minimal impact on HIV-1 transcription; however I was not able to modulate the transcription of the host genes to definitely rule out their contribution to the maintenance of HIV-1 latency as there is not sufficient data looking at regulation of transcription of these host genes. The orientation

of the host genes and HIV-1 LTR does not seem to correlate with a specific

transcriptional repression mechanism. However, in the cell lines with the convergent

orientation of promoters the increasing distance between the two promoters seems to

decrease the level of host gene transcription upon cell activation. It would be intriguing to

investigate whether increasing the distance between HIV-1 LTR and a convergent

promoter would increase their transcriptional interference by constructing a plasmid with

the HIV-1 promoter integrated at different positions in the same gene. Because there was

a range of responses to TSA treatment among CA5, BA1, 11B10, EF7 and CG3 cells one

should try another HDAC inhibitor, such as valporic acid or vorinostat. It may be

informative in future studies to look at the influence of activation with CD3+28 to see if

signaling downstream of the TCR has different effect on host genes and HIV-1

expression compared to treatment with PMA+PHA. Future studies should be also aimed

at measuring the strength of the neighboring host promoter to see if it plays a role in

repressing HIV-1 transcription. Additional experiments verifying that the host genes are

transcriptionally active need to be performed. Future studies would benefit from looking

at the presence of active RNAP II at the host genes by using an antibody specific to

phosphorylated Ser-2 of RNAP II. Future studies would ideally look at the presence of

chimeric mRNA messages spanning the upstream host gene and the viral promoter to see

if the elongating RNAP II disrupts transcriptional machinery at HIV-1 LTR. Because

HIV-1 latency in inducible cell lines is regulated by a combination of mechanisms it will

be critical to see if NELF cooperates with Blimp-1 to repress HIV-1 transcription by

inhibiting RNAP II processivity.

Significance and Implications

My data show that there are common mechanisms mediating HIV-1 latency. Blimp-1 represses RNAP II processivity to sequences downstream of the ISRE, while proviruses integrated into active neighboring host genes have RNAP II paused at their promoters. Both, the inducible cell lines as well as cells expressing high levels of Blimp-1 have accumulation of short HIV-1 mRNA transcripts showing that RNAP II pausing is a common checkpoint of HIV-1 transcription. Previous work from our laboratory showed that NELF recruits NCoR1-GPS2-HDAC3 complex to HIV-1 LTR to remodel chromatin and induce premature termination of transcripts in primary CD4$^+$ T cells (Natarajan et al., 2013). Thus, RNAP II pausing is coordinating multiple repressive pathways and targeting it may help in reactivating latent provirus.

To eradicate HIV-1 infection it would be preferable to use "shock & kill" approach, where latency reversing agents (LRA) would be used to reactivate latent virus while the patient is on HAART (**Fig. 27**). This strategy would result in elimination of latent reservoir and cell death of infected cells. Recent studies showed that broadly neutralizing antibodies (bNAbs) prevent the establishment of new infections and in combination with LRA greatly reduce the chance of viral rebound after cessation of treatment (Halper-Stromberg et al., 2014). HIV-1 gene therapy is another promising venue, where by using zinc-finger endonucleases (ZFNs) one can disrupt either the genes encoding HIV-1 co-receptors or provirus itself (Manjunath et al., 2013). The "shock & kill" strategy could benefit from including bNAbs to block new infections and gene

therapy to further interfere with HIV-1 replication (**Fig. 27**). In addition drugs targeting

NELF or Blimp-1 may aid in the release of paused RNAP II, increase RNAP II

processivity and open chromatin structure.
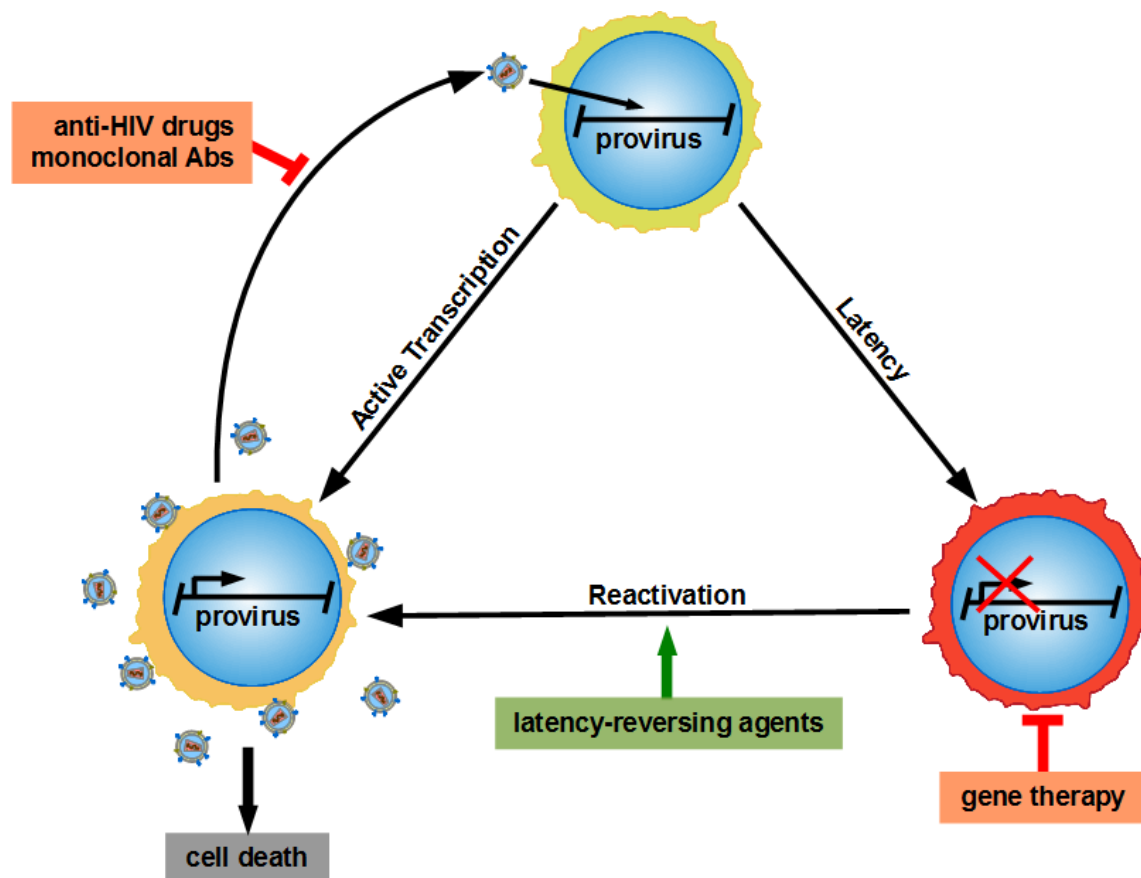
Figure 27. "Shock & Kill" Strategy to Cure HIV-1.

Eradication of HIV-1 infection will require the disruption of HIV-1 latency by using LRA. The disruption of latency should co-occur with HAART administration. The addition of bNAbs may prevent new infections, while gene therapy may aid in disruption of latent provirus that is not responsive to LRA. The goal of the "shock & kill" strategy would be reactivation of all latent provirus concurrent with depletion of all infected cells.

APPENDIX

RATIONALE

The Src kinase leukocyte-specific protein tyrosine kinase (Lck) is essential for initiating T cell receptor (TCR) signaling by activating downstream signaling proteins, such as phosphoinositide 3-kinase (PI3K) which phosphorylates PIP2 to phosphatidylinositol-3,4,5-trisphosphate (PIP3) (Cannons and Schwartzberg, 2004). The Tec kinase interleukin-2-inducible T cell kinase (Itk) is a critical mediator of cytoskeleton rearrangements induced by TCR signaling. Itk is recruited to the PM by binding to PIP3, where it is phosphorylated by Lck (Cannons and Schwartzberg, 2004).

Previously, our lab has identified Lck and Itk as being crucial in efficient HIV-1 replication. I have shown that Lck positively regulates HIV-1 release, and physically interacts with Gag (Strasner et al., 2008). In T cells Gag assembly takes place at the PM, but in Lck deficient T cells Gag accumulates in intracellular compartments (Finzi et al., 2007; Strasner et al., 2008). Itk is required for efficient viral entry, transcription, and release (Readinger et al., 2008). I wanted to confirm that Lck increases HIV-1 release by regulating multiple stages of Gag intracellular transport and processing. I was especially interested in determining whether Lck is involved in transporting Gag to the PM, or preventing endocytosis of assembling Gag from the PM.

METHODS

For fluorescence microscopy experiments I have generated GFP-tagged WT Lck

expression construct by using CT-GFP Fusion TOPO TA Expression Kit (Invitrogen).

293T and HeLa cells were plated on glass coverslips (Fisher). At 16 h post-transfection,

cells were fixed in 2% paraformaldehyde, incubated with DAPI (Invitrogen), and

mounted on glass slides. Samples were imaged using a 60x objective on Nikon

deconvolution wide-field Epifluorescence microscope. Stack of 31 images were collected

at 0.3-m spacing. Images were deconvolved using 10 iterations with NIS elements

software. The data analysis was done with NIS elements software. To look at Lck role in

HIV-1 replication I have generated Jurkat T cell lines with stable Lck knockdown using

sh-RNA (Dharmacon). For experiments looking at the viral release VLPs were purified

by pelleting collected supernatants on 20% sucrose gradient at 100,000 xg for 1.5 h at 4

°C. The HIV-1 concentration in the supernatants was measured using an Alliance HIV-1

p24 Elisa Kit (Perkin-Elmer). I have used cell fractionation to look at Lck, Itk and Gag

co-localization in different cellular compartments. Whole cell lysates were run on SDS-

PAGE (12% gel) and western blots were used to detect protein expression.

RESULTS

To test the role of Lck in HIV-1 release I have generated stable, Lck-deficient

Jurkat cell lines using Lck shRNA. Although in the past our lab had used JCaM cells,

which do not express functional Lck, the use of cells in which Lck was knockdown

represents a more plausible approach to study the effect of Lck on HIV-1 release.

Examination of virus-infected sh-Lck Jurkat cells confirmed Lck's role in viral

replication as the Lck knockdown decreased HIV-1 release by approximately 75% (**Fig. 28**).
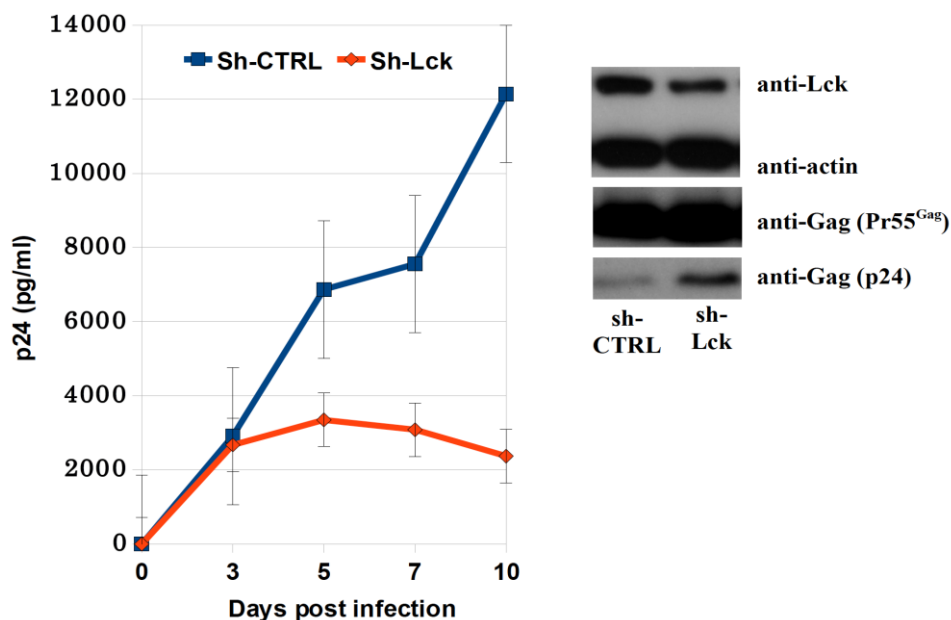
Figure 28. Lck is Required for Efficient HIV-1 Replication.

Jurkat T cells stably transduced with sh-Lck or sh-Ctrl lentivirus were infected with HIV-1-PLAP-Nef(-) and the release of HIV-1 p24 was monitored over the course of 10 days. Whole cell lystes were probed with either anti-HIV-1 p24^{gag}, anti-Lck or anti-actin Abs. Results shown are from a single experiment with each data point representing three independent infections. These data are representative of three experiments.

To determine if Lck and Itk are the primary non-receptor tyrosine kinases

mediating HIV-1 replication I have also examined the role of Fyn. Fyn and Lck are the

two Src kinases expressed in T cells. I have decreased Fyn expression in Jurkat cells

using si-Fyn. The knockdown was successful as determined by immunoblots; however,

with or without activation with anti-CD3 and -CD28 Abs , there was no significant difference in HIV-1 release between si-CTRL and si-Fyn transfected cells as measured by p24 release (**Fig. 29**). Thus, Lck is the primary Src kinase required for efficient HIV-1 particle release from infected T cells.
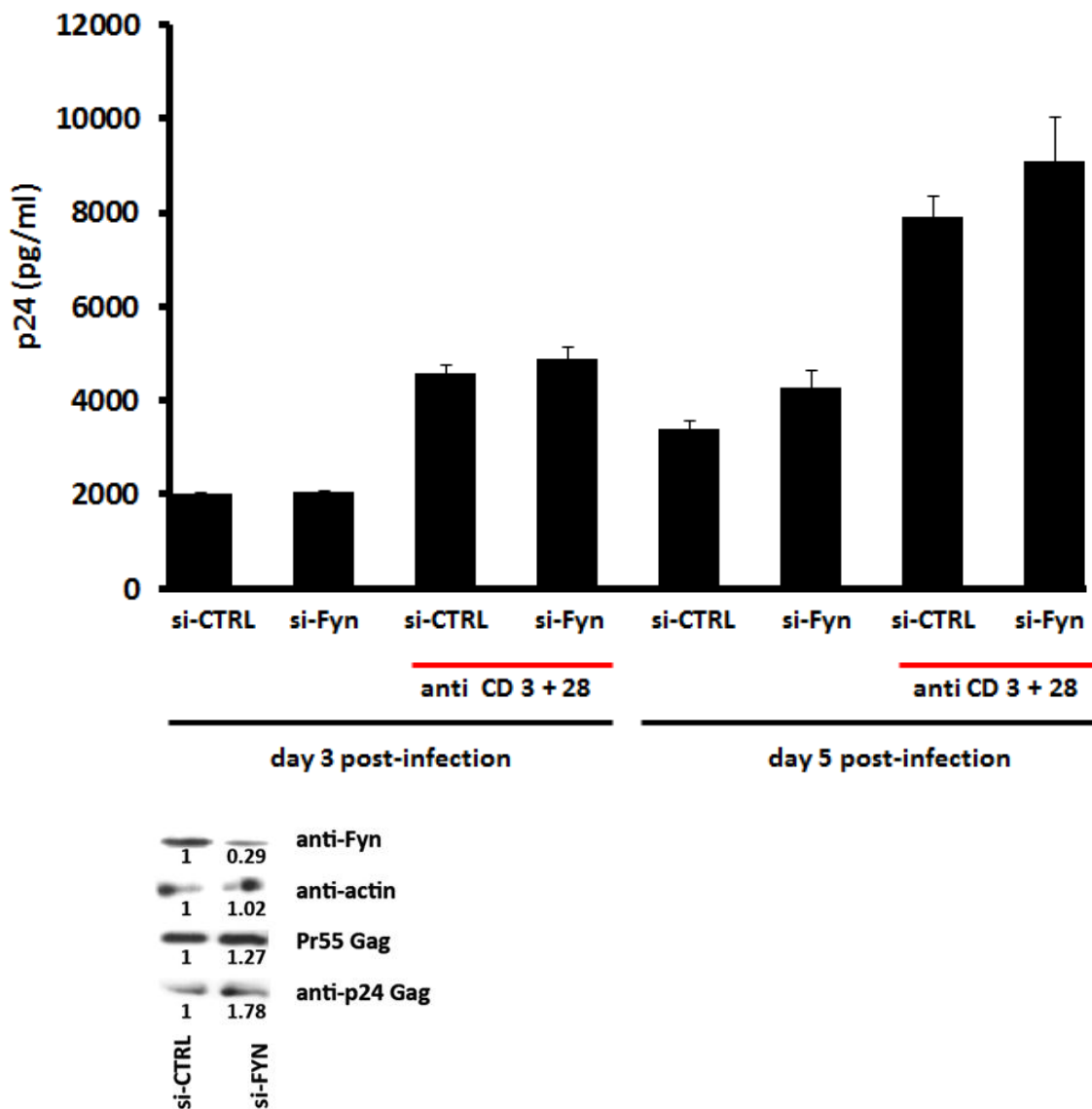
Figure 29. Fyn has Minimal Impact on HIV-1 Replication in T Cells.

Jurkat cells were electroporated with si-CTRL or si-Fyn, and infected 24 h post transfection with HIV-1-PLAP-Nef(+). Cells were activated with 0.1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 Abs for 24 h before collection. Release of $p24^{gag}$ was monitored at days 3 and 5 post-infection. Whole cell lysates were probed with anti-Fyn and anti-actin Abs.

Previously our lab has shown that Lck promotes Gag assembly at the PM (Strasner et al., 2008).I have now demonstrated that in 293T (**Fig. 30**) and in HeLa cells (**Fig. 31**) Lck co-localizes with Gag at the PM and in intracellular compartments. Mutation of the basic region in the MA domain leads to redistribution of Gag and its intracellular accumulation in late endosomes (Chukkapalli et al., 2008; Ono and Freed, 2004; Zhou et al., 1994). I speculated that expression of Lck may redirect the mutant Gag to the PM through a direct interaction with it. To test this I used Δ-MA-Gag, which has a deletion of residues 15 through 99 in the basic region of MA domain. However, using fluorescent imaging I observed that Δ-MA-Gag was located in intracellular compartments, even in the presence of Lck. This data suggest that Δ-MA-Gag has altered trafficking and thus does not interact with Lck, because it is not present in the same subcellular compartments as Lck, or that Lck binds to the MA domain of Gag.

A.

B.
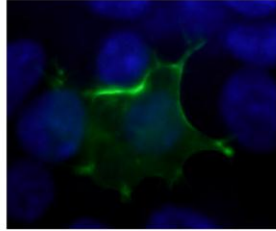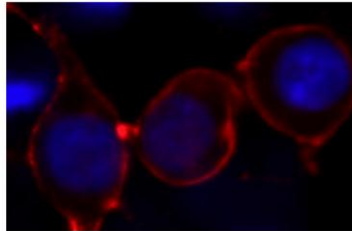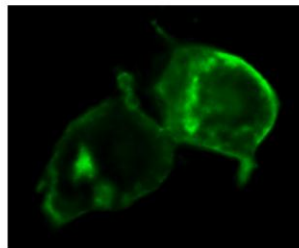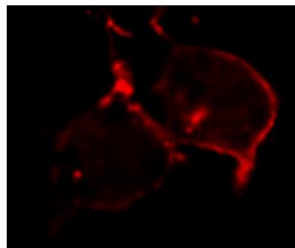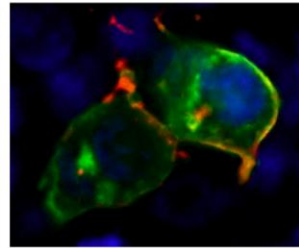
Figure 30. Lck Co-localizes with Gag at the PM and in Intracellular Compartments.

293T cells were plated onto glass coverslips and transfected (A) or cotransfected (B) with Lck, and Gag-cherry as indicated. 16H post-transfection cells were washed with PBS and then fixed with 2% paraformaldehyde and stained with antibody to Lck. The glass coverslips were mounted onto glass slides and viewed using a Nikon fluorescence microscope at 60x immersion and deconvoluted using 10 iterations with NIS elements software.

A.

B.
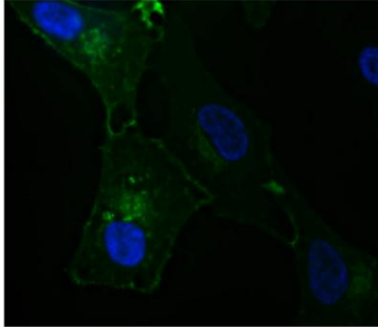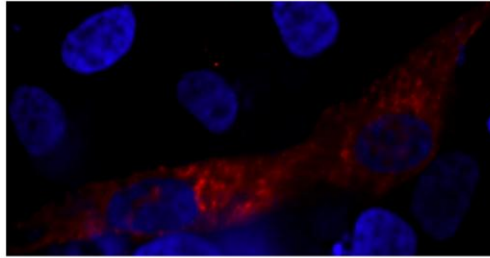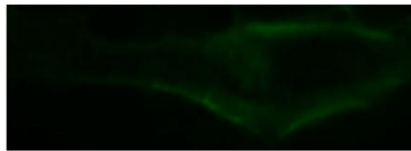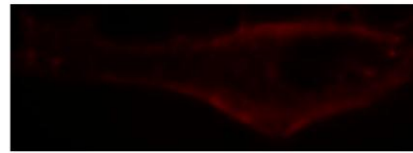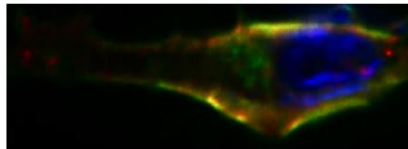
Figure 31. Lck Alters the Localization of Gag in HeLa Cells.

HeLa cells were plated onto glass coverslips and transfected (A) or cotransfected (B) with Lck-GFP and Gag-Cherry, as indicated. 72H post-transfection cells were washed with PBS then fixed with 2% paraformaldehyde. The glass coverslips were mounted onto glass slides and viewed using a Nikon fluorescence microscope at 60x oil immersion and deconvoluted using 10 iterations with NIS elements software.

A. **Gag-ΔMA-Cherry**

B. **Gag-ΔMA-Cherry**   **Lck**

Gag-ΔMA-Cherry + **Lck** + **DAPI**
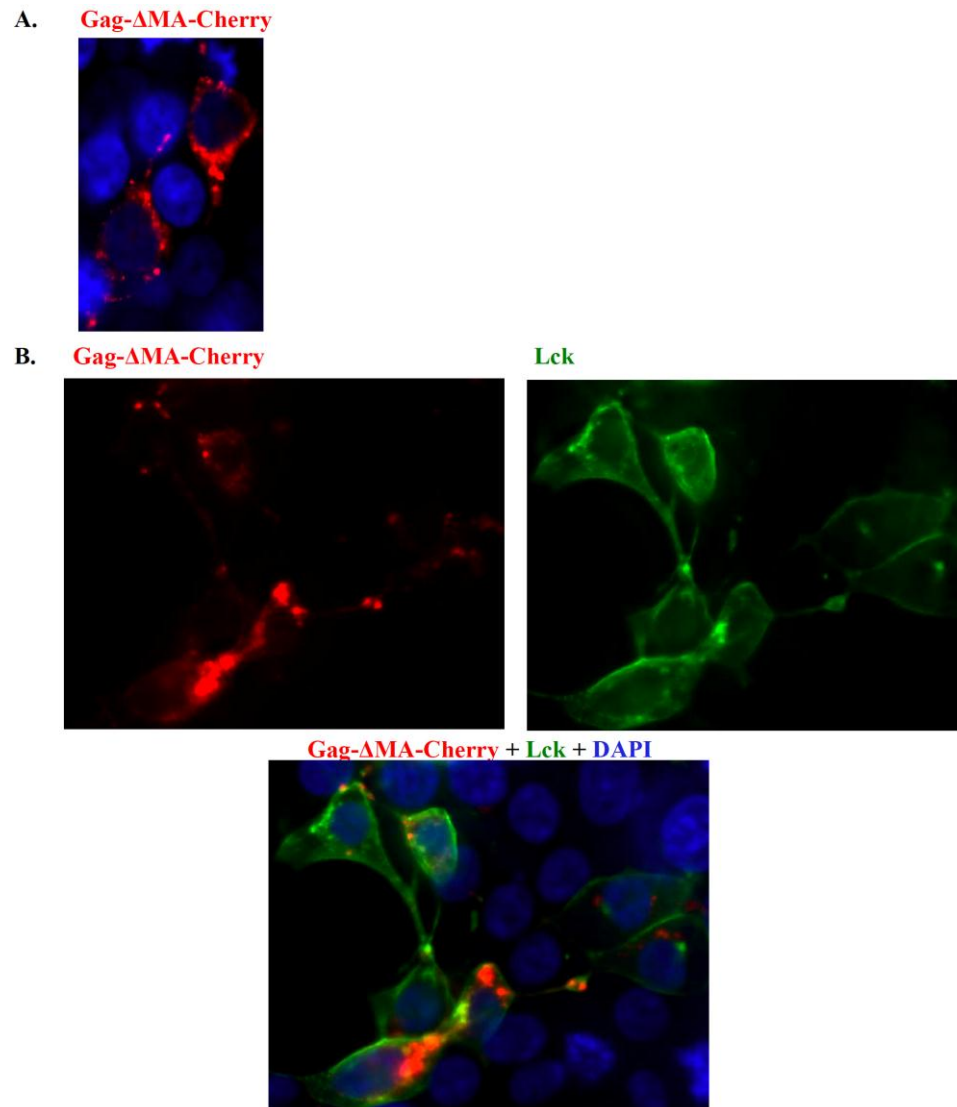
Figure 32. Lck Does Not Alter the Intracellular Accumulation of ΔMA-Gag.

293T cells were plated onto glass coverslips and transfected with ΔMA(15-99)-Gag-Cherry (A), or cotransfected with Lck and ΔMA-Gag-Cherry (B). 16H post-transfection cells were washed with PBS and then fixed with 2% prafaformaldehyde and stained with anti-Lck Ab. The glass coverslips were mounted onto glass slides and viewed using a Nikon fluorescence microscope at 60x oil immersion and deconvolved using 10 iterations with NIS elements software.

The assembly of HIV-1 occurs at the PM in T cells and macrophages, though some studies have suggested that intracellular compartments in macrophages can support virus assembly (Ono and Freed, 2004). However, as a result of frequent endocytosis Gag is also found in late endosomes in cells with preferential Gag assembly at the PM (Finzi et al., 2007; Klein et al., 2007; Ono and Freed, 2004). Clathrin- and cholesterol-dependent endocytosis of Gag along microtubules is mediated by dynamin (Cannons and Schwartzberg, 2004; Klein et al., 2007). To identify the function of Lck in preventing intracellular Gag accumulation I treated cells with Dynasore, a dynamin inhibitor, to block dynamin-dependent endocytosis. The treatment of 293T cells transfected with Gag, as well as Jurkat cell lines infected with HIV-1 with Dynasore did not change the fold induction of released p24 mediated by Lck (**Fig. 33 and 34**). These data suggests that Lck does not prevent endocytosis of Gag, or that HIV-1 is not endocytosed via a dynamin dependent pathway. Clathrin- and dynamin-dependent endocytosis are the two major endocytic pathways. To assess whether Lck prevents nascent virus endocytosis future studies would benefit from including Chlorpromazine to inhibit the clathrin- dependent endocytosis. Further studies investigating whether Lck inhibits the endocytosis of Gag should also include live cell microscopy of cells transfected with Gag-Cherry and Lck-GFP. The movement of Gag at the PM may be visualized by fluorescence recovery after photobleaching (FRAP), where regions beneath the PM associated with Gag-Cherry would be photobleached and monitored for subsequent recovery of Gag-Cherry.
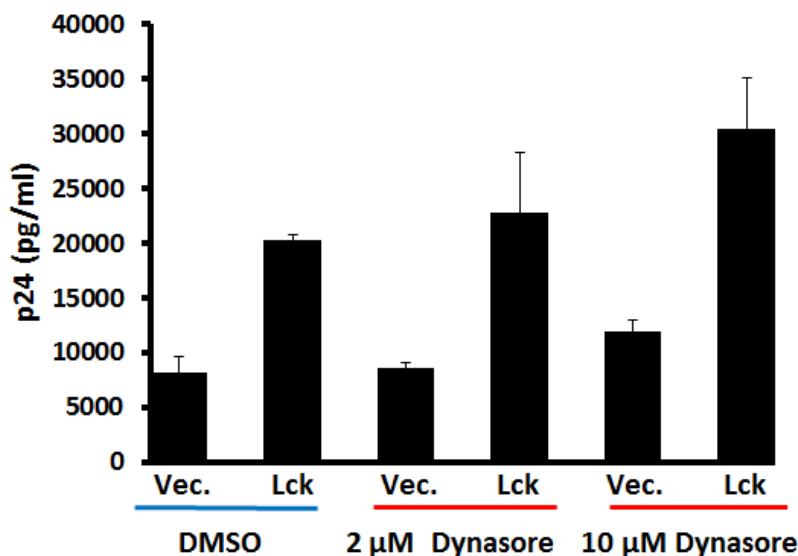
Figure 33. Lck Mediated Virus Like Particles Release is Dynamin Independent.

293T cells were transfected with Gag along with Lck or pcDNA3.1 vector control via calcium phosphate. 3 h after transfection new media with 2 and 10 µM Dynasore or DMSO was added to cells. 72 h after transfection supernatants were collected, spun on 20% sucrose cushion and analyzed for p24 via ELISA.

The free diffusion of Gag from cytosol to the PM is prevented by densely packed cytoplasm and HIV-1 has to use the cellular transport system to ensure successful exit from the cell (Naghavi and Goff, 2007). Transport of Gag RNA, as well as Pr55Gag to the PM is microtubule dependent (Mouland et al., 2001). To test whether Lck-mediated VLPs release is actin and microtubule dependent, I co-transfected 293T cells with Gag and Lck or infected stable Jurkat cell lines with HIV-1 and blocked cytoskeleton reorganization with different inhibitors. Latrunculin B- and Cytocholasin-D-mediated inhibition of actin polymerization interfered with viral release, but did not block Lck-mediated enhancement of VLPs and HIV-1 release (**Fig. 34 and 35**). The treatment of

cells with Nocodazole, a microtubules polymerization inhibitor, did not impede the

release of virus like particles, both in the presence and absence of Lck (**Fig. 34**). The 7

day treatment of Jurkat cells with Nocodazole was toxic to the cells (**Fig. 35**). These data

suggest that Lck mediated enhancement of HIV-1 release does not depend on

cytoskeleton reorganization. Additional experiments should be performed using a

microtubule depolymerization inhibitor (taxol) to rule-out the potential contribution of

microtubules in Lck-mediated HIV-1 release.
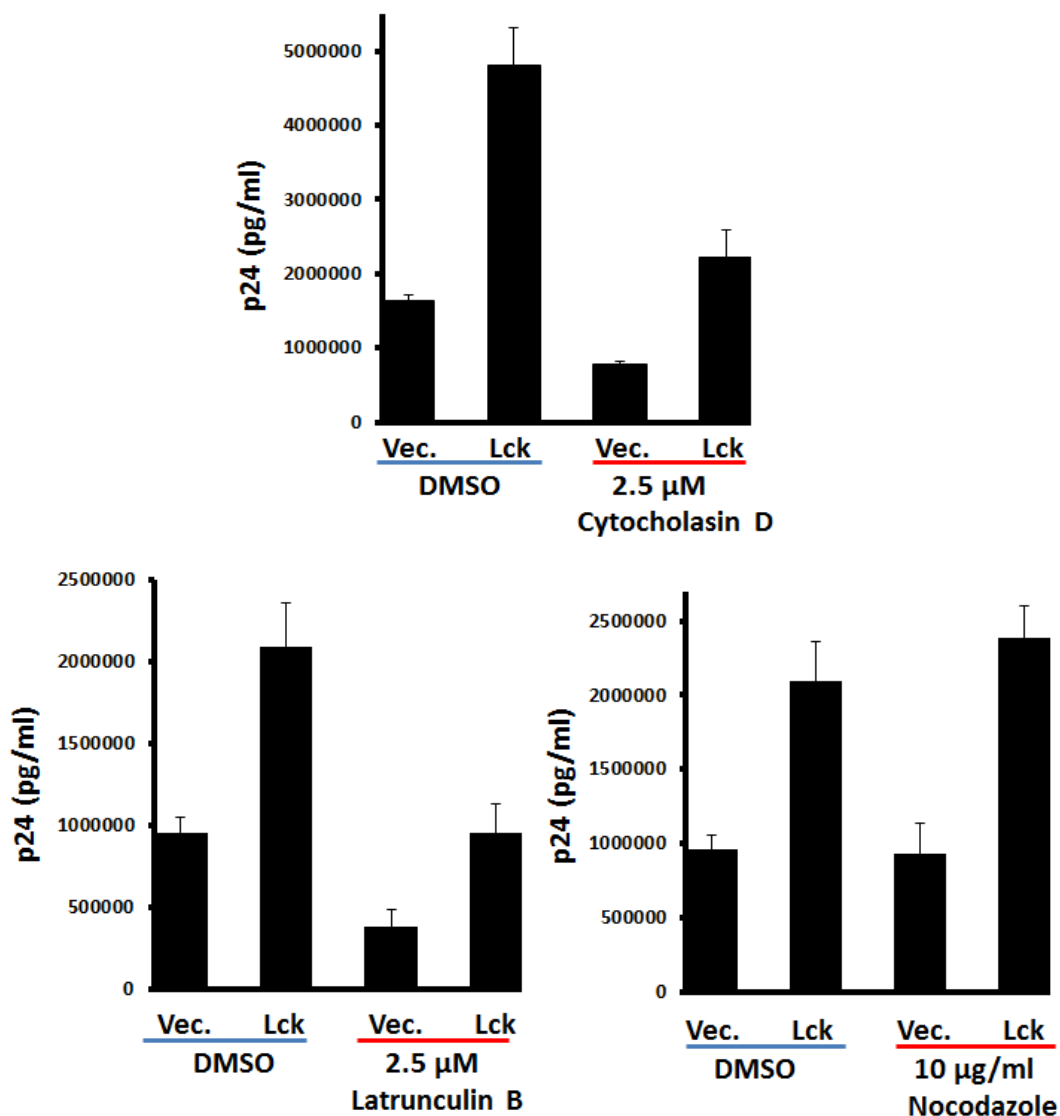
Figure 34. Lck Mediated Virus Like Particles Release Does Not Require Cytoskeleton Reorganization.

293T cells were transfected with Gag along with Lck or pcDNA3.1 vector control via calcium phosphate. 3 h after transfection new media with 2.5µM Cytocholasin D, 2.5µM Latrunculin B and 10 µg/ml Nocodazole or DMSO was added to cells. 72 h after transfection supernatants were collected and analyzed for p24 via ELISA.
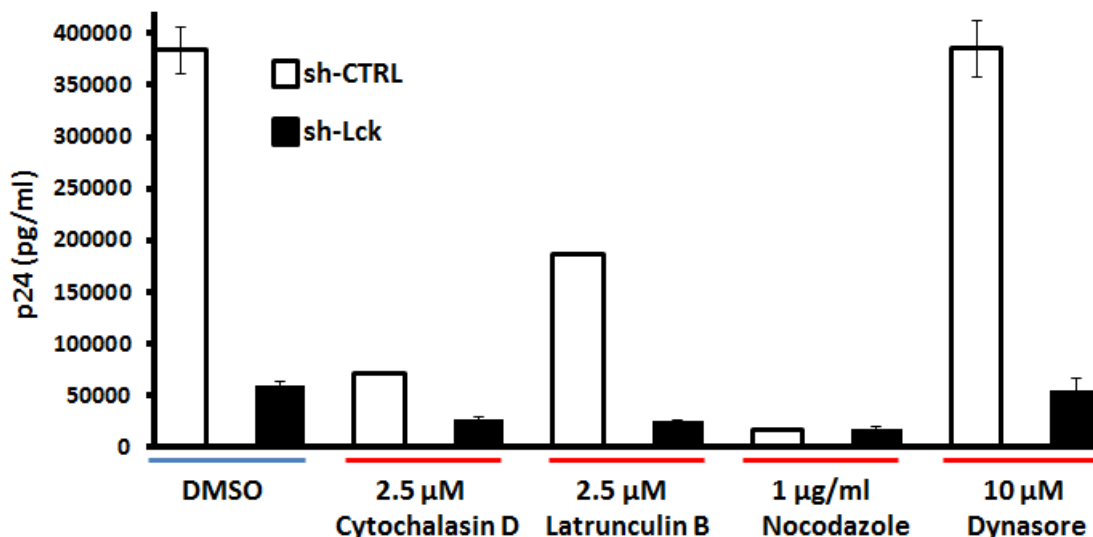
Figure 35. Lck Mediated HIV-1 Replication Is Not Abolished by Cytoskeleton and Dynamin Inhibitors.

sh-Control and sh-Lck Jurkat stable cell lines were infected with HIV-1-PLAP-Nef(+). 8 h post-infection new media with inhibitors were added to the cells. 7 days post-infection the supernatants were collected and analyzed for p24 via ELISA.

Rab proteins are small GTPases controlling vesicular trafficking (Saftig and Klumperman, 2009). One such protein, Rab11, is located on the cytosolic face of perinuclear recycling endosomes and controls the trafficking of vesicles from TGN and recycling endosomes to the PM (Saftig and Klumperman, 2009). Src kinases are present on clathrin-independent vesicles that recycle back out to the PM dependent on Rab11 and actin (Donaldson et al., 2009). The exit of Lck from Rab11 positive endosomes requires the activation of Rab11 by the adaptor protein Uncoordinated 119 (Unc119), which is present in Rab-11 positive endosomes (Gorska et al., 2009). Rab11 enhances HIV-1 release, and its deficiency results in an intracellular accumulation of Gag, and in a decreased HIV-1 release (Amet et al., 2008; Saftig and Klumperman, 2009). Because Lck

and Gag require Rab11 to be conveyed to the PM, it is conceivable that they both follow similar trafficking route (Amet et al., 2008; Gorska et al., 2009). To examine the effect of altering Lck trafficking on Gag release I have transfected 293T cells with Gag, Lck, Rab11 (Gorska et al., 2009) and Unc119 (Gorska et al., 2009) via calcium phosphate. Supernatants were collected 72 h post-transfection, VLPs purified and analyzed for p24 via ELISA. The overexpression of Rab11 alone did not affect VLPs release (**Fig. 36**), even though it has been previously shown to inhibit perinuclear accumulation of Gag (Amet et al., 2008). Additionally, over-expression of Rab11 slightly decreased Lck-mediated VLPs release (**Fig. 36**). One might expect that Unc119 increases VLPs release by inducing Lck to activate Abl leading to actin reorganization, as well as inhibiting dynamin-dependent endocytosis (Gu et al., 2009; Karim et al., 2010).However, overexpression of Unc119 also did not increase viral release and decreased Lck-mediated enhancement (**Fig. 36**). One possible explanation for these negative data may be the fact that in 293T cells Gag is transported to the PM even in the absence of Lck (**Fig. 30**). Future experiments should be performed in HeLa or preferably in T cells and use siRNAs to deplete Rab11 and Unc119 or dominant-negative forms of Rab11 and Unc119. Alternatively, Lck trafficking might be also altered by manipulating the expression of MAL protein (Anton et al., 2008).
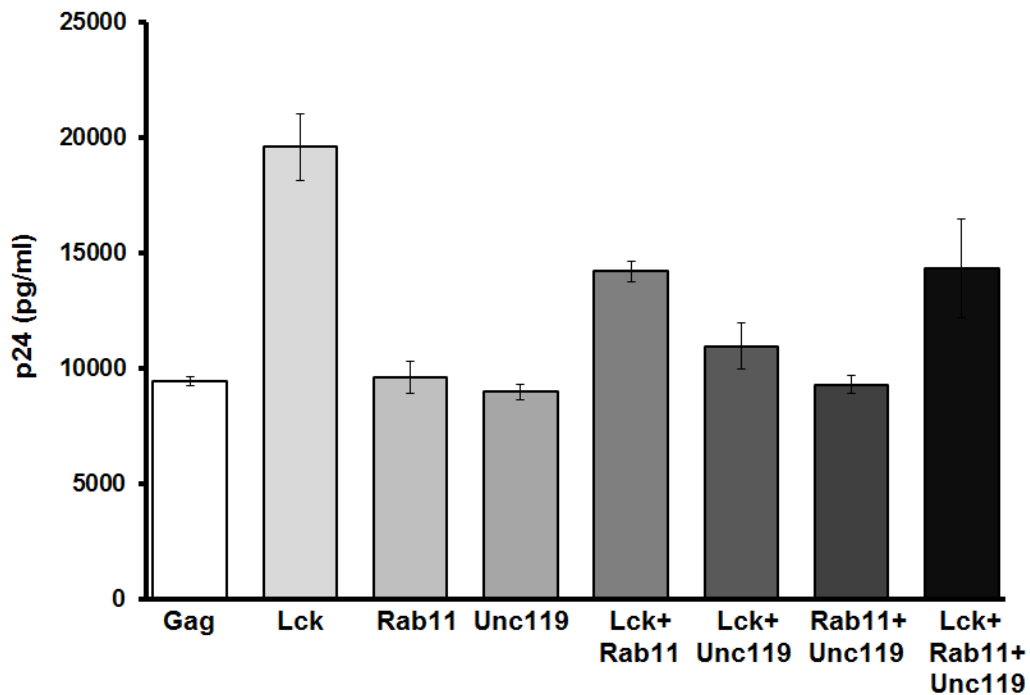
Figure 36. Rab11 and Unc119 do not Increase VLPs Release.

293T cells were transfected with Gag along with Lck, Rab11, Unc119 or pcDNA3.1 vector control via calcium phosphate. 72 h after transfection, supernatants were collected, spun on 20% sucrose cushion and analyzed for p24 via ELISA.

Lck orchestrates T cell signaling by phosphorylating downstream proteins. To determine if Lck needs to be active to enhance HIV-1 replication I have treated Jurkat cells with Damnacanthal, a specific Lck autophosphorylation inhibitor, and PP2, a Src family inhibitor. There was no significant difference between the unstimulated cells treated with Damnacanthal and PP2 compared to the control cells (**Fig. 37**). However, stimulated cells treated with Damnacanthal and PP2 showed even greater amounts of HIV-1 being released than the control cells (**Fig. 37**). This suggests that the activation of

Lck is dispensable for efficient HIV-1 replication. These results support the previous

data showing that the kinase dead Lck induces virus like particles release more efficiently

than the wild type Lck (Strasner et al., 2008), suggesting that Lck may be acting as an

adapter protein for Gag assembly.
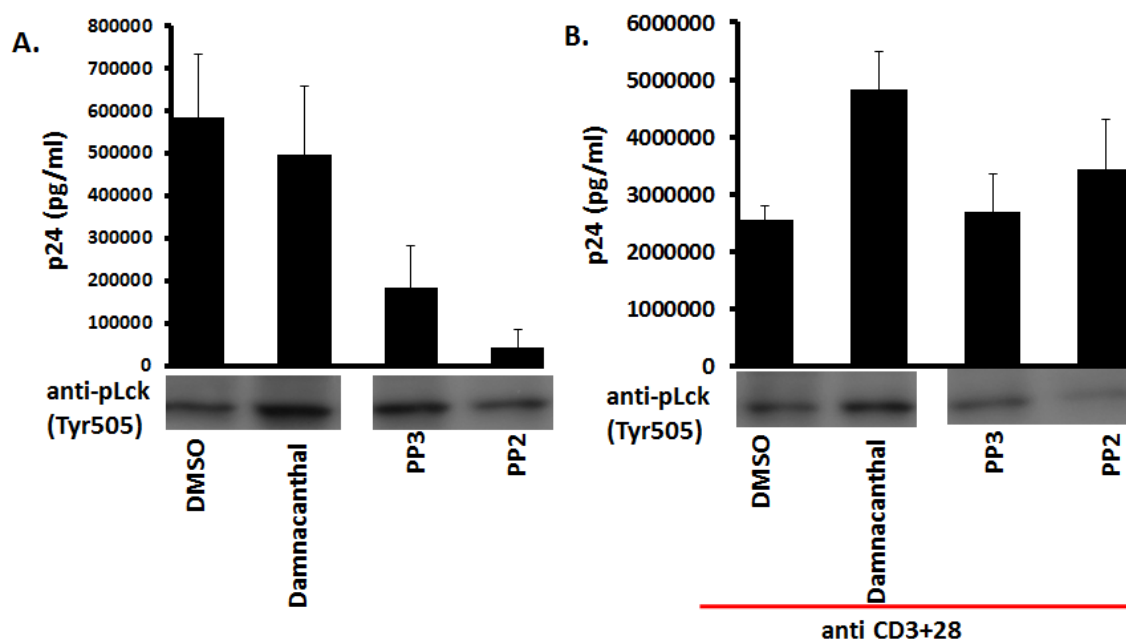
Figure 37. Activation of Lck is Not Required for Efficient HIV-1 Replication.

Jurkat cells were pre-treated with kinase inhibitors for 1 hour, and infected with HIV-1-PLAP-Nef(+) in the presence of inhibitors. 12 h post-infection the new media with inhibitors was added to the cells. The cells were left unactivated (A) or activated with anti-CD3 and 28 Abs for 24h (B). At 7 days post-infection the supernatant was collected and analyzed for p-24 via ELISA. Whole cell lysates were probed with anti-pLck (Tyr505) Ab.

Lck and Itk are both T cell specific non-receptor tyrosine kinases that participate

in T cell signaling, and enhance HIV-1 release (Readinger et al., 2008; Strasner et al.,

2008). Consistent with previous reports the non-receptor tyrosine kinases Lck and Itk

increased VLPs release (**Fig. 38**). Additionally, the co-transfection of Gag with Lck and

Itk showed that Lck and Itk cooperatively interact to enhance VLPs release (**Fig. 38**).

These data suggests that Lck and Itk functionally interact with each other to achieve

optimal HIV-1 release. The synergistic effect between Lck and Itk on HIV-1 release

should be confirmed in T cells by performing double knockdown of Lck and Itk.
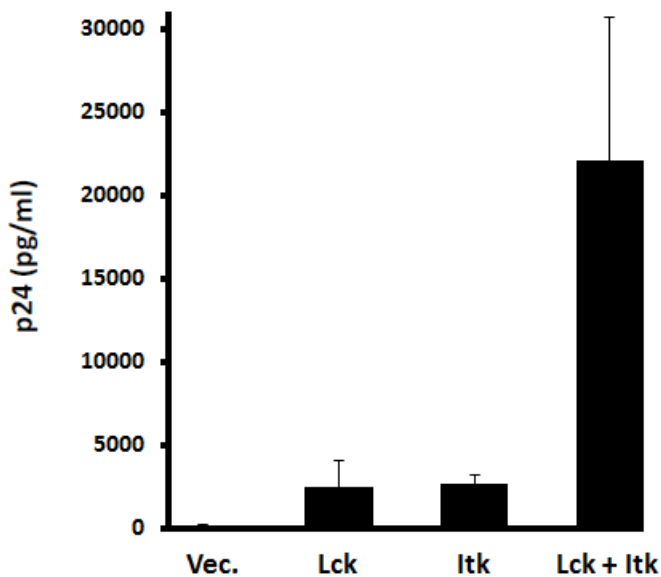
Figure 38. Lck and Itk Co-operate to Enhance Gag Release.

293T cells were transfected with Gag along with Lck, Itk or pEGFP vector control via calcium phosphate. 72 h after transfection, supernatants were collected, spun on 20% sucrose cushion and analyzed for p24 via ELISA.

To identify intracellular compartments in which Gag and Lck interact, and the

route that they follow to the PM I used subcellular fractionation. sh-Lck and sh-CTRL

Jurkat cells were infected for 24 h with HIV-1. Cells were homogenized 5 days post-

infection, and separated using subcellular fractionation into 13 fractions, with fractions 1-

5 predicted to represent lipid rafts (Triantafilou et al., 2002). Gag co-localized with Lck in fractions 2-5 (**Fig. 40**). As expected, cells stably expressing sh-Lck had overall reduced levels of Lck and Lck was present only in fractions 2-4 instead of 1-5 (**Fig. 40**). The reduction of Lck levels also resulted in decreased presence of Itk in fractions 2-5 (**Fig. 39**). These results suggest that the subcellular localization of Itk is Lck dependent. In the subcellular fraction experiment the different fractions should be probed with Abs for markers of lipid rafts (GM1), non-raft associated proteins (CD45), early endosomes (EEA1and Rab5), late endosomes (Lamp1 and TGN), as well as Rab11 and Unc119, proteins controlling the trafficking and activation of Lck.

The above experiments do not provide definitive mechanism by which Lck enhances Gag release. All experiments with inhibitors should be better controlled, account for possible cell death and preferentially the cytoskeleton should be visualized using microscopy.
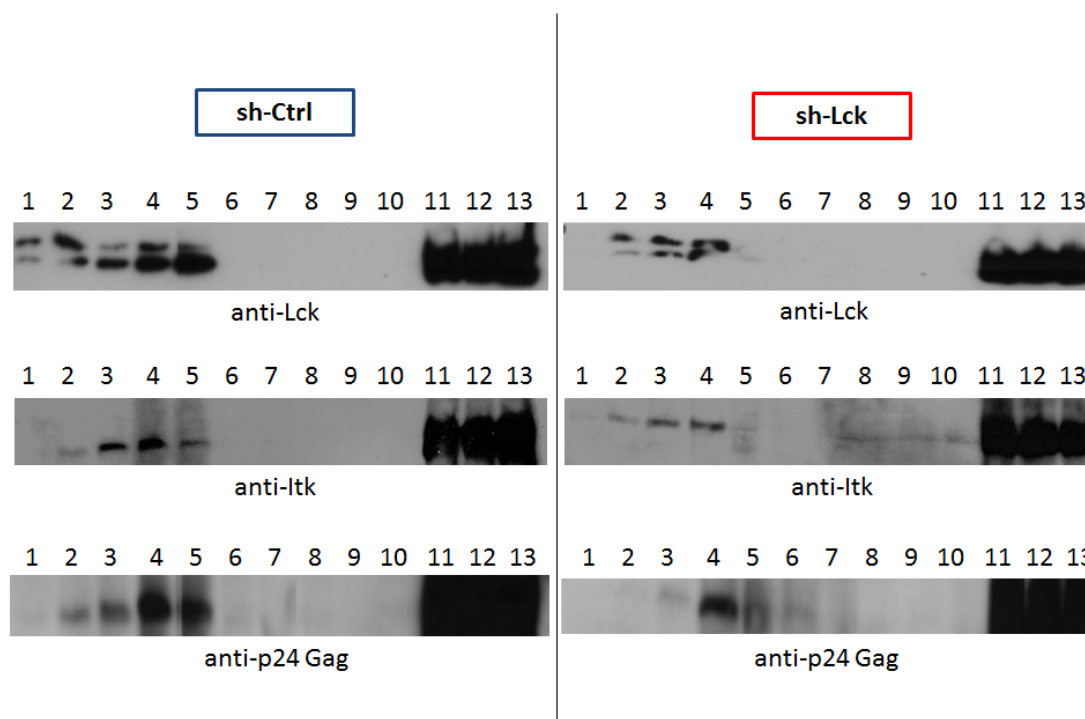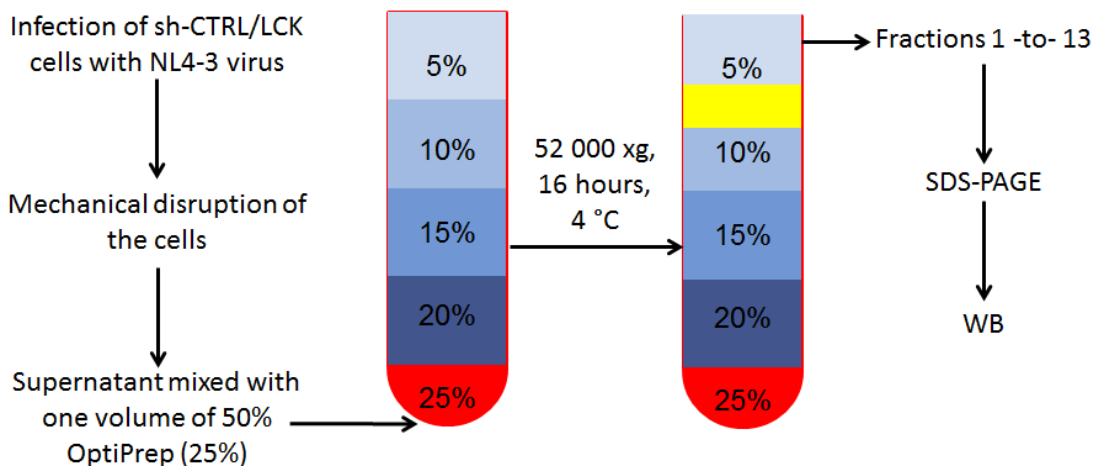
Figure 39. Lck Co-Fractionates with Gag in Lipid Rafts.

A) The experimental workflow used for subcellular fractionation of HIV-1-infected cell lines. B) Jurkat T cells stably transduced with sh-Lck or sh-Ctrl lentivirus were infected with HIV-1 for 7 days. Cells were lysed in buffer A containing 0.25% Triton X-100 at 4°C and the lysate was mechanistically disrupted. Lysates were mixed with one volume of 50% OptiPrep and centrifuged at 52,000 xg for 16 h at 4 °C. Thirteen fractions were collected from the top of the gradient, and analyzed by western blot. Fractions 1-5 are predicted to contain lipid rafts (Triantafilou et al., 2002).

REFERENCES

Agbottah, E., Deng, L., Dannenberg, L.O., Pumfery, A., and Kashanchi, F. (2006). Effect of SWI/SNF chromatin remodeling complex on HIV-1 Tat activated transcription. Retrovirology *3*, 48.

Amet, T., Nonaka, M., Dewan, M.Z., Saitoh, Y., Qi, X., Ichinose, S., Yamamoto, N., and Yamaoka, S. (2008). Statin-induced inhibition of HIV-1 release from latently infected U1 cells reveals a critical role for protein prenylation in HIV-1 replication. Microbes and infection / Institut Pasteur *10*, 471-480.

Amsen, D., Spilianakis, C.G., and Flavell, R.A. (2009). How are T(H)1 and T(H)2 effector cells made? Current opinion in immunology *21*, 153-160.

Ancelin, K., Lange, U.C., Hajkova, P., Schneider, R., Bannister, A.J., Kouzarides, T., and Surani, M.A. (2006). Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. Nature cell biology *8*, 623-630.

Angelov, D., Charra, M., Seve, M., Cote, J., Khochbin, S., and Dimitrov, S. (2000). Differential remodeling of the HIV-1 nucleosome upon transcription activators and SWI/SNF complex binding. Journal of molecular biology *302*, 315-326.

Anton, O., Batista, A., Millan, J., Andres-Delgado, L., Puertollano, R., Correas, I., and Alonso, M.A. (2008). An essential role for the MAL protein in targeting Lck to the plasma membrane of human T lymphocytes. The Journal of experimental medicine *205*, 3201-3213.

Archin, N.M., Cheema, M., Parker, D., Wiegand, A., Bosch, R.J., Coffin, J.M., Eron, J., Cohen, M., and Margolis, D.M. (2010). Antiretroviral intensification and valproic acid lack sustained effect on residual HIV-1 viremia or resting CD4+ cell infection. PloS one *5*, e9390.

Archin, N.M., Liberty, A.L., Kashuba, A.D., Choudhary, S.K., Kuruc, J.D., Crooks, A.M., Parker, D.C., Anderson, E.M., Kearney, M.F., Strain, M.C., *et al.* (2012). Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature *487*, 482-485.

Bacchus, C., Cheret, A., Avettand-Fenoel, V., Nembot, G., Melard, A., Blanc, C., Lascoux-Combe, C., Slama, L., Allegre, T., Allavena, C., *et al.* (2013). A single HIV-1 cluster and a skewed immune homeostasis drive the early spread of HIV among resting CD4+ cell subsets within one month post-infection. PloS one *8*, e64219.

Barnes, N.A., Stephenson, S.J., Tooze, R.M., and Doody, G.M. (2009). Amino acid deprivation links BLIMP-1 to the immunomodulatory enzyme indoleamine 2,3-dioxygenase. Journal of Immunology *183*, 5768-5777.

Baron, B.W., Desai, M., Baber, L.J., Paras, L., Zhang, Q., Sadhu, A., Duguay, S., Nucifora, G., McKeithan, T.W., and Zeleznik-Le, N. (1997). BCL6 can repress transcription from the human immunodeficiency virus type I promoter/enhancer region. Genes, chromosomes & cancer *19*, 14-21.

Barre-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., *et al.* (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science *220*, 868-871.

Barton, K., and Margolis, D. (2013). Selective targeting of the repressive transcription factors YY1 and cMyc to disrupt quiescent human immunodeficiency viruses. AIDS research and human retroviruses *29*, 289-298.

Bassuk, A.G., Anandappa, R.T., and Leiden, J.M. (1997). Physical interactions between Ets and NF-kappaB/NFAT proteins play an important role in their cooperative activation of the human immunodeficiency virus enhancer in T cells. Journal of virology *71*, 3563-3573.

Bentley, K., Deacon, N., Sonza, S., Zeichner, S., and Churchill, M. (2004). Mutational analysis of the HIV-1 LTR as a promoter of negative sense transcription. Archives of virology *149*, 2277-2294.

Bikoff, E.K., Morgan, M.A., and Robertson, E.J. (2009). An expanding job description for Blimp-1/PRDM1. Current opinion in genetics & development *19*, 379-385.

Birney, E., Stamatoyannopoulos, J.A., Dutta, A., Guigo, R., Gingeras, T.R., Margulies, E.H., Weng, Z., Snyder, M., Dermitzakis, E.T., Thurman, R.E., *et al.* (2007). Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature *447*, 799-816.

Bisgrove, D.A., Mahmoudi, T., Henklein, P., and Verdin, E. (2007). Conserved P-TEFb-interacting domain of BRD4 inhibits HIV transcription. Proceedings of the National Academy of Sciences of the United States of America *104*, 13690-13695.

Blazkova, J., Trejbalova, K., Gondois-Rey, F., Halfon, P., Philibert, P., Guiguen, A., Verdin, E., Olive, D., Van Lint, C., Hejnar, J., *et al.* (2009). CpG methylation controls reactivation of HIV from latency. PLoS pathogens *5*, e1000554.

Blokzijl, A., ten Dijke, P., and Ibanez, C.F. (2002). Physical and functional interaction between GATA-3 and Smad3 allows TGF-beta regulation of GATA target genes. Current biology : CB *12*, 35-45.

Blonska, M., Joo, D., Zweidler-McKay, P.A., Zhao, Q., and Lin, X. (2012). CARMA1 controls Th2 cell-specific cytokine expression through regulating JunB and GATA3 transcription factors. Journal of Immunology *188*, 3160-3168.

Boettler, T., Choi, Y.S., Salek-Ardakani, S., Cheng, Y., Moeckel, F., Croft, M., Crotty, S., and von Herrath, M. (2013). Exogenous OX40 stimulation during lymphocytic choriomeningitis virus infection impairs follicular Th cell differentiation and diverts CD4 T cells into the effector lineage by upregulating Blimp-1. Journal of Immunology *191*, 5026-5035.

Bosque, A., Famiglietti, M., Weyrich, A.S., Goulston, C., and Planelles, V. (2011). Homeostatic proliferation fails to efficiently reactivate HIV-1 latently infected central memory CD4+ T cells. PLoS pathogens *7*, e1002288.

Bosque, A., and Planelles, V. (2009). Induction of HIV-1 latency and reactivation in primary memory CD4+ T cells. Blood *113*, 58-65.

Bres, V., Gomes, N., Pickle, L., and Jones, K.A. (2005). A human splicing factor, SKIP, associates with P-TEFb and enhances transcription elongation by HIV-1 Tat. Genes & development *19*, 1211-1226.

Bres, V., Yoshida, T., Pickle, L., and Jones, K.A. (2009). SKIP interacts with c-Myc and Menin to promote HIV-1 Tat transactivation. Molecular cell *36*, 75-87.

Budhiraja, S., Famiglietti, M., Bosque, A., Planelles, V., and Rice, A.P. (2013). Cyclin T1 and CDK9 T-loop phosphorylation are downregulated during establishment of HIV-1 latency in primary resting memory CD4+ T cells. Journal of virology *87*, 1211-1220.

Budhiraja, S., Ramakrishnan, R., and Rice, A.P. (2012). Phosphatase PPM1A negatively regulates P-TEFb function in resting CD4(+) T cells and inhibits HIV-1 gene expression. Retrovirology *9*, 52.

Bunch, H., Zheng, X., Burkholder, A., Dillon, S.T., Motola, S., Birrane, G., Ebmeier, C.C., Levine, S., Fargo, D., Hu, G., *et al.* (2014). TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause release. Nature structural & molecular biology.

Burchill, M.A., Yang, J., Vogtenhuber, C., Blazar, B.R., and Farrar, M.A. (2007). IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3+ regulatory T cells. Journal of Immunology *178*, 280-290.

Buzon, M.J., Sun, H., Li, C., Shaw, A., Seiss, K., Ouyang, Z., Martin-Gayo, E., Leng, J., Henrich, T.J., Li, J.Z., *et al.* (2014). HIV-1 persistence in CD4+ T cells with stem cell-like properties. Nature medicine *20*, 139-142.

Byun, J.S., Fufa, T.D., Wakano, C., Fernandez, A., Haggerty, C.M., Sung, M.H., and Gardner, K. (2012). ELL facilitates RNA polymerase II pause site entry and release. Nature communications *3*, 633.

Calvanese, V., Chavez, L., Laurent, T., Ding, S., and Verdin, E. (2013). Dual-color HIV reporters trace a population of latently infected cells and enable their purification. Virology *446*, 283-292.

Cannons, J.L., and Schwartzberg, P.L. (2004). Fine-tuning lymphocyte regulation: what's new with tyrosine kinases and phosphatases? Current opinion in immunology *16*, 296-303.

Catalfamo, M., Di Mascio, M., Hu, Z., Srinivasula, S., Thaker, V., Adelsberger, J., Rupert, A., Baseler, M., Tagaya, Y., Roby, G., *et al.* (2008). HIV infection-associated immune activation occurs by two distinct pathways that differentially affect CD4 and CD8 T cells. Proceedings of the National Academy of Sciences of the United States of America *105*, 19851-19856.

Chan, Y.H., Chiang, M.F., Tsai, Y.C., Su, S.T., Chen, M.H., Hou, M.S., and Lin, K.I. (2009). Absence of the transcriptional repressor Blimp-1 in hematopoietic lineages reveals its role in dendritic cell homeostatic development and function. Journal of Immunology *183*, 7039-7046.

Chang, D.H., Angelin-Duclos, C., and Calame, K. (2000). BLIMP-1: trigger for differentiation of myeloid lineage. Nature immunology *1*, 169-176.

Chang, H.C., Han, L., Jabeen, R., Carotta, S., Nutt, S.L., and Kaplan, M.H. (2009). PU.1 regulates TCR expression by modulating GATA-3 activity. Journal of Immunology *183*, 4887-4894.

Chang, H.C., Sehra, S., Goswami, R., Yao, W., Yu, Q., Stritesky, G.L., Jabeen, R., McKinley, C., Ahyi, A.N., Han, L., *et al.* (2010). The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation. Nature immunology *11*, 527-534.

Chang, T.L., Mosoian, A., Pine, R., Klotman, M.E., and Moore, J.P. (2002). A soluble factor(s) secreted from CD8(+) T lymphocytes inhibits human immunodeficiency virus type 1 replication through STAT1 activation. Journal of virology *76*, 569-581.

Che, K.F., Shankar, E.M., Muthu, S., Zandi, S., Sigvardsson, M., Hinkula, J., Messmer, D., and Larsson, M. (2012). p38 Mitogen-activated protein kinase/signal transducer and activator of transcription-3 pathway signaling regulates expression of inhibitory molecules in T cells activated by HIV-1-exposed dendritic cells. Mol Med *18*, 1169-1182.

Chen, A.K., Sengupta, P., Waki, K., Van Engelenburg, S.B., Ochiya, T., Ablan, S.D., Freed, E.O., and Lippincott-Schwartz, J. (2014). MicroRNA binding to the HIV-1 Gag protein inhibits Gag assembly and virus production. Proceedings of the National Academy of Sciences of the United States of America *111*, E2676-2683.

Chen, J., Malcolm, T., Estable, M.C., Roeder, R.G., and Sadowski, I. (2005). TFII-I regulates induction of chromosomally integrated human immunodeficiency virus type 1 long terminal repeat in cooperation with USF. Journal of virology *79*, 4396-4406.

Chen, W., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., and Wahl, S.M. (2003). Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. The Journal of experimental medicine *198*, 1875-1886.

Cheng, B., Li, T., Rahl, P.B., Adamson, T.E., Loudas, N.B., Guo, J., Varzavand, K., Cooper, J.J., Hu, X., Gnatt, A., *et al.* (2012). Functional association of Gdown1 with RNA polymerase II poised on human genes. Molecular cell *45*, 38-50.

Chevalier, M.F., and Weiss, L. (2013). The split personality of regulatory T cells in HIV infection. Blood *121*, 29-37.

Chiang, M.F., Yang, S.Y., Lin, I.Y., Hong, J.B., Lin, S.J., Ying, H.Y., Chen, C.M., Wu, S.Y., Liu, F.T., and Lin, K.I. (2013). Inducible deletion of the Blimp-1 gene in adult epidermis causes granulocyte-dominated chronic skin inflammation in mice. Proceedings of the National Academy of Sciences of the United States of America *110*, 6476-6481.

Choi, Y.S., Eto, D., Yang, J.A., Lao, C., and Crotty, S. (2013). Cutting edge: STAT1 is required for IL-6-mediated Bcl6 induction for early follicular helper cell differentiation. Journal of Immunology *190*, 3049-3053.

Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F.A., Yassine-Diab, B., Boucher, G., Boulassel, M.R., Ghattas, G., Brenchley, J.M., *et al.* (2009). HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nature medicine *15*, 893-900.

Chukkapalli, V., Hogue, I.B., Boyko, V., Hu, W.S., and Ono, A. (2008). Interaction between the human immunodeficiency virus type 1 Gag matrix domain and phosphatidylinositol-(4,5)-bisphosphate is essential for efficient gag membrane binding. Journal of virology *82*, 2405-2417.

Cimmino, L., Martins, G.A., Liao, J., Magnusdottir, E., Grunig, G., Perez, R.K., and Calame, K.L. (2008). Blimp-1 attenuates Th1 differentiation by repression of ifng, tbx21, and bcl6 gene expression. Journal of Immunology *181*, 2338-2347.

Ciuffi, A., Llano, M., Poeschla, E., Hoffmann, C., Leipzig, J., Shinn, P., Ecker, J.R., and Bushman, F. (2005). A role for LEDGF/p75 in targeting HIV DNA integration. Nature medicine *11*, 1287-1289.

Coyle-Rink, J., Sweet, T., Abraham, S., Sawaya, B., Batuman, O., Khalili, K., and Amini, S. (2002). Interaction between TGFbeta signaling proteins and C/EBP controls basal and Tat-mediated transcription of HIV-1 LTR in astrocytes. Virology *299*, 240-247.

Crawford, A., Angelosanto, J.M., Kao, C., Doering, T.A., Odorizzi, P.M., Barnett, B.E., and Wherry, E.J. (2014). Molecular and transcriptional basis of CD4(+) T cell dysfunction during chronic infection. Immunity *40*, 289-302.

Cretney, E., Xin, A., Shi, W., Minnich, M., Masson, F., Miasari, M., Belz, G.T., Smyth, G.K., Busslinger, M., Nutt, S.L., *et al.* (2011). The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. Nature immunology *12*, 304-311.

Cron, R.Q., Bartz, S.R., Clausell, A., Bort, S.J., Klebanoff, S.J., and Lewis, D.B. (2000). NFAT1 enhances HIV-1 gene expression in primary human CD4 T cells. Clin Immunol *94*, 179-191.

Crotti, A., Lusic, M., Lupo, R., Lievens, P.M., Liboi, E., Della Chiara, G., Tinelli, M., Lazzarin, A., Patterson, B.K., Giacca, M., *et al.* (2007). Naturally occurring C-terminally truncated STAT5 is a negative regulator of HIV-1 expression. Blood *109*, 5380-5389.

Crotty, S., Johnston, R.J., and Schoenberger, S.P. (2010). Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. Nature immunology *11*, 114-120.

Dahabieh, M.S., Ooms, M., Malcolm, T., Simon, V., and Sadowski, I. (2011). Identification and functional analysis of a second RBF-2 binding site within the HIV-1 promoter. Virology *418*, 57-66.

Dahabieh, M.S., Ooms, M., Simon, V., and Sadowski, I. (2013). A doubly fluorescent HIV-1 reporter shows that the majority of integrated HIV-1 is latent shortly after infection. Journal of virology *87*, 4716-4727.

Dasgupta, P., Saikumar, P., Reddy, C.D., and Reddy, E.P. (1990). Myb protein binds to human immunodeficiency virus 1 long terminal repeat (LTR) sequences and transactivates LTR-mediated transcription. Proceedings of the National Academy of Sciences of the United States of America *87*, 8090-8094.

De Ingeniis, J., Ratnikov, B., Richardson, A.D., Scott, D.A., Aza-Blanc, P., De, S.K., Kazanov, M., Pellecchia, M., Ronai, Z., Osterman, A.L., *et al.* (2012). Functional specialization in proline biosynthesis of melanoma. PloS one *7*, e45190.

de Masson, A., Kirilovsky, A., Zoorob, R., Avettand-Fenoel, V., Morin, V., Oudin, A., Descours, B., Rouzioux, C., and Autran, B. (2014). Blimp-1 overexpression is associated with low HIV-1 reservoir and transcription levels in central memory CD4+ T cells from elite controllers. AIDS.

Deeks, S.G., Autran, B., Berkhout, B., Benkirane, M., Cairns, S., Chomont, N., Chun, T.W., Churchill, M., Di Mascio, M., Katlama, C., *et al.* (2012). Towards an HIV cure: a global scientific strategy. Nature reviews Immunology *12*, 607-614.

Della Chiara, G., Crotti, A., Liboi, E., Giacca, M., Poli, G., and Lusic, M. (2011). Negative regulation of HIV-1 transcription by a heterodimeric NF-kappaB1/p50 and C-terminally truncated STAT5 complex. Journal of molecular biology *410*, 933-943.

Deng, L., Wang, D., de la Fuente, C., Wang, L., Li, H., Lee, C.G., Donnelly, R., Wade, J.D., Lambert, P., and Kashanchi, F. (2001). Enhancement of the p300 HAT activity by HIV-1 Tat on chromatin DNA. Virology *289*, 312-326.

Diehl, F., Brown, M.A., van Amerongen, M.J., Novoyatleva, T., Wietelmann, A., Harriss, J., Ferrazzi, F., Bottger, T., Harvey, R.P., Tucker, P.W., *et al.* (2010). Cardiac deletion of Smyd2 is dispensable for mouse heart development. PloS one *5*, e9748.

Ding, D., Qu, X., Li, L., Zhou, X., Liu, S., Lin, S., Wang, P., Kong, C., Wang, X., Liu, L., *et al.* (2013). Involvement of histone methyltransferase GLP in HIV-1 latency through catalysis of H3K9 dimethylation. Virology *440*, 182-189.

Donahue, D.A., Bastarache, S.M., Sloan, R.D., and Wainberg, M.A. (2013). Latent HIV-1 can be reactivated by cellular superinfection in a Tat-dependent manner, which can lead to the emergence of multidrug-resistant recombinant viruses. Journal of virology *87*, 9620-9632.

Donahue, D.A., Kuhl, B.D., Sloan, R.D., and Wainberg, M.A. (2012). The viral protein Tat can inhibit the establishment of HIV-1 latency. Journal of virology *86*, 3253-3263.

Donaldson, J.G., Porat-Shliom, N., and Cohen, L.A. (2009). Clathrin-independent endocytosis: a unique platform for cell signaling and PM remodeling. Cellular signalling *21*, 1-6.

du Chene, I., Basyuk, E., Lin, Y.L., Triboulet, R., Knezevich, A., Chable-Bessia, C., Mettling, C., Baillat, V., Reynes, J., Corbeau, P., *et al.* (2007). Suv39H1 and HP1gamma are responsible for chromatin-mediated HIV-1 transcriptional silencing and post-integration latency. The EMBO journal *26*, 424-435.

Dutta, A., Miaw, S.C., Yu, J.S., Chen, T.C., Lin, C.Y., Lin, Y.C., Chang, C.S., He, Y.C., Chuang, S.H., Yen, M.I., *et al.* (2013). Altered T-bet dominance in IFN-gamma-decoupled CD4+ T cells with attenuated cytokine storm and preserved memory in influenza. Journal of Immunology *190*, 4205-4214.

Duverger, A., Jones, J., May, J., Bibollet-Ruche, F., Wagner, F.A., Cron, R.Q., and Kutsch, O. (2009). Determinants of the establishment of human immunodeficiency virus type 1 latency. Journal of virology *83*, 3078-3093.

Easley, R., Carpio, L., Dannenberg, L., Choi, S., Alani, D., Van Duyne, R., Guendel, I., Klase, Z., Agbottah, E., Kehn-Hall, K., *et al.* (2010). Transcription through the HIV-1 nucleosomes: effects of the PBAF complex in Tat activated transcription. Virology *405*, 322-333.

Elyaman, W., Bassil, R., Bradshaw, E.M., Orent, W., Lahoud, Y., Zhu, B., Radtke, F., Yagita, H., and Khoury, S.J. (2012). Notch receptors and Smad3 signaling cooperate in the induction of interleukin-9-producing T cells. Immunity *36*, 623-634.

Eszterhas, S.K., Bouhassira, E.E., Martin, D.I., and Fiering, S. (2002). Transcriptional interference by independently regulated genes occurs in any relative arrangement of the genes and is influenced by chromosomal integration position. Molecular and cellular biology *22*, 469-479.

Faria, N.R., Rambaut, A., Suchard, M.A., Baele, G., Bedford, T., Ward, M.J., Tatem, A.J., Sousa, J.D., Arinaminpathy, N., Pepin, J., *et al.* (2014). HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. Science *346*, 56-61.

Fauci, A.S., Pantaleo, G., Stanley, S., and Weissman, D. (1996). Immunopathogenic mechanisms of HIV infection. Annals of internal medicine *124*, 654-663.

Felber, B.K., Hadzopoulou-Cladaras, M., Cladaras, C., Copeland, T., and Pavlakis, G.N. (1989). rev protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA. Proceedings of the National Academy of Sciences of the United States of America *86*, 1495-1499.

Finzi, A., Orthwein, A., Mercier, J., and Cohen, E.A. (2007). Productive human immunodeficiency virus type 1 assembly takes place at the plasma membrane. Journal of virology *81*, 7476-7490.

Friedman, J., Cho, W.K., Chu, C.K., Keedy, K.S., Archin, N.M., Margolis, D.M., and Karn, J. (2011). Epigenetic silencing of HIV-1 by the histone H3 lysine 27 methyltransferase enhancer of Zeste 2. Journal of virology *85*, 9078-9089.

Fu, W., Ergun, A., Lu, T., Hill, J.A., Haxhinasto, S., Fassett, M.S., Gazit, R., Adoro, S., Glimcher, L., Chan, S., *et al.* (2012). A multiply redundant genetic switch 'locks in' the transcriptional signature of regulatory T cells. Nature immunology *13*, 972-980.

Fujinaga, K., Irwin, D., Huang, Y., Taube, R., Kurosu, T., and Peterlin, B.M. (2004). Dynamics of human immunodeficiency virus transcription: P-TEFb phosphorylates RD and dissociates negative effectors from the transactivation response element. Molecular and cellular biology *24*, 787-795.

Furia, B., Deng, L., Wu, K., Baylor, S., Kehn, K., Li, H., Donnelly, R., Coleman, T., and Kashanchi, F. (2002). Enhancement of nuclear factor-kappa B acetylation by coactivator p300 and HIV-1 Tat proteins. The Journal of biological chemistry *277*, 4973-4980.

Gaertner, B., Johnston, J., Chen, K., Wallaschek, N., Paulson, A., Garruss, A.S., Gaudenz, K., De Kumar, B., Krumlauf, R., and Zeitlinger, J. (2012). Poised RNA polymerase II changes over developmental time and prepares genes for future expression. Cell reports *2*, 1670-1683.

Galio, L., Briquet, S., Cot, S., Guillet, J.G., and Vaquero, C. (1997). Analysis of interactions between huGATA-3 transcription factor and three GATA regulatory elements of HIV-1 long terminal repeat, by surface plasmon resonance. Analytical biochemistry *253*, 70-77.

Gallo, R.C., Sarin, P.S., Gelmann, E.P., Robert-Guroff, M., Richardson, E., Kalyanaraman, V.S., Mann, D., Sidhu, G.D., Stahl, R.E., Zolla-Pazner, S., *et al.* (1983). Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science *220*, 865-867.

Garber, M.E., Wei, P., and Jones, K.A. (1998). HIV-1 Tat interacts with cyclin T1 to direct the P-TEFb CTD kinase complex to TAR RNA. Cold Spring Harbor symposia on quantitative biology *63*, 371-380.

Garcia-Bates, T.M., Baglole, C.J., Bernard, M.P., Murant, T.I., Simpson-Haidaris, P.J., and Phipps, R.P. (2009). Peroxisome proliferator-activated receptor gamma ligands enhance human B cell antibody production and differentiation. Journal of Immunology *183*, 6903-6912.

Garrus, J.E., von Schwedler, U.K., Pornillos, O.W., Morham, S.G., Zavitz, K.H., Wang, H.E., Wettstein, D.A., Stray, K.M., Cote, M., Rich, R.L., *et al.* (2001). Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. Cell *107*, 55-65.

Gattinoni, L., Lugli, E., Ji, Y., Pos, Z., Paulos, C.M., Quigley, M.F., Almeida, J.R., Gostick, E., Yu, Z., Carpenito, C., *et al.* (2011). A human memory T cell subset with stem cell-like properties. Nature medicine *17*, 1290-1297.

Gegonne, A., Bosselut, R., Bailly, R.A., and Ghysdael, J. (1993). Synergistic activation of the HTLV1 LTR Ets-responsive region by transcription factors Ets1 and Sp1. The EMBO journal *12*, 1169-1178.

Genestier, L., Taillardet, M., Mondiere, P., Gheit, H., Bella, C., and Defrance, T. (2007). TLR agonists selectively promote terminal plasma cell differentiation of B cell subsets specialized in thymus-independent responses. Journal of Immunology *178*, 7779-7786.

Giroux, M., Delisle, J.S., Gauthier, S.D., Heinonen, K.M., Hinsinger, J., Houde, B., Gaboury, L., Brochu, S., Wu, J., Hebert, M.J., *et al.* (2011). SMAD3 prevents graft-versus-host disease by restraining Th1 differentiation and granulocyte-mediated tissue damage. Blood *117*, 1734-1744.

Goila-Gaur, R., and Strebel, K. (2008). HIV-1 Vif, APOBEC, and intrinsic immunity. Retrovirology *5*, 51.

Gong, D., and Malek, T.R. (2007). Cytokine-dependent Blimp-1 expression in activated T cells inhibits IL-2 production. Journal of Immunology *178*, 242-252.

Gorska, M.M., Liang, Q., Karim, Z., and Alam, R. (2009). Uncoordinated 119 protein controls trafficking of Lck via the Rab11 endosome and is critical for immunological synapse formation. Journal of Immunology *183*, 1675-1684.

Gosselin, A., Monteiro, P., Chomont, N., Diaz-Griffero, F., Said, E.A., Fonseca, S., Wacleche, V., El-Far, M., Boulassel, M.R., Routy, J.P., *et al.* (2010). Peripheral blood CCR4+CCR6+ and CXCR3+CCR6+CD4+ T cells are highly permissive to HIV-1 infection. Journal of Immunology *184*, 1604-1616.

Grant, C., Oh, U., Fugo, K., Takenouchi, N., Griffith, C., Yao, K., Newhook, T.E., Ratner, L., and Jacobson, S. (2006). Foxp3 represses retroviral transcription by targeting both NF-kappaB and CREB pathways. PLoS pathogens *2*, e33.

Grenningloh, R., Kang, B.Y., and Ho, I.C. (2005). Ets-1, a functional cofactor of T-bet, is essential for Th1 inflammatory responses. The Journal of experimental medicine *201*, 615-626.

Gu, J.J., Ryu, J.R., and Pendergast, A.M. (2009). Abl tyrosine kinases in T-cell signaling. Immunological reviews *228*, 170-183.

Gummuluru, S., and Emerman, M. (1999). Cell cycle- and Vpr-mediated regulation of human immunodeficiency virus type 1 expression in primary and transformed T-cell lines. Journal of virology *73*, 5422-5430.

Guth, C.A., and Sodroski, J. (2014). Contribution of PDZD8 to stabilization of the human immunodeficiency virus type 1 capsid. Journal of virology *88*, 4612-4623.

Gyory, I., Fejer, G., Ghosh, N., Seto, E., and Wright, K.L. (2003). Identification of a functionally impaired positive regulatory domain I binding factor 1 transcription repressor in myeloma cell lines. Journal of Immunology *170*, 3125-3133.

Gyory, I., Wu, J., Fejer, G., Seto, E., and Wright, K.L. (2004). PRDI-BF1 recruits the histone H3 methyltransferase G9a in transcriptional silencing. Nature immunology *5*, 299-308.

Hadjur, S., Bruno, L., Hertweck, A., Cobb, B.S., Taylor, B., Fisher, A.G., and Merkenschlager, M. (2009). IL4 blockade of inducible regulatory T cell differentiation: the role of Th2 cells, Gata3 and PU.1. Immunology letters *122*, 37-43.

Halper-Stromberg, A., Lu, C.L., Klein, F., Horwitz, J.A., Bournazos, S., Nogueira, L., Eisenreich, T.R., Liu, C., Gazumyan, A., Schaefer, U., *et al.* (2014). Broadly Neutralizing Antibodies and Viral Inducers Decrease Rebound from HIV-1 Latent Reservoirs in Humanized Mice. Cell *158*, 989-999.

Hamamoto, R., Furukawa, Y., Morita, M., Iimura, Y., Silva, F.P., Li, M., Yagyu, R., and Nakamura, Y. (2004). SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. Nature cell biology *6*, 731-740.

Hasgall, P.A., Hoogewijs, D., Faza, M.B., Panse, V.G., Wenger, R.H., and Camenisch, G. (2011). The putative RNA helicase HELZ promotes cell proliferation, translation initiation and ribosomal protein S6 phosphorylation. PloS one *6*, e22107.

He, G., and Margolis, D.M. (2002). Counterregulation of chromatin deacetylation and histone deacetylase occupancy at the integrated promoter of human immunodeficiency virus type 1 (HIV-1) by the HIV-1 repressor YY1 and HIV-1 activator Tat. Molecular and cellular biology *22*, 2965-2973.

He, N., Chan, C.K., Sobhian, B., Chou, S., Xue, Y., Liu, M., Alber, T., Benkirane, M., and Zhou, Q. (2011). Human Polymerase-Associated Factor complex (PAFc) connects the Super Elongation Complex (SEC) to RNA polymerase II on chromatin. Proceedings of the National Academy of Sciences of the United States of America *108*, E636-645.

Henderson, A., Holloway, A., Reeves, R., and Tremethick, D.J. (2004). Recruitment of SWI/SNF to the human immunodeficiency virus type 1 promoter. Molecular and cellular biology *24*, 389-397.

Henderson, A.J., and Calame, K.L. (1997). CCAAT/enhancer binding protein (C/EBP) sites are required for HIV-1 replication in primary macrophages but not CD4(+) T cells. Proceedings of the National Academy of Sciences of the United States of America *94*, 8714-8719.

Henderson, A.J., Zou, X., and Calame, K.L. (1995). C/EBP proteins activate transcription from the human immunodeficiency virus type 1 long terminal repeat in macrophages/monocytes. Journal of virology *69*, 5337-5344.

Henning, M.S., Morham, S.G., Goff, S.P., and Naghavi, M.H. (2010). PDZD8 is a novel Gag-interacting factor that promotes retroviral infection. Journal of virology *84*, 8990-8995.

Henning, M.S., Stiedl, P., Barry, D.S., McMahon, R., Morham, S.G., Walsh, D., and Naghavi, M.H. (2011). PDZD8 is a novel moesin-interacting cytoskeletal regulatory protein that suppresses infection by herpes simplex virus type 1. Virology *415*, 114-121.

Henrich, T.J., and Gandhi, R.T. (2013). Early Treatment and HIV-1 Reservoirs: A Stitch in Time? The Journal of infectious diseases.

Henrich, T.J., Hanhauser, E., Marty, F.M., Sirignano, M.N., Keating, S., Lee, T.H., Robles, Y.P., Davis, B.T., Li, J.Z., Heisey, A., *et al.* (2014). Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. Annals of internal medicine *161*, 319-327.

Hill, A.L., Rosenbloom, D.I., Fu, F., Nowak, M.A., and Siliciano, R.F. (2014). Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. Proceedings of the National Academy of Sciences of the United States of America *111*, 13475-13480.

Hirschhorn-Cymerman, D., Budhu, S., Kitano, S., Liu, C., Zhao, F., Zhong, H., Lesokhin, A.M., Avogadri-Connors, F., Yuan, J., Li, Y., *et al.* (2012). Induction of tumoricidal function in CD4+ T cells is associated with concomitant memory and terminally differentiated phenotype. The Journal of experimental medicine *209*, 2113-2126.

Ho, I.C., Lo, D., and Glimcher, L.H. (1998). c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. The Journal of experimental medicine *188*, 1859-1866.

Ho, Y.C., Shan, L., Hosmane, N.N., Wang, J., Laskey, S.B., Rosenbloom, D.I., Lai, J., Blankson, J.N., Siliciano, J.D., and Siliciano, R.F. (2013). Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell *155*, 540-551.

Hodge, L.S., Ziesmer, S.C., Yang, Z.Z., Secreto, F.J., Gertz, M.A., Novak, A.J., and Ansell, S.M. (2012). IL-21 in the bone marrow microenvironment contributes to IgM secretion and proliferation of malignant cells in Waldenstrom macroglobulinemia. Blood *120*, 3774-3782.

Holmes, D., Gao, J., and Su, L. (2011). Foxp3 inhibits HDAC1 activity to modulate gene expression in human T cells. Virology *421*, 12-18.

Holmes, D., Knudsen, G., Mackey-Cushman, S., and Su, L. (2007). FoxP3 enhances HIV-1 gene expression by modulating NFkappaB occupancy at the long terminal repeat in human T cells. The Journal of biological chemistry *282*, 15973-15980.

Horie, R., Ishida, T., Maruyama-Nagai, M., Ito, K., Watanabe, M., Yoneyama, A., Higashihara, M., Kimura, S., and Watanabe, T. (2007). TRAF activation of C/EBPbeta (NF-IL6) via p38 MAPK induces HIV-1 gene expression in monocytes/macrophages. Microbes and infection / Institut Pasteur *9*, 721-728.

Hu, L., Gocke, A.R., Knapp, E., Rosenzweig, J.M., Grishkan, I.V., Baxi, E.G., Zhang, H., Margolick, J.B., Whartenby, K.A., and Calabresi, P.A. (2012). Functional blockade of the voltage-gated potassium channel Kv1.3 mediates reversion of T effector to central memory lymphocytes through SMAD3/p21cip1 signaling. The Journal of biological chemistry *287*, 1261-1268.

Hua, L., Yao, S., Pham, D., Jiang, L., Wright, J., Sawant, D., Dent, A.L., Braciale, T.J., Kaplan, M.H., and Sun, J. (2013). Cytokine-Dependent Induction of CD4+ T cells with Cytotoxic Potential during Influenza Virus Infection. Journal of virology *87*, 11884-11893.

Huss, D.J., Winger, R.C., Cox, G.M., Guerau-de-Arellano, M., Yang, Y., Racke, M.K., and Lovett-Racke, A.E. (2011). TGF-beta signaling via Smad4 drives IL-10 production in effector Th1 cells and reduces T-cell trafficking in EAE. European journal of immunology *41*, 2987-2996.

Ichii, H., Sakamoto, A., Arima, M., Hatano, M., Kuroda, Y., and Tokuhisa, T. (2007). Bcl6 is essential for the generation of long-term memory CD4+ T cells. International immunology *19*, 427-433.

Ikeda, T., Shibata, J., Yoshimura, K., Koito, A., and Matsushita, S. (2007). Recurrent HIV-1 integration at the BACH2 locus in resting CD4+ T cell populations during effective highly active antiretroviral therapy. The Journal of infectious diseases *195*, 716-725.

Imai, K., Togami, H., and Okamoto, T. (2010). Involvement of histone H3 lysine 9 (H3K9) methyltransferase G9a in the maintenance of HIV-1 latency and its reactivation by BIX01294. The Journal of biological chemistry *285*, 16538-16545.

Isel, C., and Karn, J. (1999). Direct evidence that HIV-1 Tat stimulates RNA polymerase II carboxyl-terminal domain hyperphosphorylation during transcriptional elongation. Journal of molecular biology *290*, 929-941.

Ivanov, D., Kwak, Y.T., Guo, J., and Gaynor, R.B. (2000). Domains in the SPT5 protein that modulate its transcriptional regulatory properties. Molecular and cellular biology *20*, 2970-2983.

Iwasaki, Y., Fujio, K., Okamura, T., Yanai, A., Sumitomo, S., Shoda, H., Tamura, T., Yoshida, H., Charnay, P., and Yamamoto, K. (2013). Egr-2 transcription factor is required for Blimp-1-mediated IL-10 production in IL-27-stimulated CD4+ T cells. European journal of immunology *43*, 1063-1073.

Jang, M.K., Mochizuki, K., Zhou, M., Jeong, H.S., Brady, J.N., and Ozato, K. (2005). The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. Molecular cell *19*, 523-534.

Jatzek, A., Tejera, M.M., Singh, A., Sullivan, J.A., Plisch, E.H., and Suresh, M. (2012). p27(Kip1) negatively regulates the magnitude and persistence of CD4 T cell memory. Journal of Immunology *189*, 5119-5128.

Johnston, R.J., Choi, Y.S., Diamond, J.A., Yang, J.A., and Crotty, S. (2012). STAT5 is a potent negative regulator of TFH cell differentiation. The Journal of experimental medicine *209*, 243-250.

Johnston, R.J., Poholek, A.C., DiToro, D., Yusuf, I., Eto, D., Barnett, B., Dent, A.L., Craft, J., and Crotty, S. (2009). Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science *325*, 1006-1010.

Jordan, A., Defechereux, P., and Verdin, E. (2001). The site of HIV-1 integration in the human genome determines basal transcriptional activity and response to Tat transactivation. The EMBO journal *20*, 1726-1738.

Juffroy, O., Bugault, F., Lambotte, O., Landires, I., Viard, J.P., Niel, L., Fontanet, A., Delfraissy, J.F., Theze, J., and Chakrabarti, L.A. (2010). Dual mechanism of impairment of interleukin-7 (IL-7) responses in human immunodeficiency virus infection: decreased IL-7 binding and abnormal activation of the JAK/STAT5 pathway. Journal of virology *84*, 96-108.

Kaczmarek, K., Morales, A., and Henderson, A.J. (2013). T Cell Transcription Factors and Their Impact on HIV Expression. Virology : research and treatment *2013*, 41-47.

Kallies, A., Hawkins, E.D., Belz, G.T., Metcalf, D., Hommel, M., Corcoran, L.M., Hodgkin, P.D., and Nutt, S.L. (2006). Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance. Nature immunology *7*, 466-474.

Kallies, A., Xin, A., Belz, G.T., and Nutt, S.L. (2009). Blimp-1 transcription factor is required for the differentiation of effector CD8(+) T cells and memory responses. Immunity *31*, 283-295.

Kanhere, A., Hertweck, A., Bhatia, U., Gokmen, M.R., Perucha, E., Jackson, I., Lord, G.M., and Jenner, R.G. (2012). T-bet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-specific targeting of distal regulatory elements. Nature communications *3*, 1268.

Karim, Z., Vepachedu, R., Gorska, M., and Alam, R. (2010). UNC119 inhibits dynamin and dynamin-dependent endocytic processes. Cellular signalling *22*, 128-137.

Kauder, S.E., Bosque, A., Lindqvist, A., Planelles, V., and Verdin, E. (2009). Epigenetic regulation of HIV-1 latency by cytosine methylation. PLoS pathogens *5*, e1000495.

Keedy, K.S., Archin, N.M., Gates, A.T., Espeseth, A., Hazuda, D.J., and Margolis, D.M. (2009). A limited group of class I histone deacetylases acts to repress human immunodeficiency virus type 1 expression. Journal of virology *83*, 4749-4756.

Keller, A.D., and Maniatis, T. (1991). Identification and characterization of a novel repressor of beta-interferon gene expression. Genes & development *5*, 868-879.

Kim, H.Y., Choi, B.S., Kim, S., Roh, T.Y., Park, J., and Yoon, C.H. (2014). NUCKS1, a novel Tat coactivator, plays a crucial role in HIV-1 replication by increasing Tat-mediated viral transcription on the HIV-1 LTR promoter. Retrovirology *11*, 67.

Kim, S.J., Zou, Y.R., Goldstein, J., Reizis, B., and Diamond, B. (2011). Tolerogenic function of Blimp-1 in dendritic cells. The Journal of experimental medicine *208*, 2193-2199.

Kim, Y.K., Bourgeois, C.F., Pearson, R., Tyagi, M., West, M.J., Wong, J., Wu, S.Y., Chiang, C.M., and Karn, J. (2006). Recruitment of TFIIH to the HIV LTR is a rate-limiting step in the emergence of HIV from latency. The EMBO journal *25*, 3596-3604.

Kimura, M.Y., Iwamura, C., Suzuki, A., Miki, T., Hasegawa, A., Sugaya, K., Yamashita, M., Ishii, S., and Nakayama, T. (2007). Schnurri-2 controls memory Th1 and Th2 cell numbers in vivo. Journal of Immunology *178*, 4926-4936.

Kinoshita, S., Chen, B.K., Kaneshima, H., and Nolan, G.P. (1998). Host control of HIV-1 parasitism in T cells by the nuclear factor of activated T cells. Cell *95*, 595-604.

Klein, K.C., Reed, J.C., and Lingappa, J.R. (2007). Intracellular destinies: degradation, targeting, assembly, and endocytosis of HIV Gag. AIDS reviews *9*, 150-161.

Kobayashi-Ishihara, M., Yamagishi, M., Hara, T., Matsuda, Y., Takahashi, R., Miyake, A., Nakano, K., Yamochi, T., Ishida, T., and Watanabe, T. (2012). HIV-1-encoded antisense RNA suppresses viral replication for a prolonged period. Retrovirology *9*, 38.

Kozuka, T., Sugita, M., Shetzline, S., Gewirtz, A.M., and Nakata, Y. (2011). c-Myb and GATA-3 cooperatively regulate IL-13 expression via conserved GATA-3 response element and recruit mixed lineage leukemia (MLL) for histone modification of the IL-13 locus. Journal of Immunology *187*, 5974-5982.

Kroenke, M.A., Eto, D., Locci, M., Cho, M., Davidson, T., Haddad, E.K., and Crotty, S. (2012). Bcl6 and Maf cooperate to instruct human follicular helper CD4 T cell differentiation. Journal of Immunology *188*, 3734-3744.

Krueger, B.J., Jeronimo, C., Roy, B.B., Bouchard, A., Barrandon, C., Byers, S.A., Searcey, C.E., Cooper, J.J., Bensaude, O., Cohen, E.A., *et al.* (2008). LARP7 is a stable component of the 7SK snRNP while P-TEFb, HEXIM1 and hnRNP A1 are reversibly associated. Nucleic acids research *36*, 2219-2229.

Kueck, T., and Neil, S.J. (2012). A cytoplasmic tail determinant in HIV-1 Vpu mediates targeting of tetherin for endosomal degradation and counteracts interferon-induced restriction. PLoS pathogens *8*, e1002609.

Kumar, A., Kumar Dorairaj, S., Prabhakaran, V.C., Prakash, D.R., and Chakraborty, S. (2007). Identification of genes associated with tumorigenesis of meibomian cell carcinoma by microarray analysis. Genomics *90*, 559-566.

Kumar, P.P., Purbey, P.K., Ravi, D.S., Mitra, D., and Galande, S. (2005). Displacement of SATB1-bound histone deacetylase 1 corepressor by the human immunodeficiency virus type 1 transactivator induces expression of interleukin-2 and its receptor in T cells. Molecular and cellular biology *25*, 1620-1633.

Kundu, M., Guermah, M., Roeder, R.G., Amini, S., and Khalili, K. (1997). Interaction between cell cycle regulator, E2F-1, and NF-kappaB mediates repression of HIV-1 gene transcription. The Journal of biological chemistry *272*, 29468-29474.

Kuo, C.T., Veselits, M.L., and Leiden, J.M. (1997). LKLF: A transcriptional regulator of single-positive T cell quiescence and survival. Science *277*, 1986-1990.

Lafeuillade, A. (2012). Eliminating the HIV reservoir. Current HIV/AIDS reports *9*, 121-131.

Landry, S., Halin, M., Lefort, S., Audet, B., Vaquero, C., Mesnard, J.M., and Barbeau, B. (2007). Detection, characterization and regulation of antisense transcripts in HIV-1. Retrovirology *4*, 71.

Larochelle, S., Amat, R., Glover-Cutter, K., Sanso, M., Zhang, C., Allen, J.J., Shokat, K.M., Bentley, D.L., and Fisher, R.P. (2012). Cyclin-dependent kinase control of the initiation-to-elongation switch of RNA polymerase II. Nature structural & molecular biology *19*, 1108-1115.

Lassen, K.G., Hebbeler, A.M., Bhattacharyya, D., Lobritz, M.A., and Greene, W.C. (2012). A flexible model of HIV-1 latency permitting evaluation of many primary CD4 T-cell reservoirs. PloS one *7*, e30176.

Laurence, A., Tato, C.M., Davidson, T.S., Kanno, Y., Chen, Z., Yao, Z., Blank, R.B., Meylan, F., Siegel, R., Hennighausen, L., *et al.* (2007). Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity *26*, 371-381.

Lazarevic, V., Chen, X., Shim, J.H., Hwang, E.S., Jang, E., Bolm, A.N., Oukka, M., Kuchroo, V.K., and Glimcher, L.H. (2011). T-bet represses T(H)17 differentiation by preventing Runx1-mediated activation of the gene encoding RORgammat. Nature immunology *12*, 96-104.

Le Douce, V., Colin, L., Redel, L., Cherrier, T., Herbein, G., Aunis, D., Rohr, O., Van Lint, C., and Schwartz, C. (2012). LSD1 cooperates with CTIP2 to promote HIV-1 transcriptional silencing. Nucleic acids research *40*, 1904-1915.

Ledford, H. (2014). HIV rebound dashes hope of 'Mississippi baby' cure. Nature.

Lenasi, T., Contreras, X., and Peterlin, B.M. (2008). Transcriptional interference antagonizes proviral gene expression to promote HIV latency. Cell host & microbe *4*, 123-133.

Lesbats, P., Botbol, Y., Chevereau, G., Vaillant, C., Calmels, C., Arneodo, A., Andreola, M.L., Lavigne, M., and Parissi, V. (2011). Functional coupling between HIV-1 integrase and the SWI/SNF chromatin remodeling complex for efficient in vitro integration into stable nucleosomes. PLoS pathogens *7*, e1001280.

Lewinski, M.K., Bisgrove, D., Shinn, P., Chen, H., Hoffmann, C., Hannenhalli, S., Verdin, E., Berry, C.C., Ecker, J.R., and Bushman, F.D. (2005). Genome-wide analysis of chromosomal features repressing human immunodeficiency virus transcription. Journal of virology *79*, 6610-6619.

Li, G., Bukrinsky, M., and Zhao, R.Y. (2009). HIV-1 viral protein R (Vpr) and its interactions with host cell. Current HIV research *7*, 178-183.

Li, M., Ablan, S.D., Miao, C., Zheng, Y.M., Fuller, M.S., Rennert, P.D., Maury, W., Johnson, M.C., Freed, E.O., and Liu, S.L. (2014). TIM-family proteins inhibit HIV-1 release. Proceedings of the National Academy of Sciences of the United States of America *111*, E3699-3707.

Li, Y., Li, G., Ivanova, A., Aaron, S., and Simm, M. (2008). The critical role of human transcriptional repressor CTCF mRNA up-regulation in the induction of anti-HIV-1 responses in CD4(+) T cells. Immunology letters *117*, 35-44.

Liang, C., Li, X., Quan, Y., Laughrea, M., Kleiman, L., Hiscott, J., and Wainberg, M.A. (1997). Sequence elements downstream of the human immunodeficiency virus type 1 long terminal repeat are required for efficient viral gene transcription. Journal of molecular biology *272*, 167-177.

Lieberson, R., Mowen, K.A., McBride, K.D., Leautaud, V., Zhang, X., Suh, W.K., Wu, L., and Glimcher, L.H. (2001). Tumor necrosis factor receptor-associated factor (TRAF)2 represses the T helper cell type 2 response through interaction with NFAT-interacting protein (NIP45). The Journal of experimental medicine *194*, 89-98.

Lien, C., Fang, C.M., Huso, D., Livak, F., Lu, R., and Pitha, P.M. (2010). Critical role of IRF-5 in regulation of B-cell differentiation. Proceedings of the National Academy of Sciences of the United States of America *107*, 4664-4668.

Lin, F.R., Kuo, H.K., Ying, H.Y., Yang, F.H., and Lin, K.I. (2007). Induction of apoptosis in plasma cells by B lymphocyte-induced maturation protein-1 knockdown. Cancer research *67*, 11914-11923.

Lin, K.I., Angelin-Duclos, C., Kuo, T.C., and Calame, K. (2002). Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. Molecular and cellular biology *22*, 4771-4780.

Lin, M.H., Yeh, L.T., Chen, S.J., Chiou, H.Y., Chu, C.C., Yen, L.B., Lin, K.I., Chang, D.M., and Sytwu, H.K. (2014). T cell-specific BLIMP-1 deficiency exacerbates experimental autoimmune encephalomyelitis in nonobese diabetic mice by increasing Th1 and Th17 cells. Clin Immunol *151*, 101-113.

Lin, Y., Wong, K., and Calame, K. (1997). Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation. Science *276*, 596-599.

Lindqvist, M., van Lunzen, J., Soghoian, D.Z., Kuhl, B.D., Ranasinghe, S., Kranias, G., Flanders, M.D., Cutler, S., Yudanin, N., Muller, M.I., *et al.* (2012). Expansion of HIV-specific T follicular helper cells in chronic HIV infection. The Journal of clinical investigation *122*, 3271-3280.

Liu, L., Oliveira, N.M., Cheney, K.M., Pade, C., Dreja, H., Bergin, A.M., Borgdorff, V., Beach, D.H., Bishop, C.L., Dittmar, M.T., *et al.* (2011). A whole genome screen for HIV restriction factors. Retrovirology *8*, 94.

Liu, P., Li, P., and Burke, S. (2010). Critical roles of Bcl11b in T-cell development and maintenance of T-cell identity. Immunological reviews *238*, 138-149.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25*, 402-408.

Lodie, T.A., Reiner, M., Coniglio, S., Viglianti, G., and Fenton, M.J. (1998). Both PU.1 and nuclear factor-kappa B mediate lipopolysaccharide- induced HIV-1 long terminal repeat transcription in macrophages. Journal of Immunology *161*, 268-276.

Lohoff, M., Ferrick, D., Mittrucker, H.W., Duncan, G.S., Bischof, S., Rollinghoff, M., and Mak, T.W. (1997). Interferon regulatory factor-1 is required for a T helper 1 immune response in vivo. Immunity *6*, 681-689.

Lord, C.A., Savitsky, D., Sitcheran, R., Calame, K., Wright, J.R., Ting, J.P., and Williams, K.L. (2009). Blimp-1/PRDM1 mediates transcriptional suppression of the NLR gene NLRP12/Monarch-1. Journal of Immunology *182*, 2948-2958.

Lu, H., Li, Z., Xue, Y., Schulze-Gahmen, U., Johnson, J.R., Krogan, N.J., Alber, T., and Zhou, Q. (2014a). AFF1 is a ubiquitous P-TEFb partner to enable Tat extraction of P-TEFb from 7SK snRNP and formation of SECs for HIV transactivation. Proceedings of the National Academy of Sciences of the United States of America *111*, E15-24.

Lu, P., Youngblood, B.A., Austin, J.W., Mohammed, A.U., Butler, R., Ahmed, R., and Boss, J.M. (2014b). Blimp-1 represses CD8 T cell expression of PD-1 using a feed-forward transcriptional circuit during acute viral infection. The Journal of experimental medicine *211*, 515-527.

Lugo-Villarino, G., Maldonado-Lopez, R., Possemato, R., Penaranda, C., and Glimcher, L.H. (2003). T-bet is required for optimal production of IFN-gamma and antigen-specific T cell activation by dendritic cells. Proceedings of the National Academy of Sciences of the United States of America *100*, 7749-7754.

Lusic, M., Marini, B., Ali, H., Lucic, B., Luzzati, R., and Giacca, M. (2013). Proximity to PML nuclear bodies regulates HIV-1 latency in CD4+ T cells. Cell host & microbe *13*, 665-677.

Ma, C.S., Avery, D.T., Chan, A., Batten, M., Bustamante, J., Boisson-Dupuis, S., Arkwright, P.D., Kreins, A.Y., Averbuch, D., Engelhard, D., *et al.* (2012). Functional STAT3 deficiency compromises the generation of human T follicular helper cells. Blood *119*, 3997-4008.

Magnusdottir, E., Kalachikov, S., Mizukoshi, K., Savitsky, D., Ishida-Yamamoto, A., Panteleyev, A.A., and Calame, K. (2007). Epidermal terminal differentiation depends on B lymphocyte-induced maturation protein-1. Proceedings of the National Academy of Sciences of the United States of America *104*, 14988-14993.

Malcolm, T., Kam, J., Pour, P.S., and Sadowski, I. (2008). Specific interaction of TFII-I with an upstream element on the HIV-1 LTR regulates induction of latent provirus. FEBS letters *582*, 3903-3908.

Maldarelli, F., Wu, X., Su, L., Simonetti, F.R., Shao, W., Hill, S., Spindler, J., Ferris, A.L., Mellors, J.W., Kearney, M.F., *et al.* (2014). Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science *345*, 179-183.

Manger, B., Weiss, A., Imboden, J., Laing, T., and Stobo, J.D. (1987). The role of protein kinase C in transmembrane signaling by the T cell antigen receptor complex. Effects of stimulation with soluble or immobilized CD3 antibodies. Journal of Immunology *139*, 2755-2760.

Manjunath, N., Yi, G., Dang, Y., and Shankar, P. (2013). Newer gene editing technologies toward HIV gene therapy. Viruses *5*, 2748-2766.

Marban, C., Suzanne, S., Dequiedt, F., de Walque, S., Redel, L., Van Lint, C., Aunis, D., and Rohr, O. (2007). Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. The EMBO journal *26*, 412-423.

Margolis, D.M., and Hazuda, D.J. (2013). Combined approaches for HIV cure. Current opinion in HIV and AIDS *8*, 230-235.

Margolis, D.M., Somasundaran, M., and Green, M.R. (1994). Human transcription factor YY1 represses human immunodeficiency virus type 1 transcription and virion production. Journal of virology *68*, 905-910.

Marshall, H.D., Chandele, A., Jung, Y.W., Meng, H., Poholek, A.C., Parish, I.A., Rutishauser, R., Cui, W., Kleinstein, S.H., Craft, J., *et al.* (2011). Differential expression of Ly6C and T-bet distinguish effector and memory Th1 CD4(+) cell properties during viral infection. Immunity *35*, 633-646.

Martins, G., and Calame, K. (2008). Regulation and functions of Blimp-1 in T and B lymphocytes. Annual review of immunology *26*, 133-169.

Martins, G.A., Cimmino, L., Liao, J., Magnusdottir, E., and Calame, K. (2008). Blimp-1 directly represses Il2 and the Il2 activator Fos, attenuating T cell proliferation and survival. The Journal of experimental medicine *205*, 1959-1965.

Martins, G.A., Cimmino, L., Shapiro-Shelef, M., Szabolcs, M., Herron, A., Magnusdottir, E., and Calame, K. (2006). Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nature immunology *7*, 457-465.

McElligott, D.L., Phillips, J.A., Stillman, C.A., Koch, R.J., Mosier, D.E., and Hobbs, M.V. (1997). CD4+ T cells from IRF-1-deficient mice exhibit altered patterns of cytokine expression and cell subset homeostasis. Journal of Immunology *159*, 4180-4186.

Meiering, C.D., Rubio, C., May, C., and Linial, M.L. (2001). Cell-type-specific regulation of the two foamy virus promoters. Journal of virology *75*, 6547-6557.

Meixner, A., Karreth, F., Kenner, L., and Wagner, E.F. (2004). JunD regulates lymphocyte proliferation and T helper cell cytokine expression. The EMBO journal *23*, 1325-1335.

Michael, N.L., Vahey, M.T., d'Arcy, L., Ehrenberg, P.K., Mosca, J.D., Rappaport, J., and Redfield, R.R. (1994). Negative-strand RNA transcripts are produced in human immunodeficiency virus type 1-infected cells and patients by a novel promoter downregulated by Tat. Journal of virology *68*, 979-987.

Moisan, J., Grenningloh, R., Bettelli, E., Oukka, M., and Ho, I.C. (2007). Ets-1 is a negative regulator of Th17 differentiation. The Journal of experimental medicine *204*, 2825-2835.

Moreno-Fernandez, M.E., Presicce, P., and Chougnet, C.A. (2012). Homeostasis and function of regulatory T cells in HIV/SIV infection. Journal of virology *86*, 10262-10269.

Mouland, A.J., Xu, H., Cui, H., Krueger, W., Munro, T.P., Prasol, M., Mercier, J., Rekosh, D., Smith, R., Barbarese, E., *et al.* (2001). RNA trafficking signals in human immunodeficiency virus type 1. Molecular and cellular biology *21*, 2133-2143.

Mouly, E., Chemin, K., Nguyen, H.V., Chopin, M., Mesnard, L., Leite-de-Moraes, M., Burlen-defranoux, O., Bandeira, A., and Bories, J.C. (2010). The Ets-1 transcription factor controls the development and function of natural regulatory T cells. The Journal of experimental medicine *207*, 2113-2125.

Mullen, A.C., High, F.A., Hutchins, A.S., Lee, H.W., Villarino, A.V., Livingston, D.M., Kung, A.L., Cereb, N., Yao, T.P., Yang, S.Y., *et al.* (2001). Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. Science *292*, 1907-1910.

Muranski, P., and Restifo, N.P. (2013). Essentials of Th17 cell commitment and plasticity. Blood *121*, 2402-2414.

Murphy, K.M., and Stockinger, B. (2010). Effector T cell plasticity: flexibility in the face of changing circumstances. Nature immunology *11*, 674-680.

Nabel, G., and Baltimore, D. (1987). An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature *326*, 711-713.

Naghavi, M.H., and Goff, S.P. (2007). Retroviral proteins that interact with the host cell cytoskeleton. Current opinion in immunology *19*, 402-407.

Nakata, Y., Brignier, A.C., Jin, S., Shen, Y., Rudnick, S.I., Sugita, M., and Gewirtz, A.M. (2010). c-Myb, Menin, GATA-3, and MLL form a dynamic transcription complex that plays a pivotal role in human T helper type 2 cell development. Blood *116*, 1280-1290.

Narita, T., Yamaguchi, Y., Yano, K., Sugimoto, S., Chanarat, S., Wada, T., Kim, D.K., Hasegawa, J., Omori, M., Inukai, N., *et al.* (2003). Human transcription elongation factor NELF: identification of novel subunits and reconstitution of the functionally active complex. Molecular and cellular biology *23*, 1863-1873.

Natarajan, M., Schiralli Lester, G.M., Lee, C., Missra, A., Wasserman, G.A., Steffen, M., Gilmour, D.S., and Henderson, A.J. (2013). Negative elongation factor (NELF) coordinates RNA polymerase II pausing, premature termination, and chromatin remodeling to regulate HIV transcription. The Journal of biological chemistry *288*, 25995-26003.

Neumann, C., Heinrich, F., Neumann, K., Junghans, V., Mashreghi, M.F., Ahlers, J., Janke, M., Rudolph, C., Mockel-Tenbrinck, N., Kuhl, A.A., *et al.* (2014). Role of Blimp-1 in programing Th effector cells into IL-10 producers. The Journal of experimental medicine *211*, 1807-1819.

Notani, D., Gottimukkala, K.P., Jayani, R.S., Limaye, A.S., Damle, M.V., Mehta, S., Purbey, P.K., Joseph, J., and Galande, S. (2010). Global regulator SATB1 recruits beta-catenin and regulates T(H)2 differentiation in Wnt-dependent manner. PLoS biology *8*, e1000296.

Nurieva, R.I., Podd, A., Chen, Y., Alekseev, A.M., Yu, M., Qi, X., Huang, H., Wen, R., Wang, J., Li, H.S., *et al.* (2012). STAT5 protein negatively regulates T follicular helper (Tfh) cell generation and function. The Journal of biological chemistry *287*, 11234-11239.

O'Doherty, U., Swiggard, W.J., and Malim, M.H. (2000). Human immunodeficiency virus type 1 spinoculation enhances infection through virus binding. Journal of virology *74*, 10074-10080.

Okamoto, H., Asamitsu, K., Nishimura, H., Kamatani, N., and Okamoto, T. (2000). Reciprocal modulation of transcriptional activities between HIV-1 Tat and MHC class II transactivator CIITA. Biochemical and biophysical research communications *279*, 494-499.

Ono, A., and Freed, E.O. (2004). Cell-type-dependent targeting of human immunodeficiency virus type 1 assembly to the plasma membrane and the multivesicular body. Journal of virology *78*, 1552-1563.

Oswald-Richter, K., Grill, S.M., Shariat, N., Leelawong, M., Sundrud, M.S., Haas, D.W., and Unutmaz, D. (2004). HIV infection of naturally occurring and genetically reprogrammed human regulatory T-cells. PLoS biology *2*, E198.

Otten, L.A., Tacchini-Cottier, F., Lohoff, M., Annunziato, F., Cosmi, L., Scarpellino, L., Louis, J., Steimle, V., Reith, W., and Acha-Orbea, H. (2003). Deregulated MHC class II transactivator expression leads to a strong Th2 bias in CD4+ T lymphocytes. Journal of Immunology *170*, 1150-1157.

Ouaked, N., Mantel, P.Y., Bassin, C., Burgler, S., Siegmund, K., Akdis, C.A., and Schmidt-Weber, C.B. (2009). Regulation of the foxp3 gene by the Th1 cytokines: the role of IL-27-induced STAT1. Journal of Immunology *182*, 1041-1049.

Ouyang, X., Zhang, R., Yang, J., Li, Q., Qin, L., Zhu, C., Liu, J., Ning, H., Shin, M.S., Gupta, M., *et al.* (2011). Transcription factor IRF8 directs a silencing programme for TH17 cell differentiation. Nature communications *2*, 314.

Owaki, T., Asakawa, M., Morishima, N., Hata, K., Fukai, F., Matsui, M., Mizuguchi, J., and Yoshimoto, T. (2005). A role for IL-27 in early regulation of Th1 differentiation. Journal of Immunology *175*, 2191-2200.

Owaki, T., Asakawa, M., Morishima, N., Mizoguchi, I., Fukai, F., Takeda, K., Mizuguchi, J., and Yoshimoto, T. (2008). STAT3 is indispensable to IL-27-mediated cell proliferation but not to IL-27-induced Th1 differentiation and suppression of proinflammatory cytokine production. Journal of Immunology *180*, 2903-2911.

Pacenti, M., Barzon, L., Favaretto, F., Fincati, K., Romano, S., Milan, G., Vettor, R., and Palu, G. (2006). Microarray analysis during adipogenesis identifies new genes altered by antiretroviral drugs. AIDS *20*, 1691-1705.

Pagans, S., Kauder, S.E., Kaehlcke, K., Sakane, N., Schroeder, S., Dormeyer, W., Trievel, R.C., Verdin, E., Schnolzer, M., and Ott, M. (2010). The Cellular lysine methyltransferase Set7/9-KMT7 binds HIV-1 TAR RNA, monomethylates the viral transactivator Tat, and enhances HIV transcription. Cell host & microbe *7*, 234-244.

Pagans, S., Pedal, A., North, B.J., Kaehlcke, K., Marshall, B.L., Dorr, A., Hetzer-Egger, C., Henklein, P., Frye, R., McBurney, M.W., *et al.* (2005). SIRT1 regulates HIV transcription via Tat deacetylation. PLoS biology *3*, e41.

Paiardini, M., and Muller-Trutwin, M. (2013). HIV-associated chronic immune activation. Immunological reviews *254*, 78-101.

Palmer, A.C., Ahlgren-Berg, A., Egan, J.B., Dodd, I.B., and Shearwin, K.E. (2009). Potent transcriptional interference by pausing of RNA polymerases over a downstream promoter. Molecular cell *34*, 545-555.

Panchanathan, R., Liu, H., Fang, C.M., Erickson, L.D., Pitha, P.M., and Choubey, D. (2012). Distinct regulation of murine lupus susceptibility genes by the IRF5/Blimp-1 axis. Journal of Immunology *188*, 270-278.

Parish, I.A., Marshall, H.D., Staron, M.M., Lang, P.A., Brustle, A., Chen, J.H., Cui, W., Tsui, Y.C., Perry, C., Laidlaw, B.J., *et al.* (2014). Chronic viral infection promotes sustained Th1-derived immunoregulatory IL-10 via BLIMP-1. The Journal of clinical investigation *124*, 3455-3468.

Parlato, S., Bruni, R., Fragapane, P., Salerno, D., Marcantonio, C., Borghi, P., Tataseo, P., Ciccaglione, A.R., Presutti, C., Romagnoli, G., *et al.* (2013). IFN-alpha regulates Blimp-1 expression via miR-23a and miR-125b in both monocytes-derived DC and pDC. PloS one *8*, e72833.

Peeters, A., Lambert, P.F., and Deacon, N.J. (1996). A fourth Sp1 site in the human immunodeficiency virus type 1 long terminal repeat is essential for negative-sense transcription. Journal of virology *70*, 6665-6672.

Pepper, M., and Jenkins, M.K. (2011). Origins of CD4(+) effector and central memory T cells. Nature immunology *12*, 467-471.

Pepper, M., Pagan, A.J., Igyarto, B.Z., Taylor, J.J., and Jenkins, M.K. (2011). Opposing signals from the Bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. Immunity *35*, 583-595.

Perkins, N.D., Edwards, N.L., Duckett, C.S., Agranoff, A.B., Schmid, R.M., and Nabel, G.J. (1993). A cooperative interaction between NF-kappa B and Sp1 is required for HIV-1 enhancer activation. The EMBO journal *12*, 3551-3558.

Perlman, M., and Resh, M.D. (2006). Identification of an intracellular trafficking and assembly pathway for HIV-1 gag. Traffic *7*, 731-745.

Perreau, M., Savoye, A.L., De Crignis, E., Corpataux, J.M., Cubas, R., Haddad, E.K., De Leval, L., Graziosi, C., and Pantaleo, G. (2013). Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. The Journal of experimental medicine *210*, 143-156.

Pessler, F., and Cron, R.Q. (2004). Reciprocal regulation of the nuclear factor of activated T cells and HIV-1. Genes and immunity *5*, 158-167.

Peterlin, B.M., and Price, D.H. (2006). Controlling the elongation phase of transcription with P-TEFb. Molecular cell *23*, 297-305.

Pincetic, A., Kuang, Z., Seo, E.J., and Leis, J. (2010). The interferon-induced gene ISG15 blocks retrovirus release from cells late in the budding process. Journal of virology *84*, 4725-4736.

Piskurich, J.F., Lin, K.I., Lin, Y., Wang, Y., Ting, J.P., and Calame, K. (2000). BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. Nature immunology *1*, 526-532.

Pollard, V.W., and Malim, M.H. (1998). The HIV-1 Rev protein. Annual review of microbiology *52*, 491-532.

Postler, T.S., and Desrosiers, R.C. (2013). The tale of the long tail: the cytoplasmic domain of HIV-1 gp41. Journal of virology *87*, 2-15.

Programme, W.H.O.J.U.N., and (UNAIDS), o.H.A. (2014). THE GAP REPORT.

Rabbi, M.F., Al-Harthi, L., and Roebuck, K.A. (1997a). TNFalpha cooperates with the protein kinase A pathway to synergistically increase HIV-1 LTR transcription via downstream TRE-like cAMP response elements. Virology *237*, 422-429.

Rabbi, M.F., Saifuddin, M., Gu, D.S., Kagnoff, M.F., and Roebuck, K.A. (1997b). U5 region of the human immunodeficiency virus type 1 long terminal repeat contains TRE-like cAMP-responsive elements that bind both AP-1 and CREB/ATF proteins. Virology *233*, 235-245.

Rafati, H., Parra, M., Hakre, S., Moshkin, Y., Verdin, E., and Mahmoudi, T. (2011). Repressive LTR nucleosome positioning by the BAF complex is required for HIV latency. PLoS biology *9*, e1001206.

Rahim, M.M., Chrobak, P., Hu, C., Hanna, Z., and Jolicoeur, P. (2009). Adult AIDS-like disease in a novel inducible human immunodeficiency virus type 1 Nef transgenic mouse model: CD4+ T-cell activation is Nef dependent and can occur in the absence of lymphophenia. Journal of virology *83*, 11830-11846.

Rahl, P.B., Lin, C.Y., Seila, A.C., Flynn, R.A., McCuine, S., Burge, C.B., Sharp, P.A., and Young, R.A. (2010). c-Myc regulates transcriptional pause release. Cell *141*, 432-445.

Ranger, A.M., Oukka, M., Rengarajan, J., and Glimcher, L.H. (1998). Inhibitory function of two NFAT family members in lymphoid homeostasis and Th2 development. Immunity *9*, 627-635.

Readinger, J.A., Schiralli, G.M., Jiang, J.K., Thomas, C.J., August, A., Henderson, A.J., and Schwartzberg, P.L. (2008). Selective targeting of ITK blocks multiple steps of HIV replication. Proceedings of the National Academy of Sciences of the United States of America *105*, 6684-6689.

Ren, B., Chee, K.J., Kim, T.H., and Maniatis, T. (1999). PRDI-BF1/Blimp-1 repression is mediated by corepressors of the Groucho family of proteins. Genes & development *13*, 125-137.

Ribeiro de Almeida, C., Heath, H., Krpic, S., Dingjan, G.M., van Hamburg, J.P., Bergen, I., van de Nobelen, S., Sleutels, F., Grosveld, F., Galjart, N., *et al.* (2009). Critical role for the transcription regulator CCCTC-binding factor in the control of Th2 cytokine expression. Journal of Immunology *182*, 999-1010.

Richman, D.D., Margolis, D.M., Delaney, M., Greene, W.C., Hazuda, D., and Pomerantz, R.J. (2009). The challenge of finding a cure for HIV infection. Science *323*, 1304-1307.

Riou, C., Yassine-Diab, B., Van grevenynghe, J., Somogyi, R., Greller, L.D., Gagnon, D., Gimmig, S., Wilkinson, P., Shi, Y., Cameron, M.J., *et al.* (2007). Convergence of TCR and cytokine signaling leads to FOXO3a phosphorylation and drives the survival of CD4+ central memory T cells. The Journal of experimental medicine *204*, 79-91.

Robichaud, G.A., Barbeau, B., Fortin, J.F., Rothstein, D.M., and Tremblay, M.J. (2002). Nuclear factor of activated T cells is a driving force for preferential productive HIV-1 infection of CD45RO-expressing CD4+ T cells. The Journal of biological chemistry *277*, 23733-23741.

Rodriguez-Garcia, M., Barr, F.D., Crist, S.G., Fahey, J.V., and Wira, C.R. (2014). Phenotype and susceptibility to HIV infection of CD4 Th17 cells in the human female reproductive tract. Mucosal immunology.

Roebuck, K.A., Gu, D.S., and Kagnoff, M.F. (1996). Activating protein-1 cooperates with phorbol ester activation signals to increase HIV-1 expression. AIDS *10*, 819-826.

Rohr, O., Lecestre, D., Chasserot-Golaz, S., Marban, C., Avram, D., Aunis, D., Leid, M., and Schaeffer, E. (2003). Recruitment of Tat to heterochromatin protein HP1 via interaction with CTIP2 inhibits human immunodeficiency virus type 1 replication in microglial cells. J Virol *77*, 5415-5427.

Romerio, F., Gabriel, M.N., and Margolis, D.M. (1997). Repression of human immunodeficiency virus type 1 through the novel cooperation of human factors YY1 and LSF. Journal of virology *71*, 9375-9382.

Rosen, C.A., Sodroski, J.G., and Haseltine, W.A. (1985). The location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. Cell *41*, 813-823.

Routy, J.P., Tremblay, C.L., Angel, J.B., Trottier, B., Rouleau, D., Baril, J.G., Harris, M., Trottier, S., Singer, J., Chomont, N., *et al.* (2012). Valproic acid in association with highly active antiretroviral therapy for reducing systemic HIV-1 reservoirs: results from a multicentre randomized clinical study. HIV medicine *13*, 291-296.

Ruan, Q., Kameswaran, V., Tone, Y., Li, L., Liou, H.C., Greene, M.I., Tone, M., and Chen, Y.H. (2009). Development of Foxp3(+) regulatory t cells is driven by the c-Rel enhanceosome. Immunity *31*, 932-940.

Rutishauser, R.L., Martins, G.A., Kalachikov, S., Chandele, A., Parish, I.A., Meffre, E., Jacob, J., Calame, K., and Kaech, S.M. (2009). Transcriptional repressor Blimp-1 promotes CD8(+) T cell terminal differentiation and represses the acquisition of central memory T cell properties. Immunity *31*, 296-308.

Saftig, P., and Klumperman, J. (2009). Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nature reviews Molecular cell biology *10*, 623-635.

Saifuddin, M., Roebuck, K.A., Chang, C., Ting, J.P., and Spear, G.T. (2000). Cutting edge: activation of HIV-1 transcription by the MHC class II transactivator. Journal of Immunology *164*, 3941-3945.

Sakane, N., Kwon, H.S., Pagans, S., Kaehlcke, K., Mizusawa, Y., Kamada, M., Lassen, K.G., Chan, J., Greene, W.C., Schnoelzer, M., *et al.* (2011). Activation of HIV transcription by the viral Tat protein requires a demethylation step mediated by lysine-specific demethylase 1 (LSD1/KDM1). PLoS pathogens *7*, e1002184.

Sallusto, F., Geginat, J., and Lanzavecchia, A. (2004). Central memory and effector memory T cell subsets: function, generation, and maintenance. Annual review of immunology *22*, 745-763.

Sarai, N., Nimura, K., Tamura, T., Kanno, T., Patel, M.C., Heightman, T.D., Ura, K., and Ozato, K. (2013). WHSC1 links transcription elongation to HIRA-mediated histone H3.3 deposition. The EMBO journal *32*, 2392-2406.

Sathe, A., Patgaonkar, M.S., Bashir, T., and Reddy, K.V. (2014). MicroRNA let-7f: a novel regulator of innate immune response in human endocervical cells. Am J Reprod Immunol *71*, 137-153.

Sato, K., Miyoshi, F., Yokota, K., Araki, Y., Asanuma, Y., Akiyama, Y., Yoh, K., Takahashi, S., Aburatani, H., and Mimura, T. (2011). Marked induction of c-Maf protein during Th17 cell differentiation and its implication in memory Th cell development. The Journal of biological chemistry *286*, 14963-14971.

Savitsky, D., Cimmino, L., Kuo, T., Martins, G.A., and Calame, K. (2007). Multiple roles for Blimp-1 in B and T lymphocytes. Advances in experimental medicine and biology *596*, 9-30.

Sawant, D.V., Wu, H., Kaplan, M.H., and Dent, A.L. (2013). The Bcl6 target gene microRNA-21 promotes Th2 differentiation by a T cell intrinsic pathway. Molecular immunology *54*, 435-442.

Schiralli Lester, G.M., and Henderson, A.J. (2012). Mechanisms of HIV Transcriptional Regulation and Their Contribution to Latency. Molecular biology international *2012*, 614120.

Seddiki, N., Phetsouphanh, C., Swaminathan, S., Xu, Y., Rao, S., Li, J., Sutcliffe, E.L., Denyer, G., Finlayson, R., Gelgor, L., *et al.* (2013). The microRNA-9/B-lymphocyte-induced maturation protein-1/IL-2 axis is differentially regulated in progressive HIV infection. European journal of immunology *43*, 510-520.

Sekimata, M., Perez-Melgosa, M., Miller, S.A., Weinmann, A.S., Sabo, P.J., Sandstrom, R., Dorschner, M.O., Stamatoyannopoulos, J.A., and Wilson, C.B. (2009). CCCTC-binding factor and the transcription factor T-bet orchestrate T helper 1 cell-specific structure and function at the interferon-gamma locus. Immunity *31*, 551-564.

Selliah, N., Zhang, M., DeSimone, D., Kim, H., Brunner, M., Ittenbach, R.F., Rui, H., Cron, R.Q., and Finkel, T.H. (2006). The gammac-cytokine regulated transcription factor, STAT5, increases HIV-1 production in primary CD4 T cells. Virology *344*, 283-291.

Selliah, N., Zhang, M., White, S., Zoltick, P., Sawaya, B.E., Finkel, T.H., and Cron, R.Q. (2008). FOXP3 inhibits HIV-1 infection of CD4 T-cells via inhibition of LTR transcriptional activity. Virology *381*, 161-167.

Seth, A., Hodge, D.R., Thompson, D.M., Robinson, L., Panayiotakis, A., Watson, D.K., and Papas, T.S. (1993). ETS family proteins activate transcription from HIV-1 long terminal repeat. AIDS research and human retroviruses *9*, 1017-1023.

Severa, M., Islam, S.A., Waggoner, S.N., Jiang, Z., Kim, N.D., Ryan, G., Kurt-Jones, E., Charo, I., Caffrey, D.R., Boyartchuk, V.L., *et al.* (2014). The transcriptional repressor BLIMP1 curbs host defenses by suppressing expression of the chemokine CCL8. Journal of Immunology *192*, 2291-2304.

Sforza, F., Nicoli, F., Gallerani, E., Finessi, V., Reali, E., Cafaro, A., Caputo, A., Ensoli, B., and Gavioli, R. (2014). HIV-1 Tat affects the programming and functionality of human CD8(+) T cells by modulating the expression of T-box transcription factors. AIDS *28*, 1729-1738.

Sgarbanti, M., Borsetti, A., Moscufo, N., Bellocchi, M.C., Ridolfi, B., Nappi, F., Marsili, G., Marziali, G., Coccia, E.M., Ensoli, B., *et al.* (2002). Modulation of human immunodeficiency virus 1 replication by interferon regulatory factors. The Journal of experimental medicine *195*, 1359-1370.

Shaffer, A.L., Lin, K.I., Kuo, T.C., Yu, X., Hurt, E.M., Rosenwald, A., Giltnane, J.M., Yang, L., Zhao, H., Calame, K., *et al.* (2002). Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. Immunity *17*, 51-62.

Shan, L., Yang, H.C., Rabi, S.A., Bravo, H.C., Shroff, N.S., Irizarry, R.A., Zhang, H., Margolick, J.B., Siliciano, J.D., and Siliciano, R.F. (2011). Influence of host gene transcription level and orientation on HIV-1 latency in a primary-cell model. Journal of virology *85*, 5384-5393.

Shankar, E.M., Che, K.F., Messmer, D., Lifson, J.D., and Larsson, M. (2011). Expression of a broad array of negative costimulatory molecules and Blimp-1 in T cells following priming by HIV-1 pulsed dendritic cells. Mol Med *17*, 229-240.

Shapiro-Shelef, M., Lin, K.I., McHeyzer-Williams, L.J., Liao, J., McHeyzer-Williams, M.G., and Calame, K. (2003). Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. Immunity *19*, 607-620.

Sharp, P.M., and Hahn, B.H. (2011). Origins of HIV and the AIDS pandemic. Cold Spring Harbor perspectives in medicine *1*, a006841.

Sheridan, P.L., Sheline, C.T., Cannon, K., Voz, M.L., Pazin, M.J., Kadonaga, J.T., and Jones, K.A. (1995). Activation of the HIV-1 enhancer by the LEF-1 HMG protein on nucleosome-assembled DNA in vitro. Genes & development *9*, 2090-2104.

Sherrill-Mix, S., Lewinski, M.K., Famiglietti, M., Bosque, A., Malani, N., Ocwieja, K.E., Berry, C.C., Looney, D., Shan, L., Agosto, L.M., *et al.* (2013). HIV latency and integration site placement in five cell-based models. Retrovirology *10*, 90.

Shin, H., Blackburn, S.D., Intlekofer, A.M., Kao, C., Angelosanto, J.M., Reiner, S.L., and Wherry, E.J. (2009). A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. Immunity *31*, 309-320.

Shin, H.M., Kapoor, V.N., Guan, T., Kaech, S.M., Welsh, R.M., and Berg, L.J. (2013). Epigenetic modifications induced by Blimp-1 Regulate CD8(+) T cell memory progression during acute virus infection. Immunity *39*, 661-675.

Siegel, A.M., Heimall, J., Freeman, A.F., Hsu, A.P., Brittain, E., Brenchley, J.M., Douek, D.C., Fahle, G.H., Cohen, J.I., Holland, S.M., *et al.* (2011a). A critical role for STAT3 transcription factor signaling in the development and maintenance of human T cell memory. Immunity *35*, 806-818.

Siegel, A.M., Rangaswamy, U.S., Napier, R.J., and Speck, S.H. (2010). Blimp-1-dependent plasma cell differentiation is required for efficient maintenance of murine gammaherpesvirus latency and antiviral antibody responses. Journal of virology *84*, 674-685.

Siegel, R., Eskdale, J., and Gallagher, G. (2011b). Regulation of IFN-lambda1 promoter activity (IFN-lambda1/IL-29) in human airway epithelial cells. Journal of Immunology *187*, 5636-5644.

Siekevitz, M., Josephs, S.F., Dukovich, M., Peffer, N., Wong-Staal, F., and Greene, W.C. (1987). Activation of the HIV-1 LTR by T cell mitogens and the trans-activator protein of HTLV-I. Science *238*, 1575-1578.

Sierra, S., Kupfer, B., and Kaiser, R. (2005). Basics of the virology of HIV-1 and its replication. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology *34*, 233-244.

Sieweke, M.H., Tekotte, H., Jarosch, U., and Graf, T. (1998). Cooperative interaction of ets-1 with USF-1 required for HIV-1 enhancer activity in T cells. The EMBO journal *17*, 1728-1739.

Siliciano, R.F., and Greene, W.C. (2011). HIV latency. Cold Spring Harbor perspectives in medicine *1*, a007096.

Skawran, B., Steinemann, D., Becker, T., Buurman, R., Flik, J., Wiese, B., Flemming, P., Kreipe, H., Schlegelberger, B., and Wilkens, L. (2008). Loss of 13q is associated with genes involved in cell cycle and proliferation in dedifferentiated hepatocellular carcinoma. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc *21*, 1479-1489.

Smith, M.A., Maurin, M., Cho, H.I., Becknell, B., Freud, A.G., Yu, J., Wei, S., Djeu, J., Celis, E., Caligiuri, M.A., *et al.* (2010). PRDM1/Blimp-1 controls effector cytokine production in human NK cells. Journal of Immunology *185*, 6058-6067.

Smith, M.A., Wright, G., Wu, J., Tailor, P., Ozato, K., Chen, X., Wei, S., Piskurich, J.F., Ting, J.P., and Wright, K.L. (2011). Positive regulatory domain I (PRDM1) and IRF8/PU.1 counter-regulate MHC class II transactivator (CIITA) expression during dendritic cell maturation. The Journal of biological chemistry *286*, 7893-7904.

Son, J.S., Sahoo, A., Chae, C.S., Hwang, J.S., Park, Z.Y., and Im, S.H. (2011). JunB and c-Rel cooperatively enhance Foxp3 expression during induced regulatory T cell differentiation. Biochemical and biophysical research communications *407*, 141-147.

Stevenson, M. (2013). CROI 2013: Basic science review. Topics in antiviral medicine *21*, 42-46.

Strasner, A.B., Natarajan, M., Doman, T., Key, D., August, A., and Henderson, A.J. (2008). The Src kinase Lck facilitates assembly of HIV-1 at the plasma membrane. Journal of Immunology *181*, 3706-3713.

Su, S.T., Ying, H.Y., Chiu, Y.K., Lin, F.R., Chen, M.Y., and Lin, K.I. (2009). Involvement of histone demethylase LSD1 in Blimp-1-mediated gene repression during plasma cell differentiation. Molecular and cellular biology *29*, 1421-1431.

Sundaravaradan, V., Mehta, R., Harris, D.T., Zack, J.A., and Ahmad, N. (2010). Differential expression and interaction of host factors augment HIV-1 gene expression in neonatal mononuclear cells. Virology *400*, 32-43.

Sung, M.H., and Simon, R. (2004). Genomewide conserved epitope profiles of HIV-1 predicted by biophysical properties of MHC binding peptides. Journal of computational biology : a journal of computational molecular cell biology *11*, 125-145.

Swider, A., Siegel, R., Eskdale, J., and Gallagher, G. (2014). Regulation of interferon lambda-1 (IFNL1/IFN-lambda1/IL-29) expression in human colon epithelial cells. Cytokine *65*, 17-23.

Tagieva, N.E., and Vaquero, C. (1997). Expression of naturally occurring antisense RNA inhibits human immunodeficiency virus type 1 heterologous strain replication. The Journal of general virology *78 (Pt 10)*, 2503-2511.

Takatori, H., Nakajima, H., Kagami, S., Hirose, K., Suto, A., Suzuki, K., Kubo, M., Yoshimura, A., Saito, Y., and Iwamoto, I. (2005). Stat5a inhibits IL-12-induced Th1 cell differentiation through the induction of suppressor of cytokine signaling 3 expression. Journal of Immunology *174*, 4105-4112.

Taki, S., Sato, T., Ogasawara, K., Fukuda, T., Sato, M., Hida, S., Suzuki, G., Mitsuyama, M., Shin, E.H., Kojima, S., *et al.* (1997). Multistage regulation of Th1-type immune responses by the transcription factor IRF-1. Immunity *6*, 673-679.

Tesmer, V.M., Rajadhyaksha, A., Babin, J., and Bina, M. (1993). NF-IL6-mediated transcriptional activation of the long terminal repeat of the human immunodeficiency virus type 1. Proceedings of the National Academy of Sciences of the United States of America *90*, 7298-7302.

Tone, Y., Furuuchi, K., Kojima, Y., Tykocinski, M.L., Greene, M.I., and Tone, M. (2008). Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nature immunology *9*, 194-202.

Torresilla, C., Larocque, E., Landry, S., Halin, M., Coulombe, Y., Masson, J.Y., Mesnard, J.M., and Barbeau, B. (2013). Detection of the HIV-1 minus-strand-encoded antisense protein and its association with autophagy. Journal of virology *87*, 5089-5105.

Triantafilou, M., Miyake, K., Golenbock, D.T., and Triantafilou, K. (2002). Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-induced cell activation. Journal of cell science *115*, 2603-2611.

Tsitsikov, E.N., Wright, D.A., and Geha, R.S. (1997). CD30 induction of human immunodeficiency virus gene transcription is mediated by TRAF2. Proceedings of the National Academy of Sciences of the United States of America *94*, 1390-1395.

Tsukumo, S., Unno, M., Muto, A., Takeuchi, A., Kometani, K., Kurosaki, T., Igarashi, K., and Saito, T. (2013a). Bach2 maintains T cells in a naive state by suppressing effector memory-related genes. Proceedings of the National Academy of Sciences of the United States of America *110*, 10735-10740.

Tsukumo, S.I., Unno, M., Muto, A., Takeuchi, A., Kometani, K., Kurosaki, T., Igarashi, K., and Saito, T. (2013b). Bach2 maintains T cells in a naive state by suppressing effector memory-related genes. Proceedings of the National Academy of Sciences of the United States of America.

Turner, C.A., Jr., Mack, D.H., and Davis, M.M. (1994). Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. Cell *77*, 297-306.

Tyagi, M., and Bukrinsky, M. (2012). Human immunodeficiency virus (HIV) latency: the major hurdle in HIV eradication. Mol Med *18*, 1096-1108.

Tyagi, M., and Karn, J. (2007). CBF-1 promotes transcriptional silencing during the establishment of HIV-1 latency. The EMBO journal *26*, 4985-4995.

van Grevenynghe, J., Procopio, F.A., He, Z., Chomont, N., Riou, C., Zhang, Y., Gimmig, S., Boucher, G., Wilkinson, P., Shi, Y., *et al.* (2008). Transcription factor FOXO3a controls the persistence of memory CD4(+) T cells during HIV infection. Nature medicine *14*, 266-274.

van Leeuwen, E.M., Sprent, J., and Surh, C.D. (2009). Generation and maintenance of memory CD4(+) T Cells. Current opinion in immunology *21*, 167-172.

Van Lint, C., Emiliani, S., Ott, M., and Verdin, E. (1996). Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. The EMBO journal *15*, 1112-1120.

van Loosdregt, J., Vercoulen, Y., Guichelaar, T., Gent, Y.Y., Beekman, J.M., van Beekum, O., Brenkman, A.B., Hijnen, D.J., Mutis, T., Kalkhoven, E., *et al.* (2010). Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization. Blood *115*, 965-974.

Verdin, E., Paras, P., Jr., and Van Lint, C. (1993). Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. The EMBO journal *12*, 3249-3259.

Wada, T., Takagi, T., Yamaguchi, Y., Ferdous, A., Imai, T., Hirose, S., Sugimoto, S., Yano, K., Hartzog, G.A., Winston, F., *et al.* (1998a). DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. Genes & development *12*, 343-356.

Wada, T., Takagi, T., Yamaguchi, Y., Watanabe, D., and Handa, H. (1998b). Evidence that P-TEFb alleviates the negative effect of DSIF on RNA polymerase II-dependent transcription in vitro. The EMBO journal *17*, 7395-7403.

Wagschal, A., Rousset, E., Basavarajaiah, P., Contreras, X., Harwig, A., Laurent-Chabalier, S., Nakamura, M., Chen, X., Zhang, K., Meziane, O., *et al.* (2012). Microprocessor, Setx, Xrn2, and Rrp6 co-operate to induce premature termination of transcription by RNAPII. Cell *150*, 1147-1157.

Wang, Y.H., Voo, K.S., Liu, B., Chen, C.Y., Uygungil, B., Spoede, W., Bernstein, J.A., Huston, D.P., and Liu, Y.J. (2010). A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. The Journal of experimental medicine *207*, 2479-2491.

Watanabe, Y., Onodera, A., Kanai, U., Ichikawa, T., Obata-Ninomiya, K., Wada, T., Kiuchi, M., Iwamura, C., Tumes, D.J., Shinoda, K., *et al.* (2014). Trithorax complex component Menin controls differentiation and maintenance of T helper 17 cells. Proceedings of the National Academy of Sciences of the United States of America *111*, 12829-12834.

Watson, K., and Edwards, R.J. (1999). HIV-1-trans-activating (Tat) protein: both a target and a tool in therapeutic approaches. Biochemical pharmacology *58*, 1521-1528.

Wei, L., Laurence, A., and O'Shea, J.J. (2008). New insights into the roles of Stat5a/b and Stat3 in T cell development and differentiation. Seminars in cell & developmental biology *19*, 394-400.

Wei, P., Garber, M.E., Fang, S.M., Fischer, W.H., and Jones, K.A. (1998). A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. Cell *92*, 451-462.

Weiss, A., and Imboden, J.B. (1987). Cell surface molecules and early events involved in human T lymphocyte activation. Advances in immunology *41*, 1-38.

Whitney, J.B., Hill, A.L., Sanisetty, S., Penaloza-MacMaster, P., Liu, J., Shetty, M., Parenteau, L., Cabral, C., Shields, J., Blackmore, S.*, et al.* (2014). Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature *512*, 74-77.

Williams, S.A., Chen, L.F., Kwon, H., Ruiz-Jarabo, C.M., Verdin, E., and Greene, W.C. (2006). NF-kappaB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation. The EMBO journal *25*, 139-149.

Wu, Y., Borde, M., Heissmeyer, V., Feuerer, M., Lapan, A.D., Stroud, J.C., Bates, D.L., Guo, L., Han, A., Ziegler, S.F.*, et al.* (2006). FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell *126*, 375-387.

Wurster, A.L., and Pazin, M.J. (2008). BRG1-mediated chromatin remodeling regulates differentiation and gene expression of T helper cells. Molecular and cellular biology *28*, 7274-7285.

Xiao, S., Jin, H., Korn, T., Liu, S.M., Oukka, M., Lim, B., and Kuchroo, V.K. (2008). Retinoic acid increases Foxp3+ regulatory T cells and inhibits development of Th17 cells by enhancing TGF-beta-driven Smad3 signaling and inhibiting IL-6 and IL-23 receptor expression. Journal of Immunology *181*, 2277-2284.

Yamada, T., Yamaguchi, Y., Inukai, N., Okamoto, S., Mura, T., and Handa, H. (2006). P-TEFb-mediated phosphorylation of hSpt5 C-terminal repeats is critical for processive transcription elongation. Molecular cell *21*, 227-237.

Yamaguchi, Y., Inukai, N., Narita, T., Wada, T., and Handa, H. (2002). Evidence that negative elongation factor represses transcription elongation through binding to a DRB sensitivity-inducing factor/RNA polymerase II complex and RNA. Molecular and cellular biology *22*, 2918-2927.

Yamaguchi, Y., Takagi, T., Wada, T., Yano, K., Furuya, A., Sugimoto, S., Hasegawa, J., and Handa, H. (1999). NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation. Cell *97*, 41-51.

Yamamoto, T., de Crombrugghe, B., and Pastan, I. (1980). Identification of a functional promoter in the long terminal repeat of Rous sarcoma virus. Cell *22*, 787-797.

Yamane, H., and Paul, W.E. (2013). Early signaling events that underlie fate decisions of naive CD4(+) T cells toward distinct T-helper cell subsets. Immunological reviews *252*, 12-23.

Yamashita, M., Kuwahara, M., Suzuki, A., Hirahara, K., Shinnaksu, R., Hosokawa, H., Hasegawa, A., Motohashi, S., Iwama, A., and Nakayama, T. (2008). Bmi1 regulates memory CD4 T cell survival via repression of the Noxa gene. The Journal of experimental medicine *205*, 1109-1120.

Yan, N., and Chen, Z.J. (2012). Intrinsic antiviral immunity. Nature immunology *13*, 214-222.

Yan, N., Regalado-Magdos, A.D., Stiggelbout, B., Lee-Kirsch, M.A., and Lieberman, J. (2010). The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1. Nature immunology *11*, 1005-1013.

Yang, W., Ng, P., Zhao, M., Wong, T.K., Yiu, S.M., and Lau, Y.L. (2008a). Promoter-sharing by different genes in human genome--CPNE1 and RBM12 gene pair as an example. BMC genomics *9*, 456.

Yang, X., Chen, Y., and Gabuzda, D. (1999). ERK MAP kinase links cytokine signals to activation of latent HIV-1 infection by stimulating a cooperative interaction of AP-1 and NF-kappaB. The Journal of biological chemistry *274*, 27981-27988.

Yang, X.O., Nurieva, R., Martinez, G.J., Kang, H.S., Chung, Y., Pappu, B.P., Shah, B., Chang, S.H., Schluns, K.S., Watowich, S.S., *et al.* (2008b). Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity *29*, 44-56.

Yang, Z., and Engel, J.D. (1993). Human T cell transcription factor GATA-3 stimulates HIV-1 expression. Nucleic acids research *21*, 2831-2836.

Yang, Z., and Greene, W.C. (2014). A new activity for SAMHD1 in HIV restriction. Nature medicine *20*, 808-809.

Yang, Z., Yik, J.H., Chen, R., He, N., Jang, M.K., Ozato, K., and Zhou, Q. (2005). Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. Molecular cell *19*, 535-545.

Yao, C., Hirata, T., Soontrapa, K., Ma, X., Takemori, H., and Narumiya, S. (2013). Prostaglandin E(2) promotes Th1 differentiation via synergistic amplification of IL-12 signalling by cAMP and PI3-kinase. Nature communications *4*, 1685.

Yin, Q., Sester, D.P., Tian, Y., Hsiao, Y.S., Lu, A., Cridland, J.A., Sagulenko, V., Thygesen, S.J., Choubey, D., Hornung, V., *et al.* (2013). Molecular mechanism for p202-mediated specific inhibition of AIM2 inflammasome activation. Cell reports *4*, 327-339.

Yu, D., Rao, S., Tsai, L.M., Lee, S.K., He, Y., Sutcliffe, E.L., Srivastava, M., Linterman, M., Zheng, L., Simpson, N., *et al.* (2009). The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity *31*, 457-468.

Yu, J., Angelin-Duclos, C., Greenwood, J., Liao, J., and Calame, K. (2000). Transcriptional repression by blimp-1 (PRDI-BF1) involves recruitment of histone deacetylase. Molecular and cellular biology *20*, 2592-2603.

Zhang, F., and Boothby, M. (2006). T helper type 1-specific Brg1 recruitment and remodeling of nucleosomes positioned at the IFN-gamma promoter are Stat4 dependent. The Journal of experimental medicine *203*, 1493-1505.

Zhang, H.S., and Wu, M.R. (2009). SIRT1 regulates Tat-induced HIV-1 transactivation through activating AMP-activated protein kinase. Virus research *146*, 51-57.

Zhang, M., Clausell, A., Robinson, T., Yin, J., Chen, E., Johnson, L., Weiss, G., Sabbaj, S., Lowe, R.M., Wagner, F.H., *et al.* (2012). Host factor transcriptional regulation contributes to preferential expression of HIV type 1 in IL-4-producing CD4 T cells. Journal of Immunology *189*, 2746-2757.

Zhang, Q., Cui, F., Fang, L., Hong, J., Zheng, B., and Zhang, J.Z. (2013). TNF-alpha impairs differentiation and function of TGF-beta-induced Treg cells in autoimmune diseases through Akt and Smad3 signaling pathway. Journal of molecular cell biology *5*, 85-98.

Zhang, Z., Klatt, A., Gilmour, D.S., and Henderson, A.J. (2007). Negative elongation factor NELF represses human immunodeficiency virus transcription by pausing the RNA polymerase II complex. The Journal of biological chemistry *282*, 16981-16988.

Zheng, W., and Flavell, R.A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell *89*, 587-596.

Zhou, W., Parent, L.J., Wills, J.W., and Resh, M.D. (1994). Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 Gag protein which interacts with acidic phospholipids. Journal of virology *68*, 2556-2569.

Zhu, J., Cote-Sierra, J., Guo, L., and Paul, W.E. (2003). Stat5 activation plays a critical role in Th2 differentiation. Immunity *19*, 739-748.

Zhu, J., Yamane, H., and Paul, W.E. (2010). Differentiation of effector CD4 T cell populations (*). Annual review of immunology *28*, 445-489.

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Publications

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K. Kaczmarek, M. Natarajan, Z. Euler, G. Alter, G. Viglianti, A.J. Henderson, Blimp-1, an Intrinsic Factor that Represses HIV-1 Proviral Transcription in Memory CD4$^+$ T Cells, *Manuscript submitted to JI.*

K. Kaczmarek, A. Morales, A.J. Henderson, T Cell Transcription Factors and Their Impact on HIV-1 Expression, *Virol. Res. Treat.* **2013**, 4, 41–47

Abstracts & Presentations

K. Kaczmarek, M. Natarajan, Z. Euler, G. Alter, G. Viglianti, A.J. Henderson, Repression of HIV-1 Transcription in Memory CD4$^+$ T Cells by Blimp-1, Scholars Day, Boston University, Boston, MA, April 15, 2014

K. Kaczmarek, G. Schiralli Lester, A. Woerner, M. Natarajan, Z. Euler, G. Alter, G. Viglianti, A.J. Henderson, Mechanisms that Negatively Regulate HIV-1 Transcription in CD4+ T Memory Cells, The 2014 Palm Springs Symposium on HIV-1/AIDS, "HIV-1 Pathogenesis: Molecules to Man", Palm Springs, CA, 2014

K. Kaczmarek, M. Natarajan, Z. Euler, G. Alter, G. Viglianti, A.J. Henderson, Repression of HIV-1 Transcription in Memory CD4$^+$ T Cells by Blimp-1, CROI, Boston, MA, 2014

G. Schiralli Lester, <u>K. Kaczmarek</u>, M. Natarajan, A.J. Henderson, Mechanisms by Which Negative Elongation Factor (NELF) Establishes and Maintains HIV-1 Latency, CROI, Boston, MA, 2014

<u>K. Kaczmarek</u>, M. Natarajan, Z. Euler, G. Alter, G. Viglianti, A.J. Henderson, Repression of HIV-1 Transcription in Memory CD4$^+$ T Cells by Blimp-1, Sixth International Workshop on HIV-1 Persistence during Therapy, Miami, FL, 2013

<u>K. Kaczmarek</u>, M. Natarajan, A.J. Henderson, Repression of HIV-1 Transcription by Blimp-1, Evans Department of Medicine Research Days, Boston University, Boston, MA, 2013

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<u>K. Kaczmarek</u>, G. Schiralli Lester, A.J. Henderson, The Role of Lck in HIV-1 Assembly and Release, Evans Department of Medicine Research Days, Boston University, Boston, MA, 2010

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