

Acknowledgments

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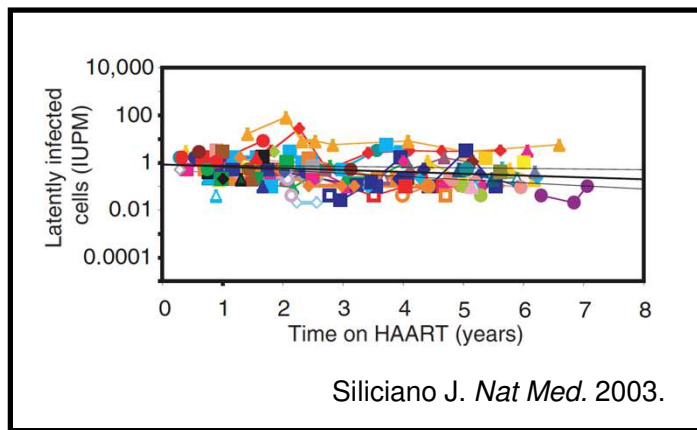
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SCOPE Study Participants

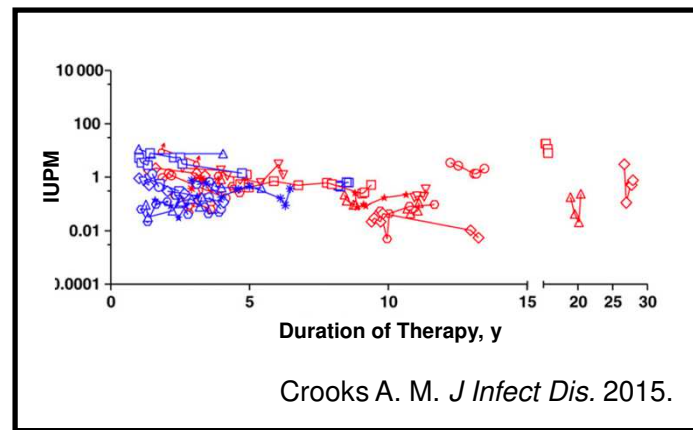


HIV Persistence in CD4 T Cells

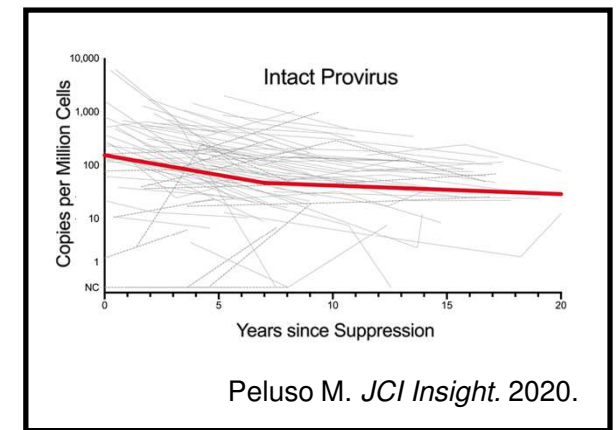
HIV-infected CD4 T cell reservoir decay (blood)



$t_{1/2} = 44$ months



$t_{1/2} = 3.6$ years



$t_{1/2} = 48$ months*

What is the biology of these cells?

*Calculation for years 0-7 since suppression

The “Latent Reservoir”

- Infected cells with reversible quiescence of HIV gene expression
- Associated with molecular blocks to HIV gene transcription and translation
- No substantial reservoir size reduction in clinical studies using therapeutic latency reversal

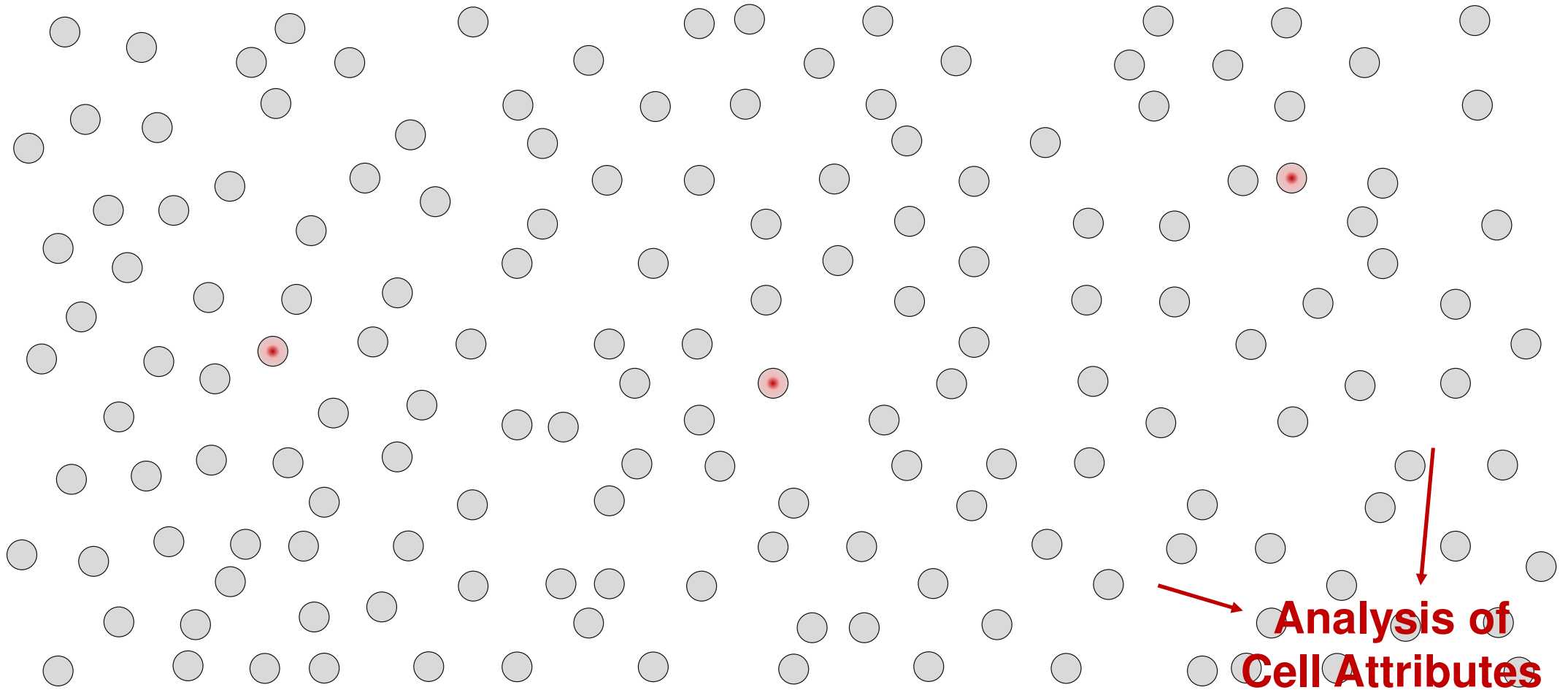
What are we missing?

Using CD4 T Cell Subsets to Study Reservoirs

- Maturation state
 - ~90% of integrated HIV DNA found in CD27⁺ memory cells (Chomont, *Nat Med* 2009)
 - ~70% of intact HIV DNA found in CD27⁻ memory cells (Hiener, *Cell Rep* 2017)
- Functional profile
 - Replication-competent HIV enriched in CXCR3⁺ cells (Banga, *Front Immunol* 2018)
 - Intact, clonal HIV DNA sequences found in Th1 cells (Lee, *JCI* 2017)
- Antigen specificity
 - HIV-specific cells ~2-5x enriched for HIV DNA vs. total memory cells (Douek, *Nature* 2002)
 - Antigen-responsive clones harbor HIV DNA (Mendoza, *J Exp Med* 2020; Simonetti, *J Clin Invest* 2020)
- Anatomic localization
 - Frequencies of HIV DNA in memory cells in ileum > blood (Yukl, *J Inf Dis* 2013)
 - Replication-competent HIV enriched in T_{FH} cells (Banga, *Nat Med* 2016)

**Infected cells scattered among subsets
→ Reservoirs are *diverse***

Goal: To Describe Infected Cells in Their Natural State



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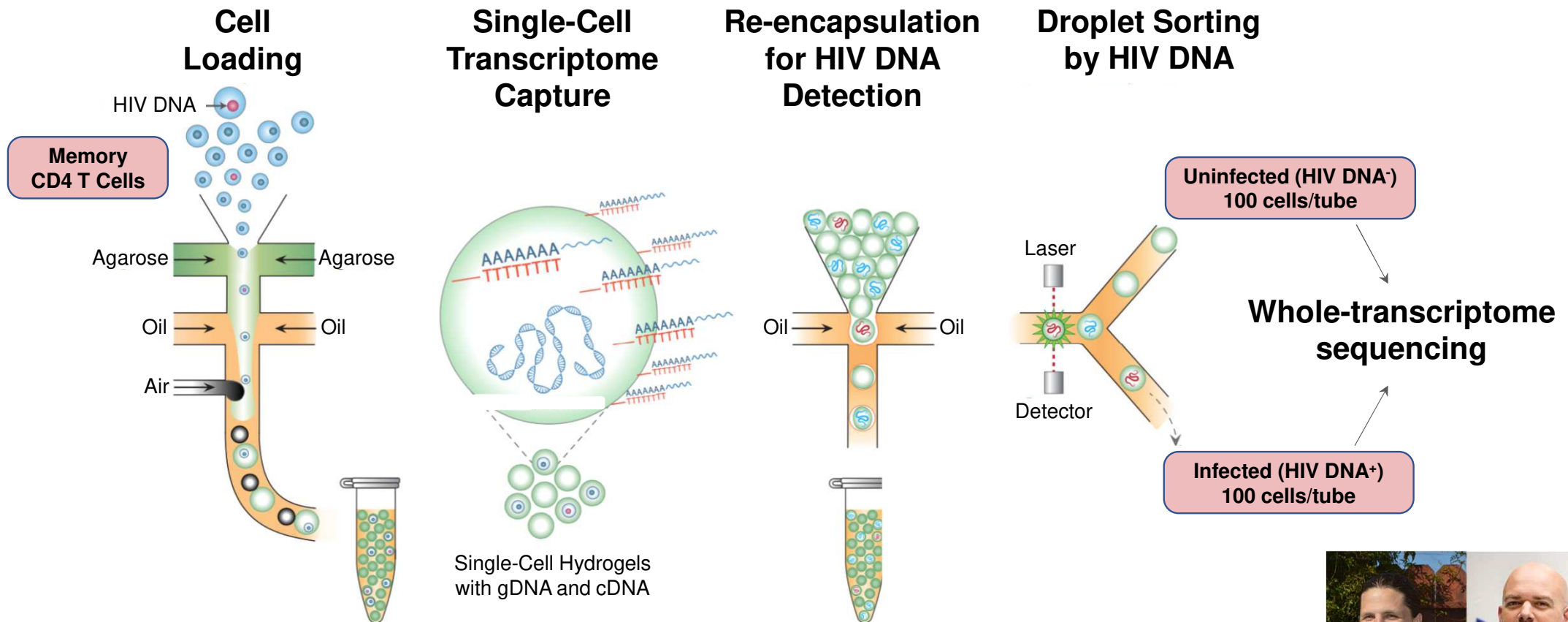
Must include latently-infected cells



The diagram illustrates a population of cells. Most cells are represented by light gray circles with black outlines, scattered across the left and center of the image. On the right side, there are three distinct cells represented by light red circles with a darker red dot in the center. Two red arrows point from the text 'Analysis of Cell Attributes' to these red cells. One arrow points to the middle red cell, and the other points to the bottom-right red cell.

**Analysis of
Cell Attributes**

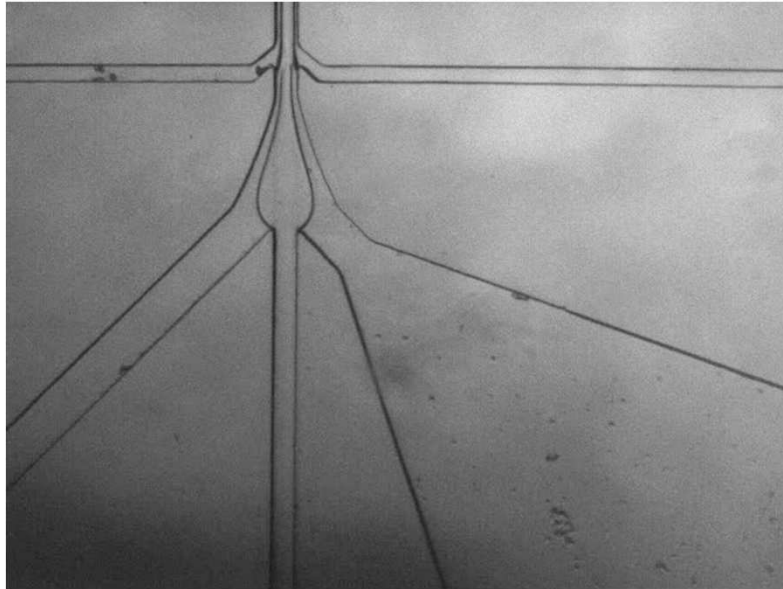
Host Gene Expression Profiling by FIND-seq



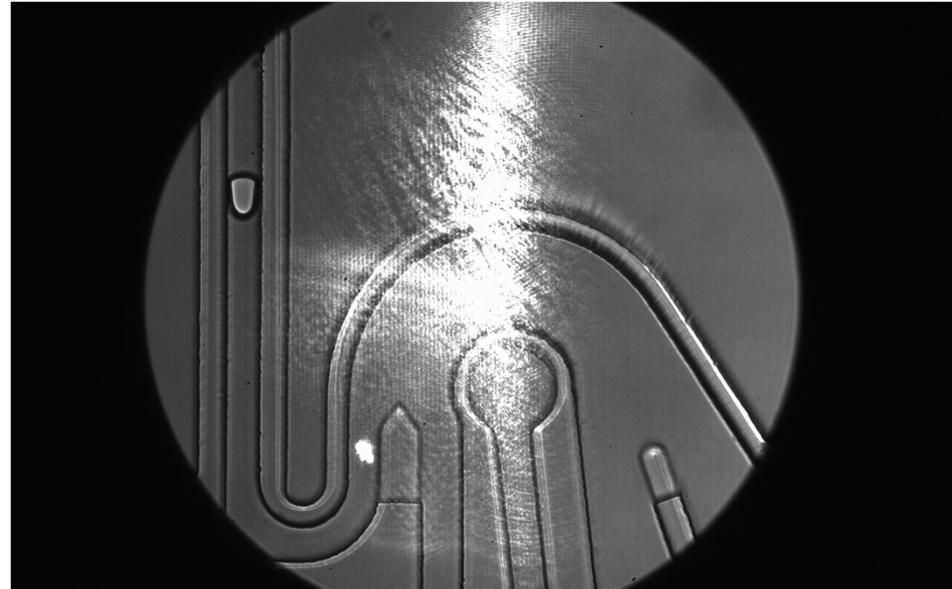
FIND-Seq = Focused Interrogation of Cells by Nucleic Acid Detection and Sequencing

FIND-seq Microfluidic Devices

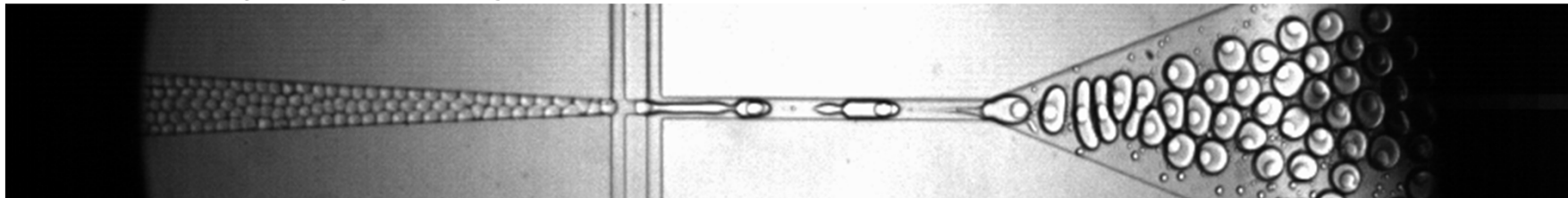
Device 1: Cell Loading



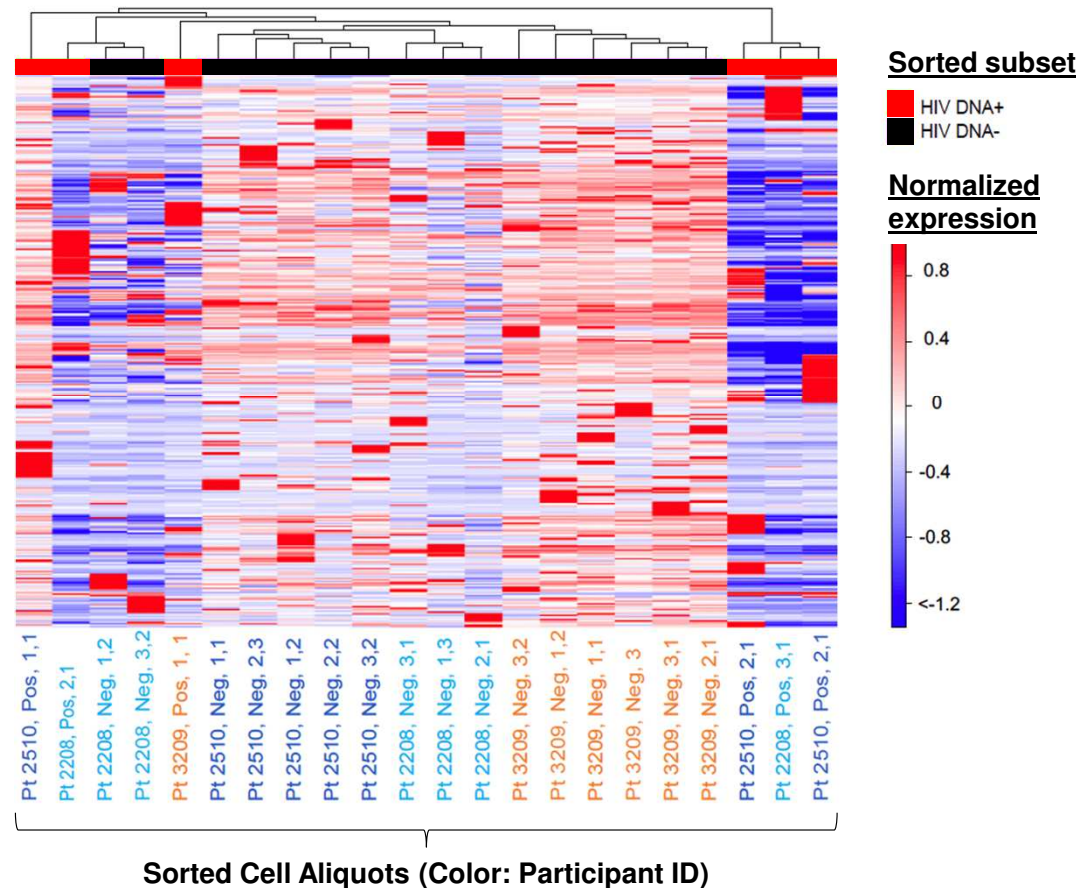
Device 3: Droplet Sorting



Device 2: Hydrogel Reinjection



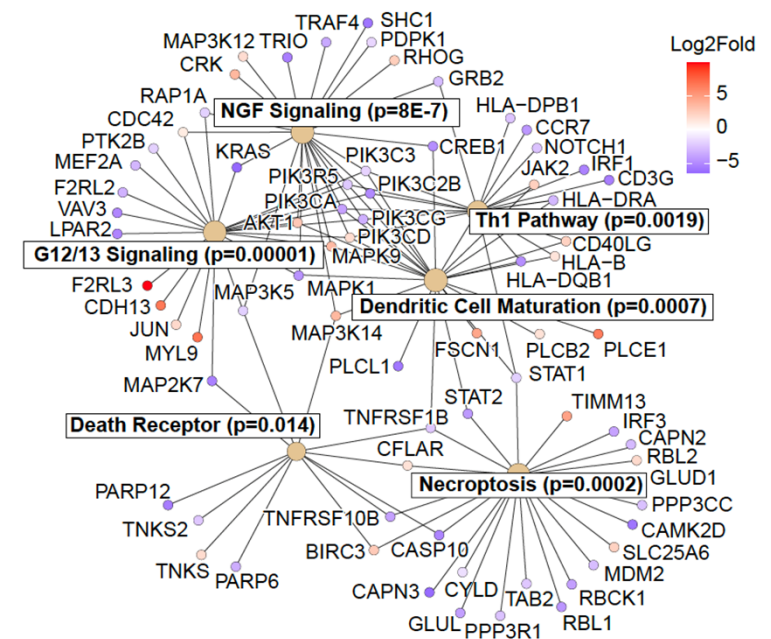
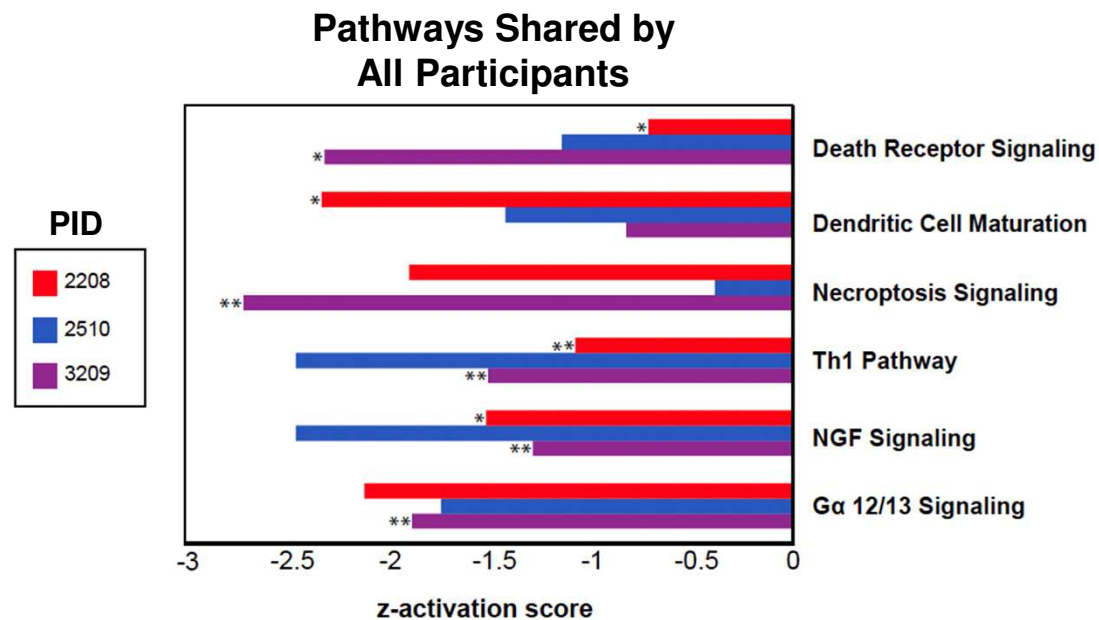
HIV-Infected Cell Transcriptomic Clustering



Partial segregation of infected vs. uninfected cells



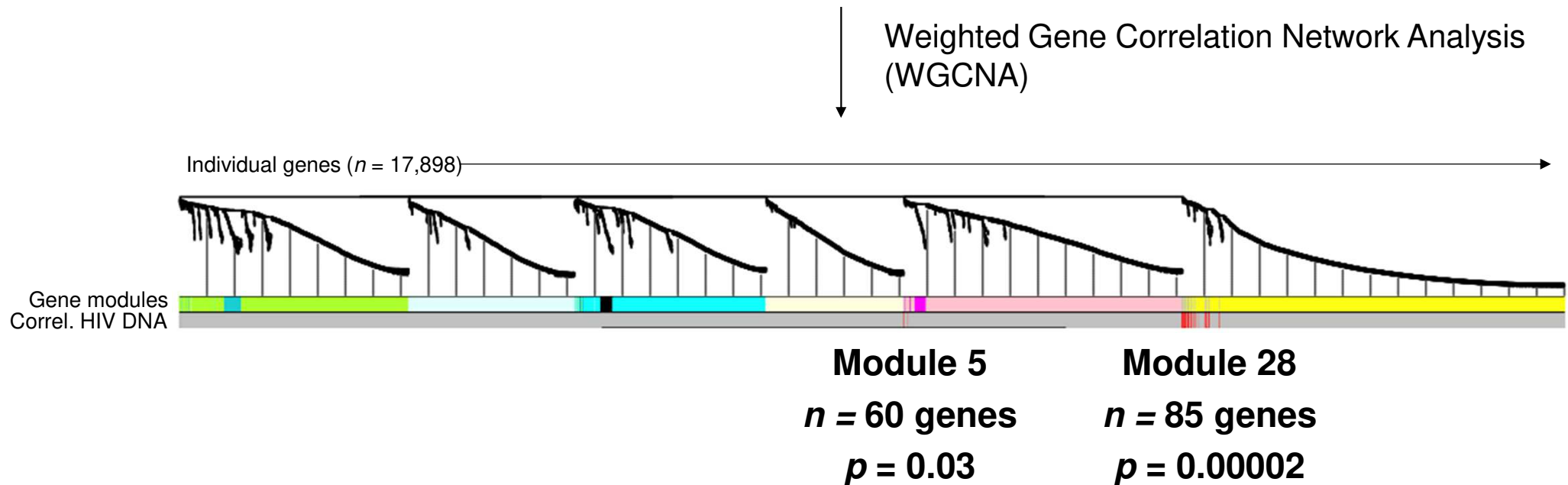
Differential Gene Expression by HIV-Infected Cells



Inhibition of cell death and antiproliferative pathways in infected cells

Transcriptomic Signatures of HIV-Infected Cells

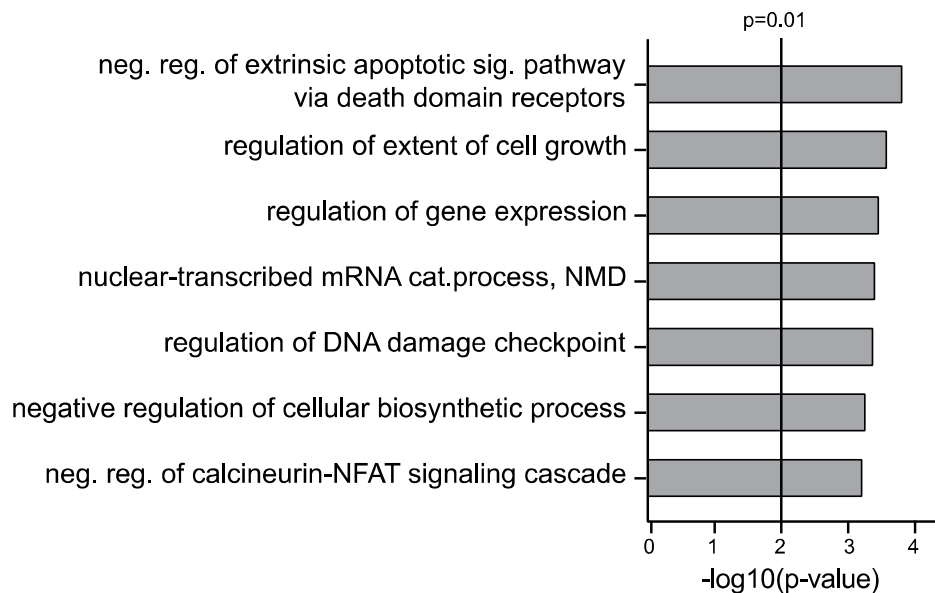
Infected and Uninfected Cell Transcriptomes



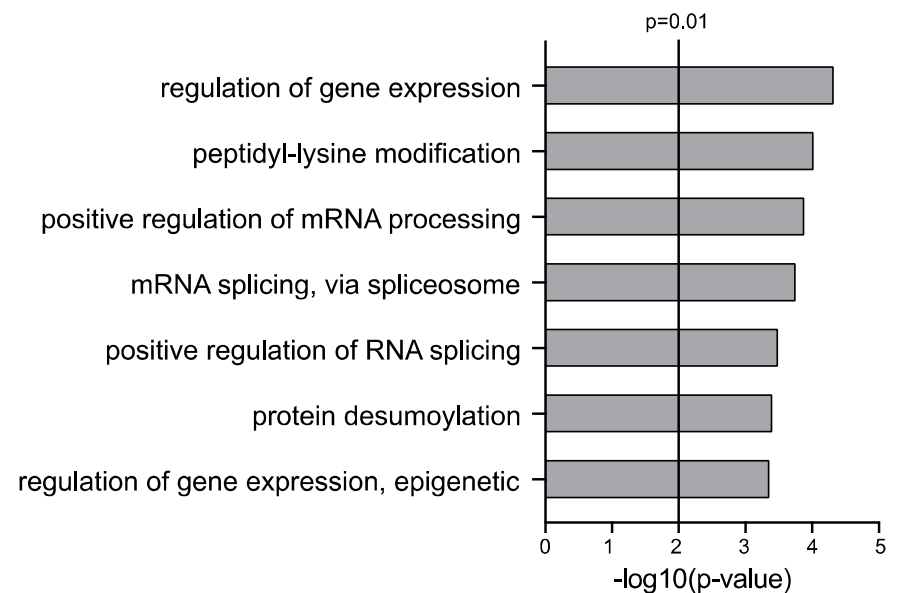
Two small groups of genes associated with infected cells

HIV-Infected Cell Signatures - Gene Ontology

Module 5



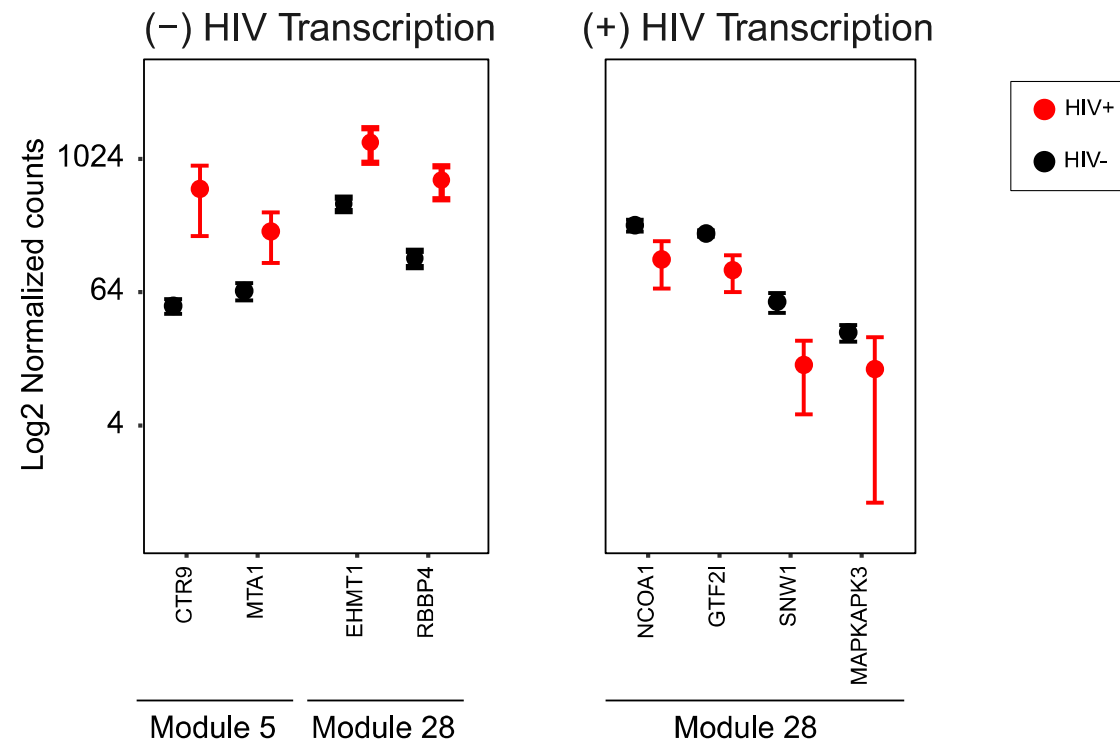
Module 28



Gene regulatory, RNA processing, and cell state gene signatures

HIV-Infected Cell Signatures - Selected Genes

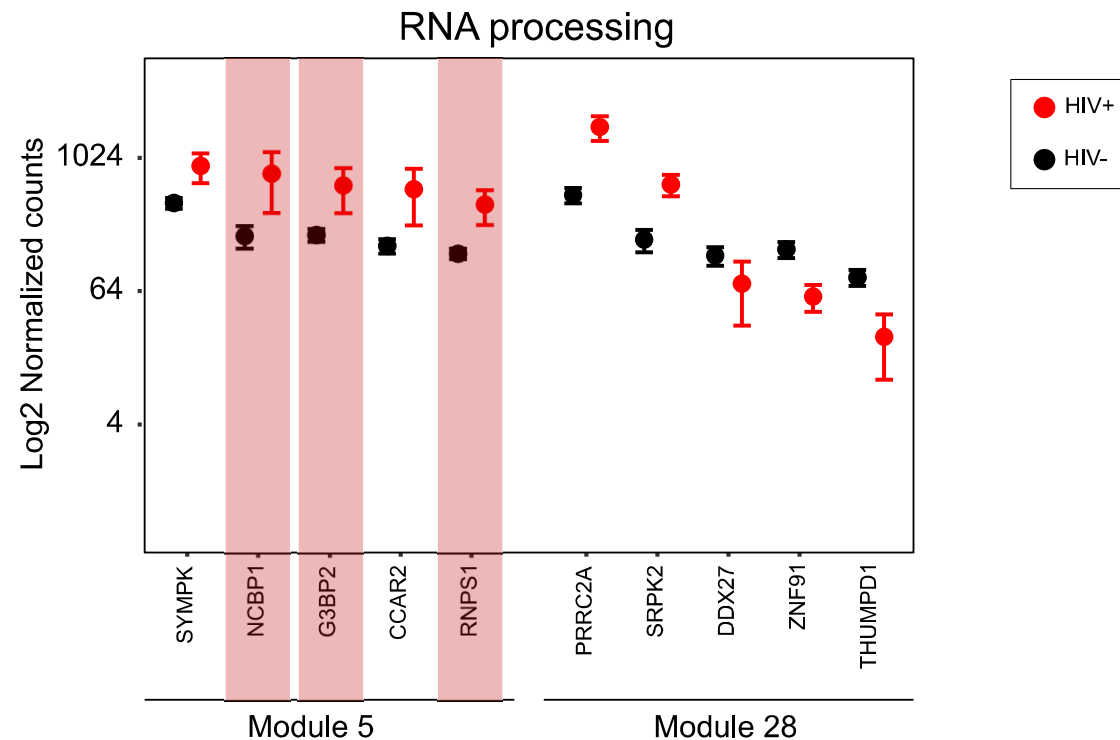
Genes with average difference ≥ 2 -fold ($n = 44$)



HIV transcriptional silencing signature

HIV-Infected Cell Signatures - Selected Genes

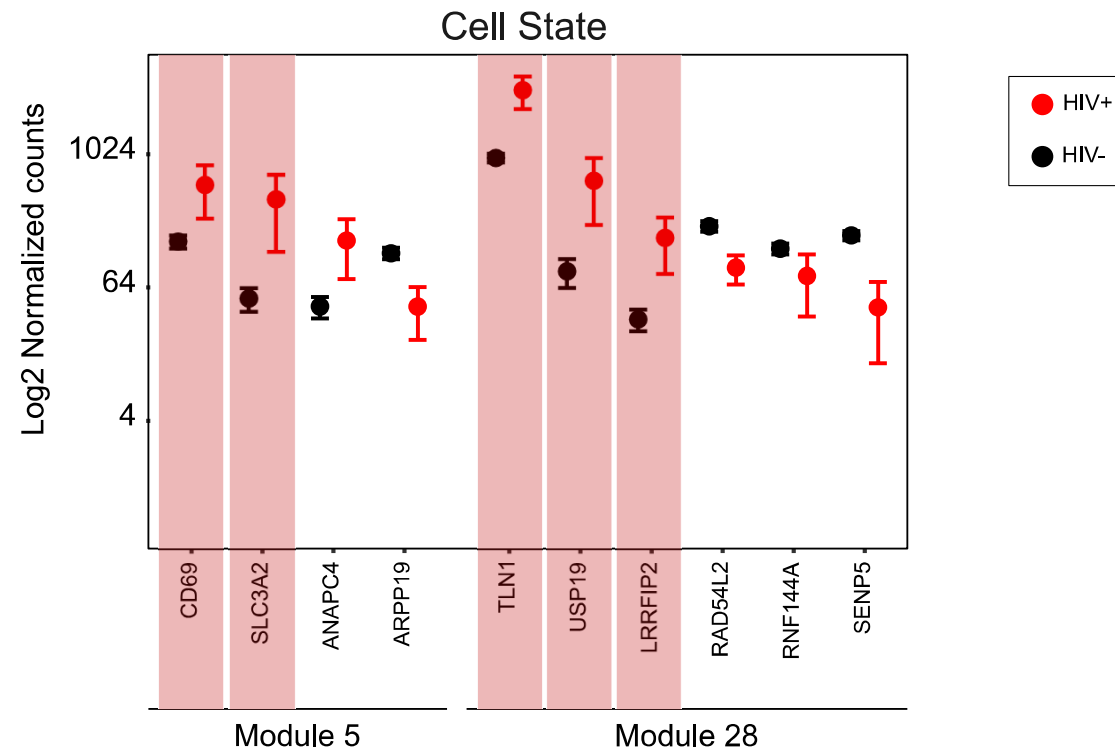
Genes with average difference ≥ 2 -fold ($n = 44$)



Nonsense-mediated decay, stress granules - post-transcriptional HIV silencing?

HIV-Infected Cell Signatures - Selected Genes

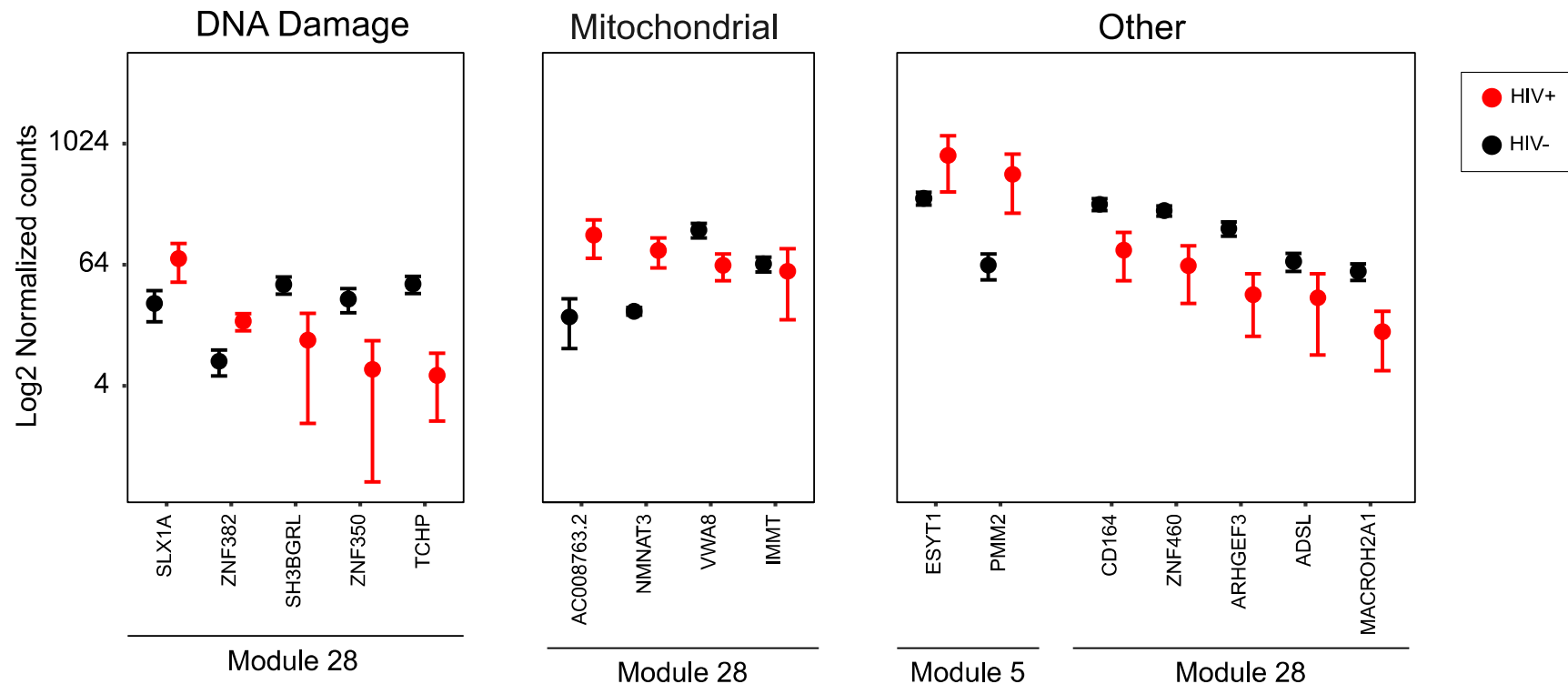
Genes with average difference ≥ 2 -fold ($n = 44$)



Cell activation, proliferation, and survival

HIV-Infected Cell Signatures - Selected Genes

Genes with average difference ≥ 2 -fold ($n = 44$)



DNA damage response - induced by infection?

Summary of Findings

- New technology for whole transcriptome sequence analysis of HIV-infected cells was developed
- Gene expression patterns of HIV-infected memory CD4 T cells under ART were distinct from those of uninfected memory CD4 T cells
- HIV-infected memory CD4 T cell transcriptomes demonstrated inhibition of cell death and antiproliferative signaling
- HIV-infected memory CD4 T cells expressed transcriptomic signatures of HIV silencing

Findings concordant with known barriers to cure

Possible Mechanisms

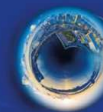
In vivo selection among cells

- Ex., Module 5 signature partially enriched in CCR6⁻ pT_{FH} cells (GSEA enrichment score = 1.49, FDR $p = 0.037$)

Consequence(s) of infection

- Cell response to infection
 - DNA damage
 - Other
- Effect of HIV expression product(s)

Opportunity to define new targets for cure strategies



COMMUNITY SUMMARY

- **Key question(s)** being asked
 - Do HIV-infected CD4 T cells in people receiving ART have distinctive patterns of gene expression, as compared to uninfected cells?
 - Could such patterns help account for HIV persistence during ART?
- **Key finding(s)** and take-home message
 - Gene expression patterns of HIV-infected CD4 T cells indicated a reduced sensitivity to cell death signals and an increased capacity to maintain HIV in a “latent” state
- What are the **next steps**?
 - Studies of combination therapies targeting latency ± cell death
 - Further efforts to understand the biology of HIV-infected CD4 T cells