**Methods:** To further our understanding of the molecular mechanisms of latency, we employed a primary cell model of HIV latency in which infected cells adopt heterogeneous transcriptional fates with a subset of infected cells establishing viral latency. We characterized this model using assay of Transposon-Accessible Chromatin sequencing (ATACseq).

**Results:** We observed that loss of viral gene expression is a stable and heritable phenotype that is maintained through multiple rounds of stimulation and expansion, suggesting a role for epigenetic maintenance of latency. Using ATACseq we found that cells in which latency is established exhibit a significantly more closed chromatin conformation, both within the HIV genome and across the host cell genome, indicating that latency is correlated with a global process of epigenomic modification and heterochromatin expansion. We also observed that latency reversing agents (LRAs) induced distinct patterns of chromatin openning in both the HIV and host cell genomes. Furthermore, we observed that latently infected cells exhibited elevated levels of specific repressive histone modifications, including H3K27me3.

**Conclusions:** Altogether, these data demonstrate that latency establishment in primary CD4 T cells occurs preferentially in a subset of cells that exhibit expanded H3K27me3-associated heterochromatin, and that viral silencing is connected to global cellular epigenomic reprogramming. A deeper understanding of this process will likely lead to new therapeutic strategies for blocking the initiation or maintenance of latency.

## PP 1.25

### Proteasomal degradation of PML protein is a stress response to HIV-1 replication and reactivation

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**Background:** Promyelocytic leukemia protein (PML) is one main structural and functional component of the nucleus. PML has been described as a potential HIV-1 latency marker (Lusic et al.2013). Moreover, PML has been recently validated in vivoas a potential therapeutic target to induce a functional cure of the infection through the use of arsenic trioxide (Yang et al.2019). We investigated the impact of metabolic changes induced by HIV-1 replication and latency reactivation on PML expression.

**Methods:** Development of latency in vitrowas studied usingprimary CD4+ T-cells. Latency reactivation was analyzed in three different J-Lat clones. Transcriptomic profiles were analyzed by RNA-Seq. Expression of PML was investigated by western blot and immunofluorescence coupled to 3D immuno-DNA- or RNA-FISH.

**Results:** RNA-Seq analysis showed that HIV-1 replication in primary CD4+ T-cells was associated with increased expression of Nrf-2 regulated antioxidant genes and enhanced iron import capacity (through transferrin receptor-1 upregulation). Upregulation of antioxidant gene expression was also observed upon HIV-1 reactivation in J-Lat cells. Both HIV-1 replication and latency reactivation were associated with PML degradation. This effect was inhibited by antioxidant supplementation (through NAC or resveratrol) or by iron chelation (through deferiprone or deferoxamine). Conversely, PML degradation could be induced by pharmacologically increasing oxidative stress or iron content. Moreover, PML degradation was dependent on proteasome function and could be inhibited by the proteasome inhibitors MG132 and bortezomib (Figure 1).

**Conclusions:** HIV-1 induced PML degradation is dependent on proteasome function and can be induced by increasing oxidative stress and/or intracellular iron content. Combined treatments converging on PML degradation may be able to increase the the anti-latency efficacy of arsenic trioxide.

## PP 1.26

### Expression of CircRNAs in HIV-1 latently infected cells from an *in vitro* model

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**Background:** The major barrier preventing the eradication of HIV-1 is a small reservoir of latently infected CD4+ T-cells that persist after antiretroviral therapy (ART) and can spawn new waves of infection after ART cessation. These rare latently infected cells have a slow division rate, and characterization of them will guide future HIV cure strategies. Circular RNAs (circRNAs) are single stranded covalently closed RNAs which lack the characteristics of linear mRNA such as 5′cap and 3′poly-A tails. CircRNAs are formed as byproduct of post-transcriptional 'back-splicing' of coding-genes. Most transcriptomic studies in HIV-1 focus on RNA species, like mRNA or miRNA, while the expression of circRNAs remains to be elucidated. The expression patterns of circRNA have been reported to be sensitive to viral infection and cell division rates, which allow us to hypothesize that circRNAs could be found in HIV-1 latently infected cells.

**Methods:** We re-analyzed publicly available total RNA-seq data from an *in vitro* HIV-1 latency model. The dataset consisted of four conditions (LI: latently infected; LU: latently uninfected; LIR: latently infected reactivated; LUR: latently uninfected reactivated) from four healthy donors. Reads were mapped to identify gene expression with Hisat2 and Stringtie, then unmapped reads were used to identify circRNAs with CIRCExplorer2 and quantified with CLEAR. differentially expressed (DE) was calculated with edgeR and Results: were validated with RT-qPCR.

**Results:** More than 3,000 high confidence circRNAs were identified from all samples; reactivated samples showed a significant decrease in the number of circRNAs while the number of expressed genes remained similar amongst the four conditions. We focused on the comparison between LI and LU and identified 2 DE circRNAs (circPDE3B and circGTDC1) and 52 DE genes. Interestingly, the two circRNAs were significantly upregulated in LI but their corresponding gene of origin were not among the list of 52 DE expressed genes. These Results: were validated through RT-qPCR.

**Conclusions:** The Results: presented here greater our knowledge of the transcriptional state of persistently infected CD4+ T-cells. We have further shown that some circRNAs are differentially expressed in latent cells from an *in vitro* HIV-1 latency model. Additional studies on circRNAs in HIV-1 persistence studies are warranted.

**Conflict of interest:** Funding: Martin Delaney BELIEVE Collaboratory (NIH grant 1UM1AI26617); which was supported by the following NIH Co-Funding and Participating Institutes and Centers: NIAID, NCI, NICHD, NHLBI, NIDA, NIMH, NIA, FIC, and OAR. Miguel de Mulder Rougvie is funded in part by the Department of Medicine, Fund for the Future program at Weill Cornell Medicine.

## PP 1.27

### Cleavage and polyadenylation specific factor 6 is required for HIV latency reversal

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**Background:** HIV can establish a status of latency in the early phase of infection both *in vitro* and *in vivo*. The mechanisms behind the HIV latency establishment and maintenance are not totally deciphered yet. To further understand the roles of host factors playing in HIV latency, we tested a preliminary panel of candidate genes which are suspected to be involved in controlling HIV-1 transcription. Cleavage and polyadenylation specific factor 6 (CPSF6) is one of our candidates, previously known to play a role in 3′ RNA cleavage and