# Effects of HIV infection on single cell epigenome and transcriptome

**Sara Morón-López, PhD**

Associate Researcher

Retrovirology and Clinical Studies Group

# Why are single-cell technologies necessary to understand HIV infection and persistence?

- HIV infected cells are **rare** within a mixed population of cells (<0.1% HIV RNA+ cells)

  → Signatures from infected cells may be masked by uninfected cells

- CD4+ T cells are highly **heterogeneous** in nature

Activation

Polarization      Memory

Proliferation capacity

| Polarization | Memory |
|---|---|
| Th1 | Naïve |
| Th2 | Central memory |
| Th17 | Transitional memory |
| Treg | Effector memory |
| | Effector |

Exhaustion

Cytokine response

Antigen specificity

"Needle in the needle stack" quote from Katie Bar
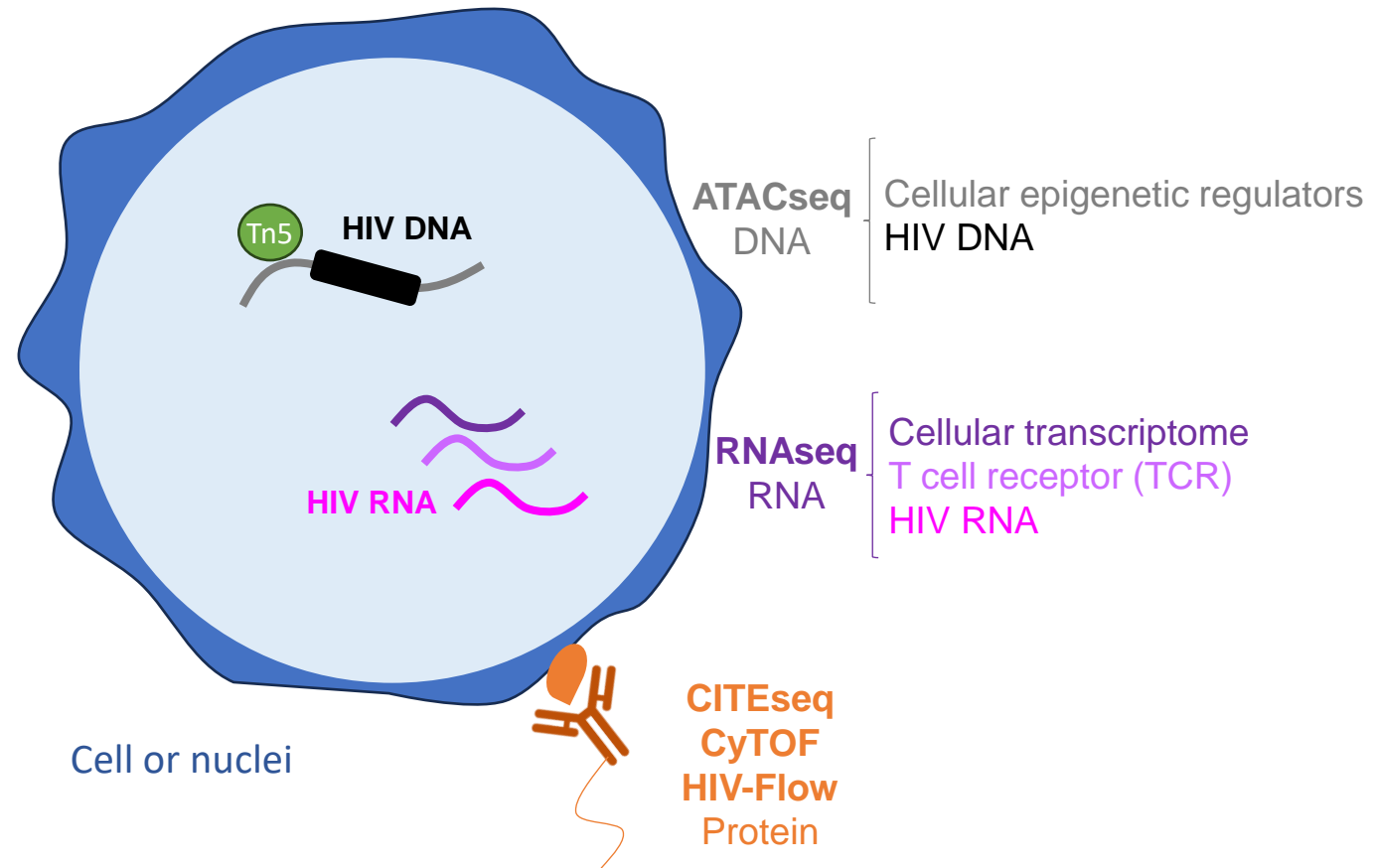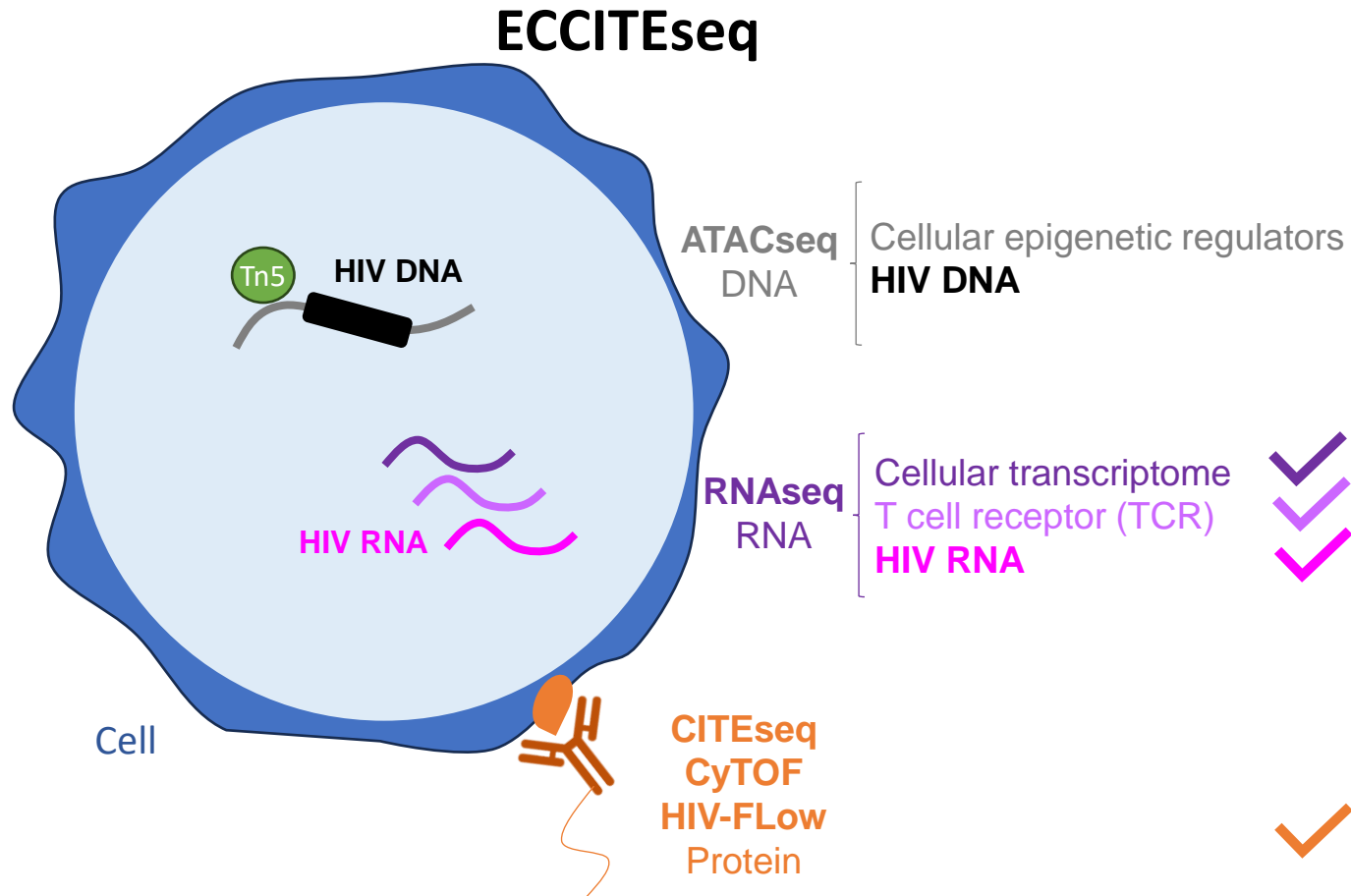
# Why single-cell multiomics might be helpful?

- It resolves the **heterogeneity** of cells

- It identifies the **rare** cells of interest

- It provides genomic, transcriptomic and proteomic wide **discovery of potential mechanisms or therapeutic targets** that can be validated

26/10/2023 S.Morón-López

# Current single-cell high throughput strategies to study HIV

# Current knowledge of single-cell multiomics to study HIV

**ECCITEseq**



**ATACseq**
DNA

Cellular epigenetic regulators
**HIV DNA**

**RNAseq**
RNA

Cellular transcriptome
T cell receptor (TCR)
**HIV RNA**

**CITEseq
CyTOF
HIV-FLow**
Protein

Cell

**Publications:**

**Single-cell multiomics reveals persistence of HIV-1 in expanded cytotoxic T cell clones**
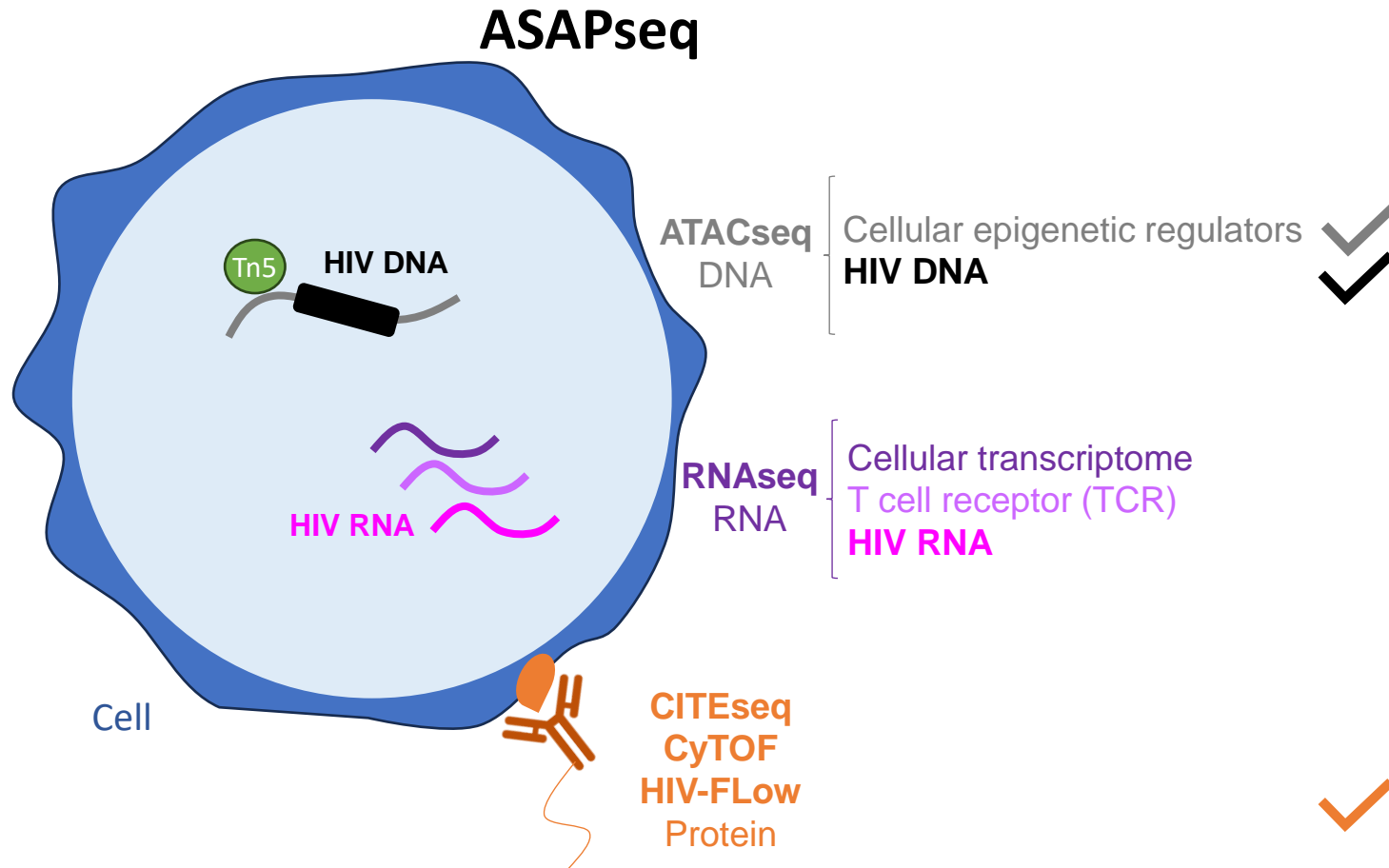Collora *et al.*, Immunity 2022

**Distinct gene expression by expanded clones of quiescent memory CD4+ T cells harboring intact latent HIV-1 proviruses**
Weymar *et al.*, Cell Reports 2022

Using HIV RNA as a surrogate, ECCITE-seq identified enrichment of HIV-infected cells in clonally expanded cytotoxic CD4+ T cells and, enrichment of latent cells carrying intact HIV-1 proviruses in clonally expanded quiescent memory CD4+ T cells
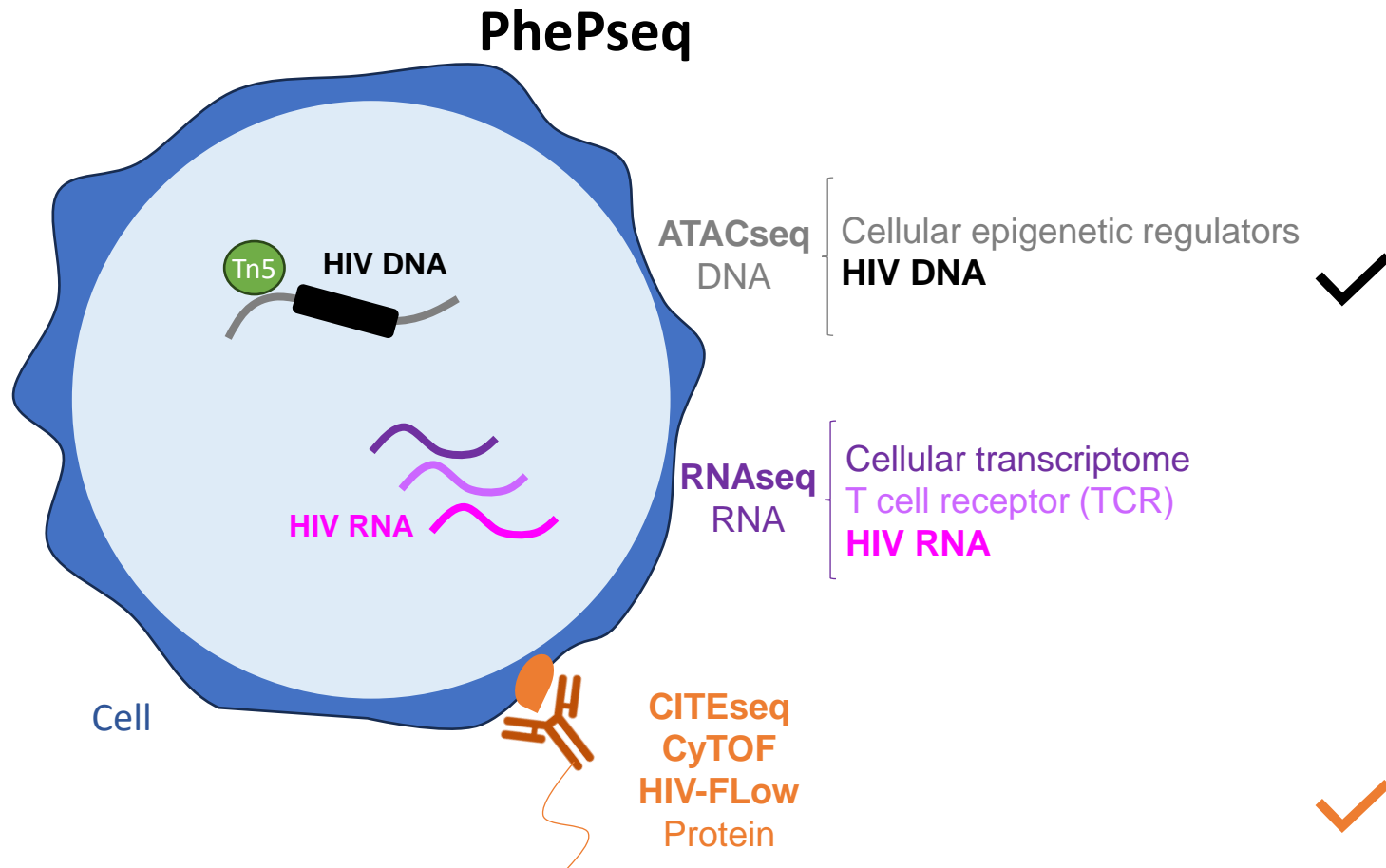
# Current knowledge of single-cell multiomics to study HIV

**ASAPseq**



Cell

**ATACseq**
DNA

Cellular epigenetic regulators
**HIV DNA**

**RNAseq**
RNA

Cellular transcriptome
T cell receptor (TCR)
**HIV RNA**

**CITEseq**
**CyTOF**
**HIV-FLow**
Protein

**Publication:**

**Profound phenotypic and epigenetic heterogeneity of HIV-1-infected CD4+ T cell reservoir**
Wu *et al.*, Nature Immunology 2022

Using ATAC-seq to identify HIV DNA, ASAP-seq captured transcription factor activity and surface protein expression of HIV DNA+ cells

# Current knowledge of single-cell multiomics to study HIV

**PhePseq**



**ATACseq** — Cellular epigenetic regulators
DNA — **HIV DNA** ✓

**RNAseq** — Cellular transcriptome
RNA — T cell receptor (TCR)
**HIV RNA**

Cell

HIV DNA

Tn5

HIV RNA

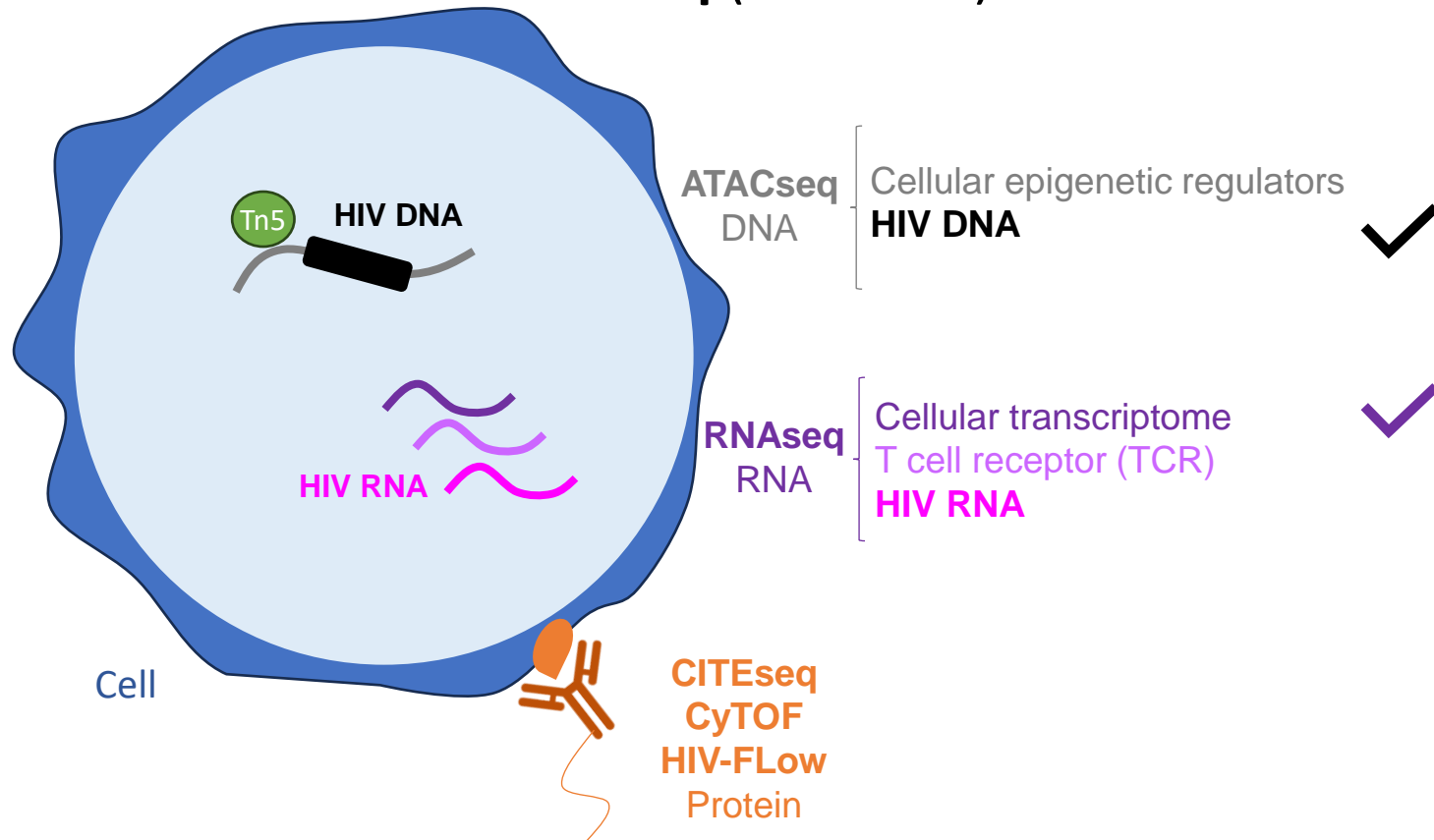**CITEseq
CyTOF
HIV-FLow**
Protein ✓

**Publication:**

**Phenotypic signatures of immune selection in HIV-1 reservoir cells**
Sun *et al.*, Nature Medicine 2023

Using targeted HIV DNA amplification, PheP-seq identified surface protein expression of intact versus defective HIV-infected cells

# Current knowledge of single-cell multiomics to study HIV

**FINDseq** (≈100 cell)



**ATACseq**
DNA

Cellular epigenetic regulators
**HIV DNA**

✓

**RNAseq**
RNA

Cellular transcriptome
T cell receptor (TCR)
**HIV RNA**

✓

**CITEseq**
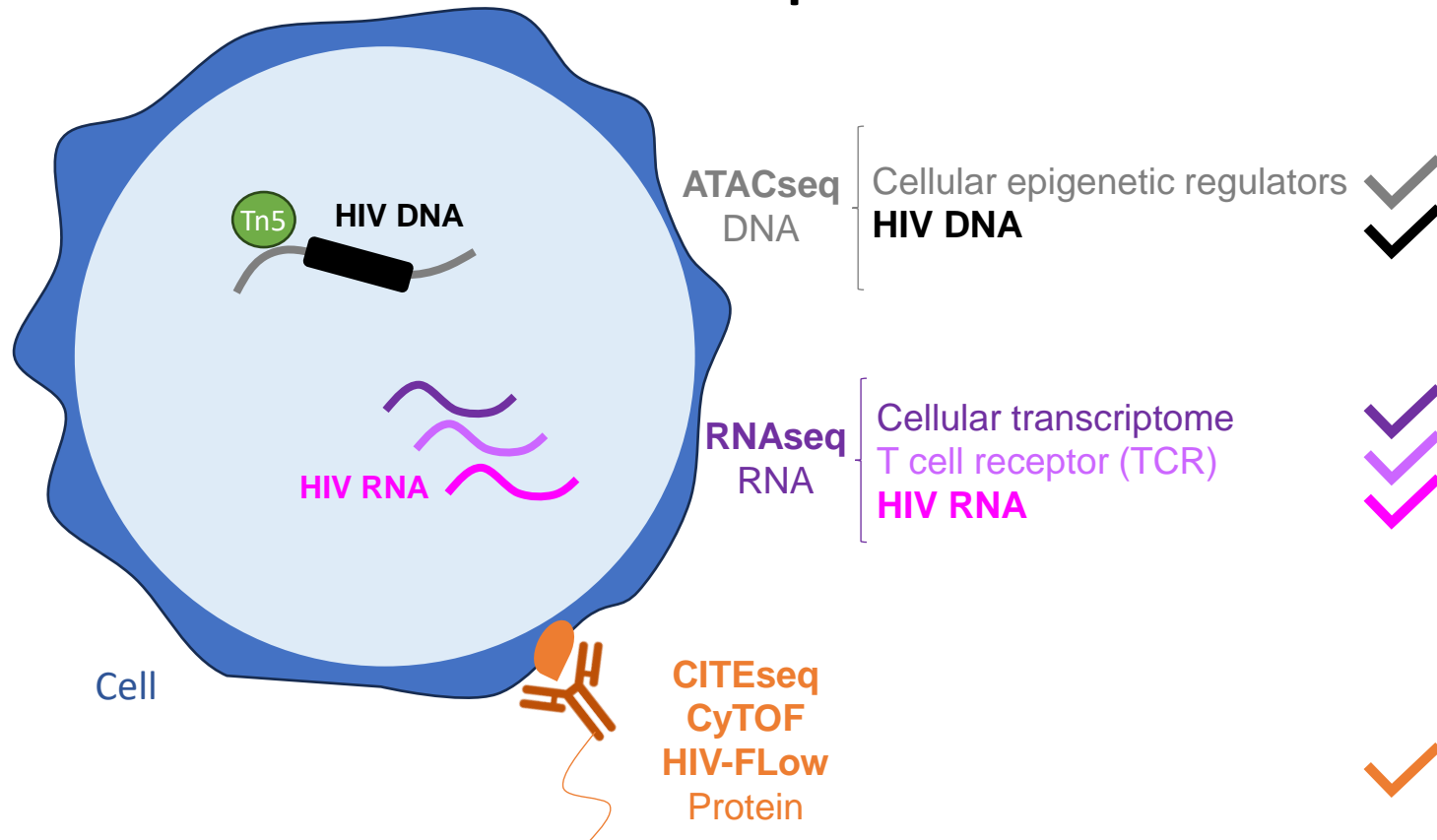**CyTOF**
**HIV-FLow**
Protein

**Publication:**

**HIV silencing and cell survival signatures in infected T cell reservoirs**
Clark *et al.*, Nature 2023

Using HIV DNA PCR-activated microfluidic sorting, FIND-seq captured the bulk transcriptome of HIV DNA cells

# Current knowledge of single-cell multiomics to study HIV

**DOGMAseq**



**ATACseq**
DNA
- Cellular epigenetic regulators ✓
- **HIV DNA** ✓

**RNAseq**
RNA
- Cellular transcriptome ✓
- T cell receptor (TCR) ✓
- **HIV RNA** ✓

**CITEseq
CyTOF
HIV-FLow**
Protein ✓

**Publication:**

**Single-cell epigenetic, transcriptional, and protein states of HIV reservoir**
Wei *et al.*, Oral Abstract #142 CROI 2023

Combining HIV mapping by ATAC-seq and HIV RNA mapping by RNA-seq, DOGMA-seq captured the epigenetic, transcriptional, and surface protein expression of latent and transcriptionally active HIV-infected cells

**Facts**

- HIV infection changes host epigenetic, transcriptomic and proteomic cellular landscapes

**Challenges**

- Previous studies compared **independent cohorts** of people with and without HIV

- **Expensive** techniques that cannot be used to analyze a large number of samples

**Inter-individual variability**

# Longitudinal follow-up of HIV-infection and ART effects at single-cell level

# Longitudinal follow-up of HIV-infection and ART effects at single-cell level
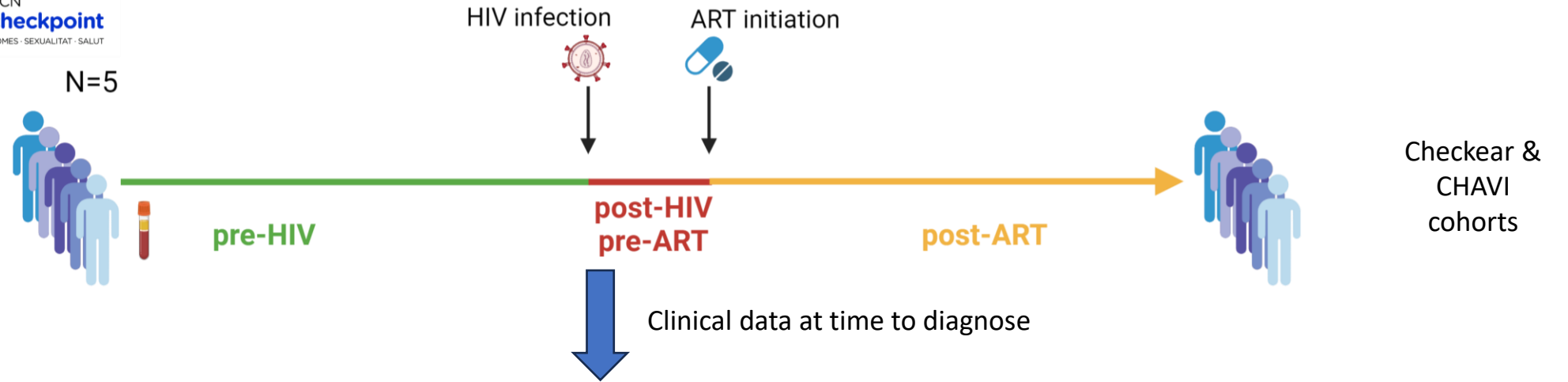


| Participant ID | Age at sample (years) | Estimated time to seroconversion (days) | Log$_{10}$VL at sample (copies/ml) | CD4 at sample (cells/µl) |
|:---:|:---:|:---:|:---:|:---:|
| 1 | 43 | 43 | 5.3 | 244 |
| 2 | 31 | 43 | 5.5 | 556 |
| 3 | 58 | 1 | 6.4 | 422 |
| 4 | 33 | 22 | 5.1 | 403 |
| 5 | 36 | 132 | 5.2 | 1207 |

# Simultaneous detection of cellular epigenetic regulators, cellular transcriptome and HIV DNA/RNA

**10X Genomics Single-cell Multiome**
**(ATAQ-seq + Gene Expression)**
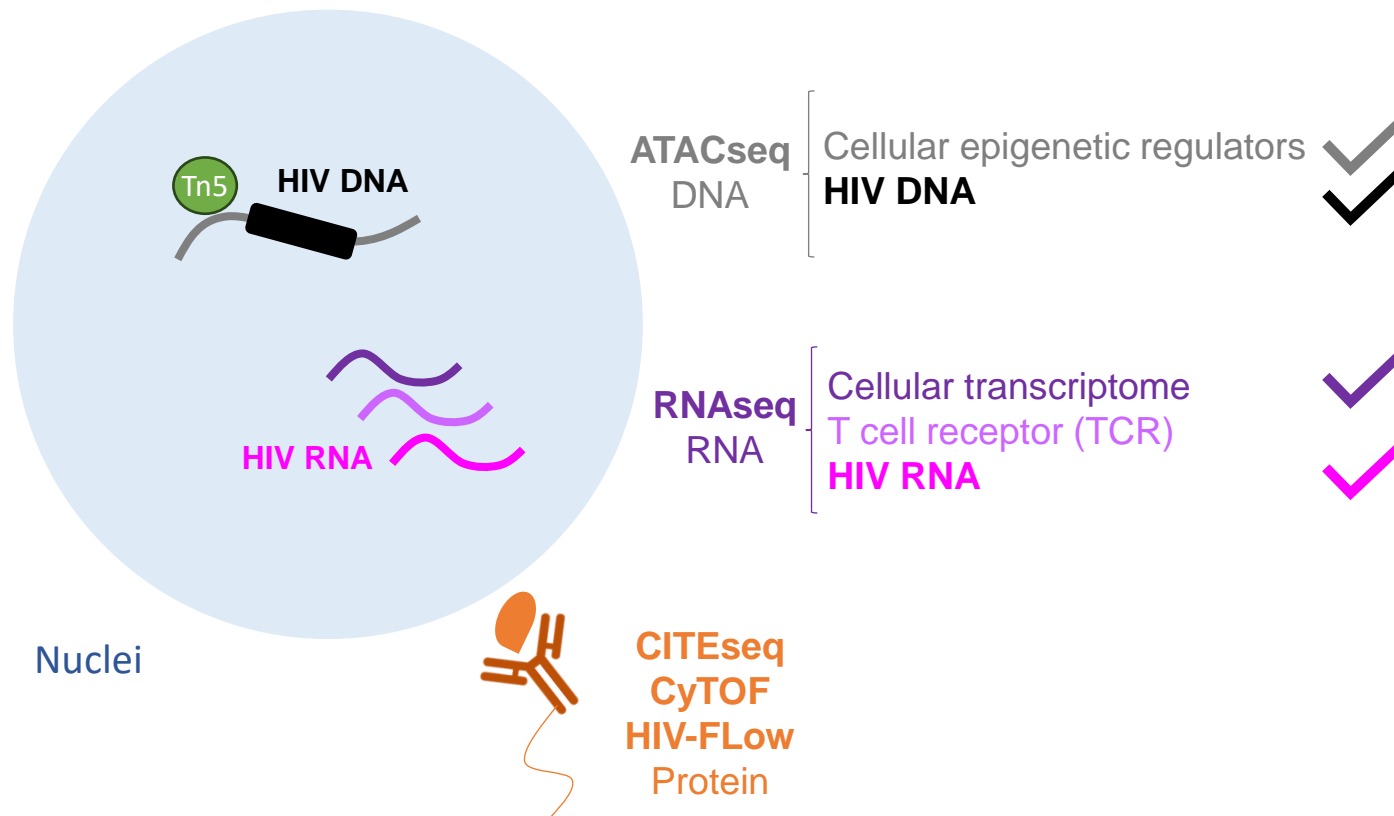


Combining HIV DNA mapping by ATAC-seq and HIV RNA mapping by RNA-seq, this technique might capture the epigenetic and transcriptional changes of latent and transcriptionally active HIV-infected cells

# Analysis of cellular epigenetic regulators, cellular transcriptome and HIV DNA/RNA

## GEX/ATAC Analysis

**Alignment**
CellRanger-ARC
(Human genome|hg38)

**Quality Control**
Seurat
(GEX/ATAC filtering,
doublet removal,
empty gems,
% mitochondrial RNA,
features,
UMI counts, etc.)

**Downstream Analysis**
Seurat
Signac
(Normalization/scaling,
dimensional reduction,
clustering,
cluster annotation,
DE/DA changes, etc.)

**Seurat Object/UMAP**
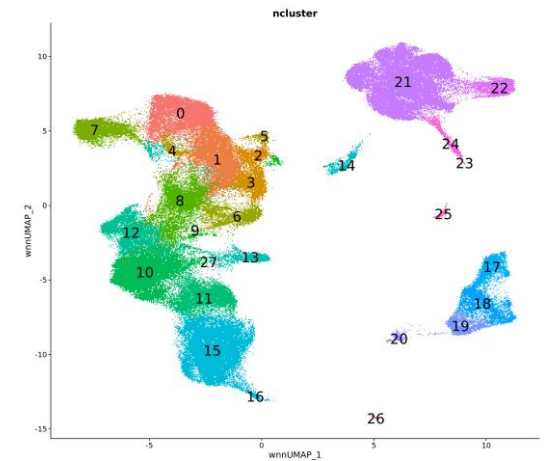


## HIV+ cell detection

**Alignment**
CellRanger-ARC
(Hybrid reference: Human genome|hg38 +
Los Alamos|1306 HIV subtype B sequences)

&

***k*-mer based matching**
Kraken2
(Los Alamos|
1306 HIV subtype B sequences)

# Simultaneous detection of HIV DNA+ and HIV RNA+ cells

- Use of [Kraken2](#) instead of classical alignment based methods

  - High sequence variability of HIV genome

  - Reference: 1306 HIV subtype B European sequences (Los Alamos)

  - This tool allows to quickly identify HIV+ cells, rather than recovering the mapping location within the HIV genome

  - How it works?

Query read

*k*-mers
+ HIV

Hash table
*Refseq*/HIV pairs

Assignment to the HIV

*k*-mers
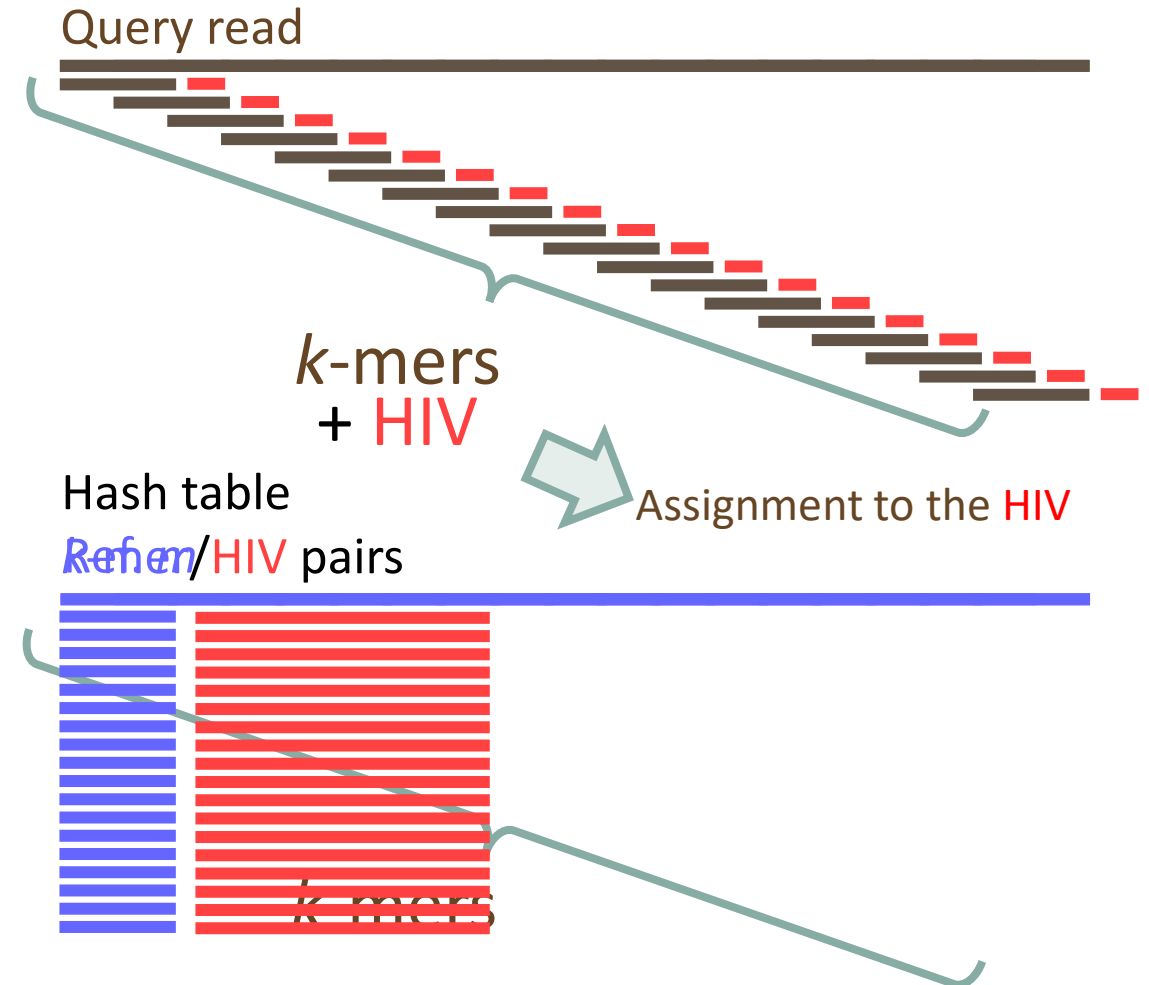
# Identification of specific cell-type signatures

**15 samples**
≈10,000 cells/sample
**150,060 single cells**
- **52,504 pre-HIV**
- **47,868 post-HIV**
- **49,688 post-ART**

Cell annotation based on:
- Azimuth reference (levels 1 and 2)
- VNPs reference (Ángel Bayón)
- Differentially expressed genes between clusters



0: CD4 TN
1: CD4 TCM
2: CD4 TTM
3: CD4 TEM
4: CD4 Treg naive
5: CD4 Treg memory
6: MAIT
7: CD8 TN
8: CD8 TCM
9: CD8 TTM
10: CD8 TEM
11: CD8 TEF
12: CD8 activated
13: CD8 CD14+
14: CD8 gd
15: NK
16: NK CD56bright
17: B naive
18: B intermediate
19: B memory
20: Plasmablast
21: CD14 Monocyte
22: CD16 Monocyte
23: cDC1
24: cDC2
25: pDC
26: HSPC

# Identification of cell changes in PBMC after HIV infection and ART
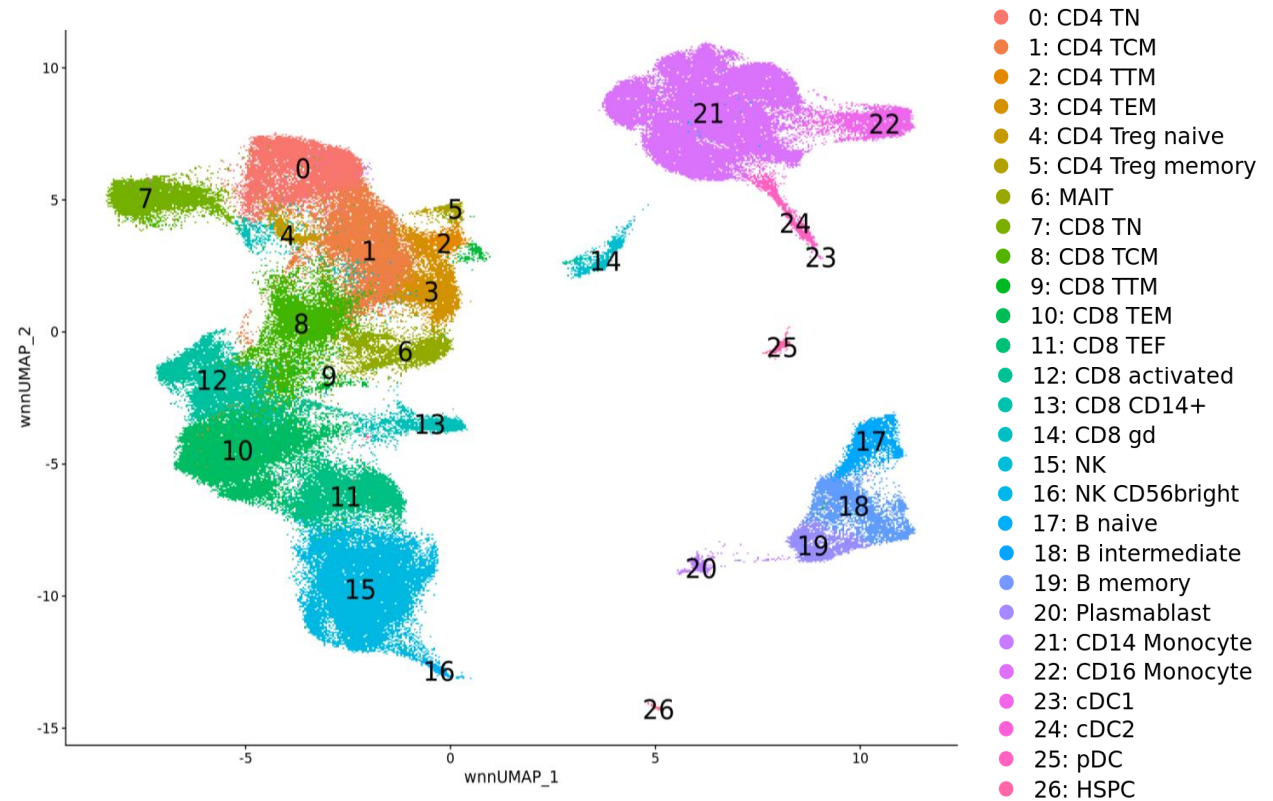


**Pre-HIV**   **Post-HIV**   **Post-ART**
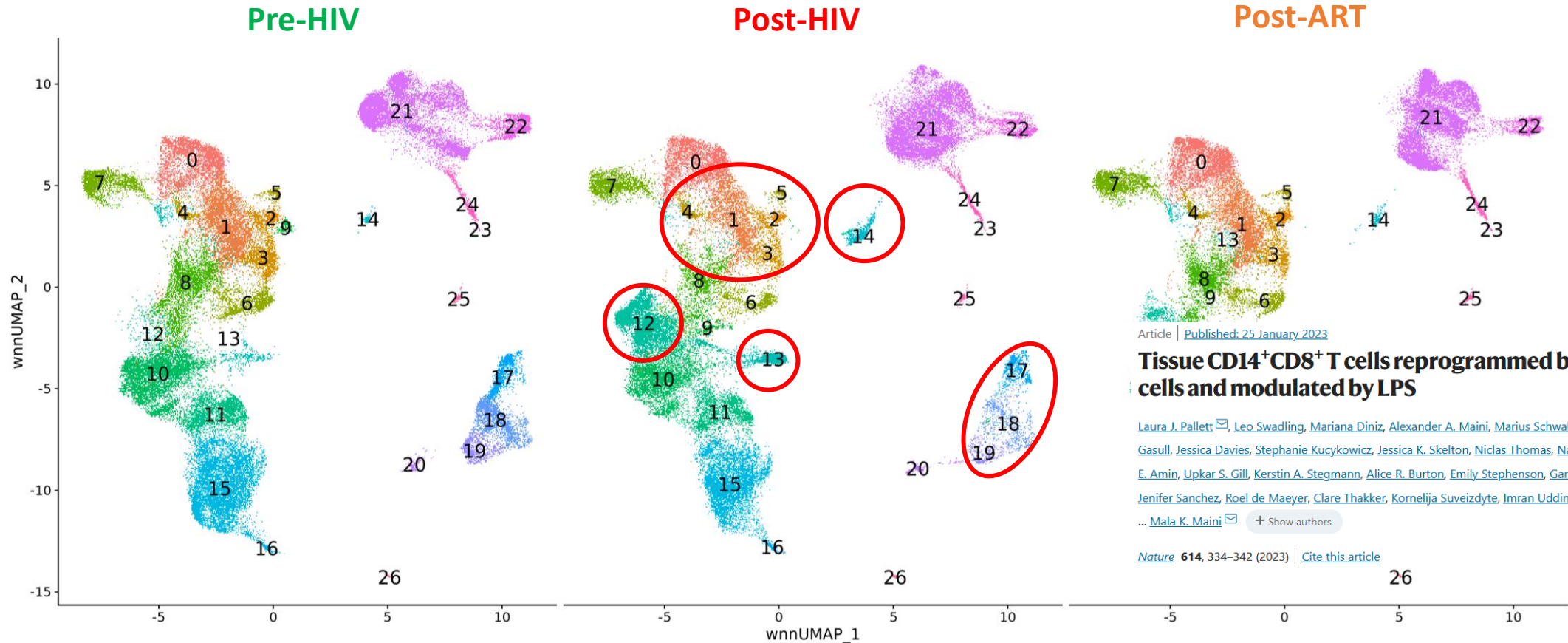
Article | Published: 25 January 2023

**Tissue CD14$^+$CD8$^+$ T cells reprogrammed by myeloid cells and modulated by LPS**

Laura J. Pallett, Leo Swadling, Mariana Diniz, Alexander A. Maini, Marius Schwabenland, Adrià Dalmau Gasull, Jessica Davies, Stephanie Kucykowicz, Jessica K. Skelton, Niclas Thomas, Nathalie M. Schmidt, Oliver E. Amin, Upkar S. Gill, Kerstin A. Stegmann, Alice R. Burton, Emily Stephenson, Gary Reynolds, Matt Whelan, Jenifer Sanchez, Roel de Maeyer, Clare Thakker, Kornelija Suveizdyte, Imran Uddin, Ana M. Ortega-Prieto, ... Mala K. Maini + Show authors

*Nature* **614**, 334–342 (2023) | Cite this article

**15 samples**
≈10,000 cells/sample
**150,060 single cells**
- **52,504 pre-HIV**
- **47,868 post-HIV**
- **49,688 post-ART**

| | | | | |
|---|---|---|---|---|
| 0: CD4 TN | 6: MAIT | 12: CD8 activated | 18: B intermediate | 24: cDC2 |
| 1: CD4 TCM | 7: CD8 TN | 13: CD8 CD14+ | 19: B memory | 25: pDC |
| 2: CD4 TTM | 8: CD8 TCM | 14: CD8 gd | 20: Plasmablast | 26: HSPC |
| 3: CD4 TEM | 9: CD8 TTM | 15: NK | 21: CD14 Monocyte | |
| 4: CD4 Treg naive | 10: CD8 TEM | 16: NK CD56bright | 22: CD16 Monocyte | |
| 5: CD4 Treg memory | 11: CD8 TEF | 17: B naive | 23: cDC1 | |

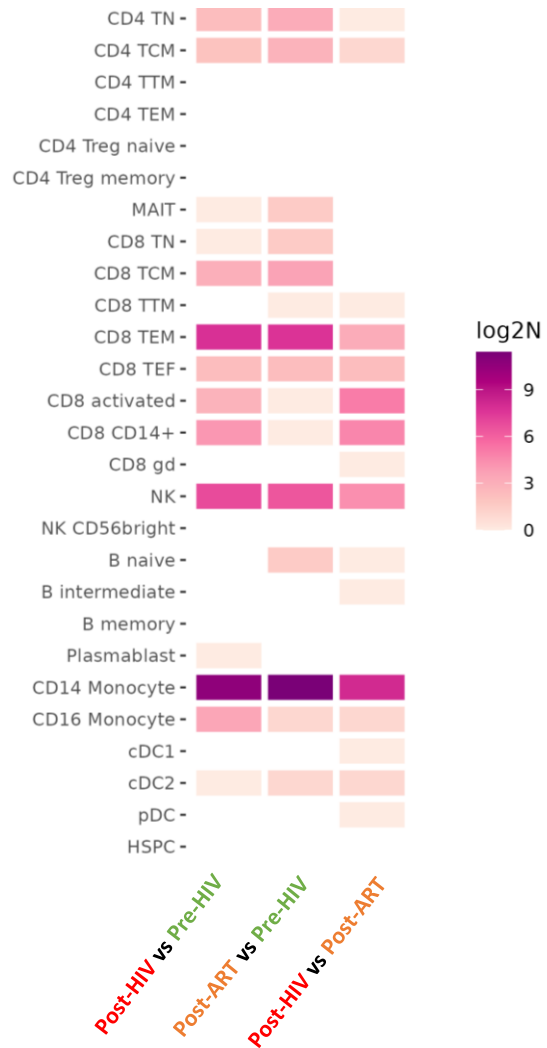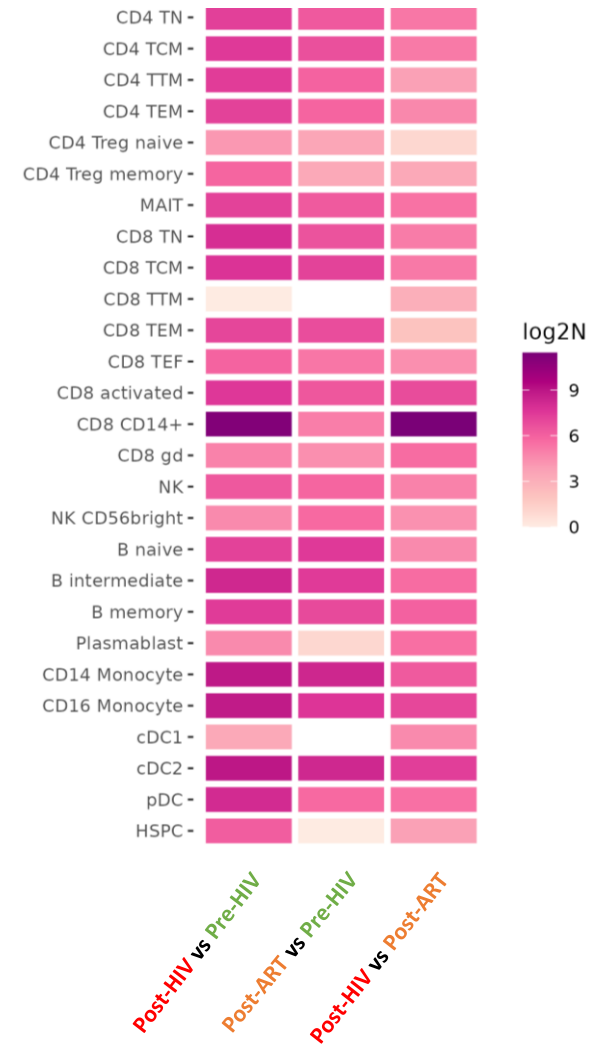# Identification of cell changes in PBMC after HIV infection and ART
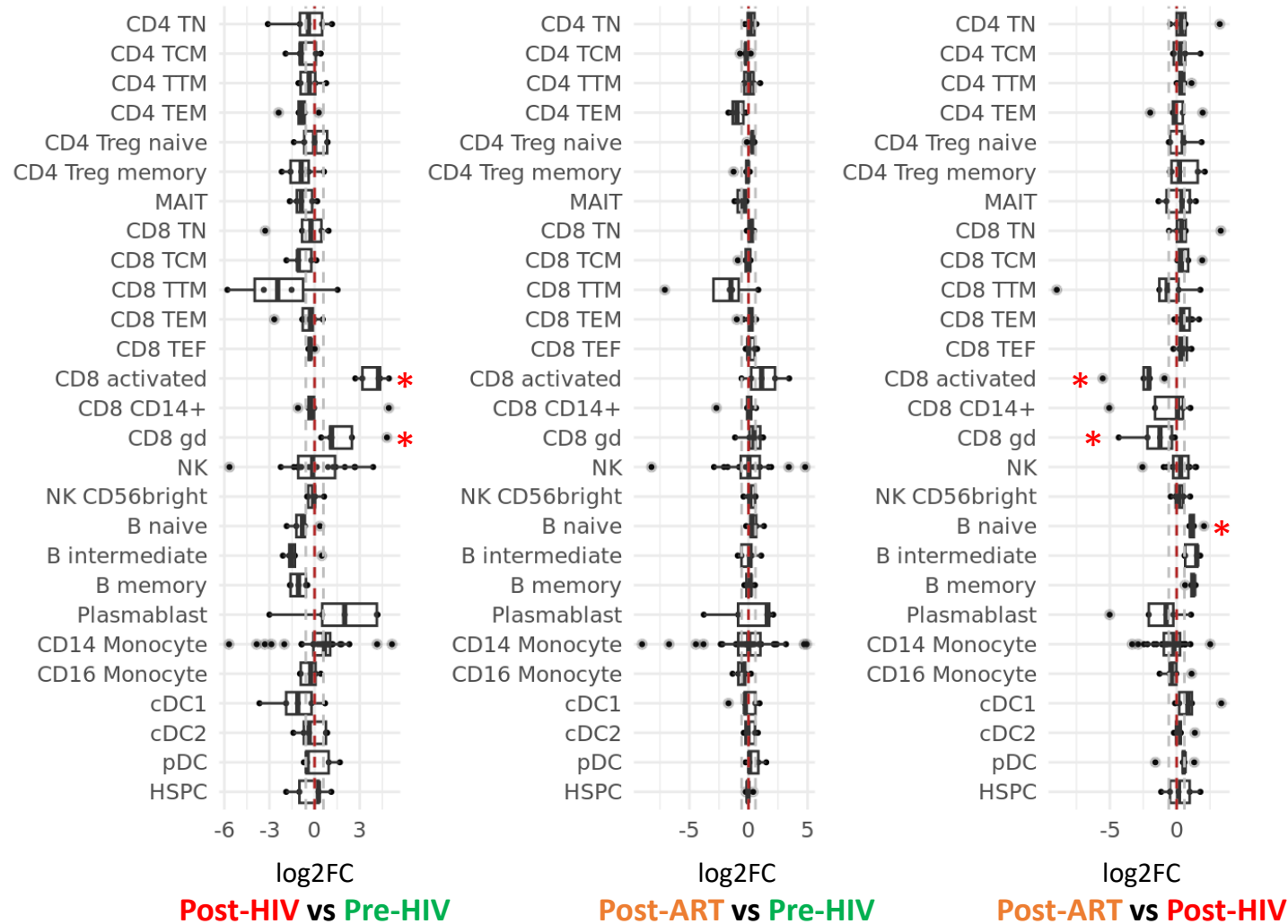


Cell abundance changes reverse after a year of suppressive ART

Logistic regression, FDR correction

# Identification of epigenetic and transcriptomic changes after HIV infection and ART
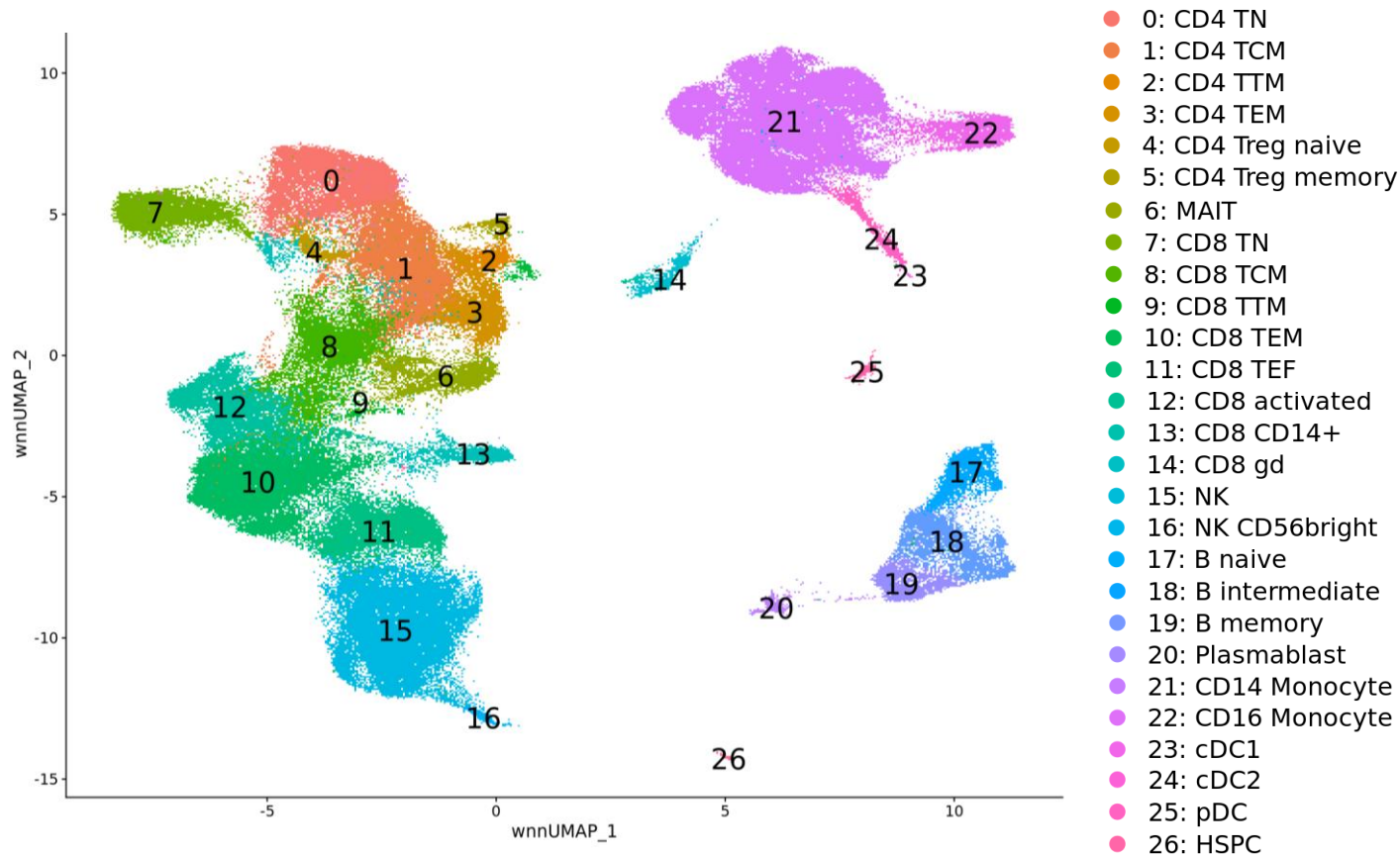


ATACseq – DNA accessibility

RNAseq – Cellular transcriptome

HIV infection causes epigenetic and transcriptomic changes, which do not reverse after a year of suppressive ART

Logistic regression, FDR correction (ATACseq $p_{adj}>0.1$, $log_2FC<0.25$; RNAseq $p_{adj}<0.05$, $log_2FC<0.25$)

# Identification of HIV DNA+ and RNA+ cells



0: CD4 TN
1: CD4 TCM
2: CD4 TTM
3: CD4 TEM
4: CD4 Treg naive
5: CD4 Treg memory
6: MAIT
7: CD8 TN
8: CD8 TCM
9: CD8 TTM
10: CD8 TEM
11: CD8 TEF
12: CD8 activated
13: CD8 CD14+
14: CD8 gd
15: NK
16: NK CD56bright
17: B naive
18: B intermediate
19: B memory
20: Plasmablast
21: CD14 Monocyte
22: CD16 Monocyte
23: cDC1
24: cDC2
25: pDC
26: HSPC

**Alignment-based**
CellRanger-ARC
(Hybrid reference: Human genome|hg38 +
Los Alamos|1306 HIV subtype B sequences)

***k*-mer based matching**
Kraken2
(Los Alamos|1306 HIV subtype B sequences)

# Identification of HIV DNA+ and RNA+ cells



**Cell Ranger-ARC**
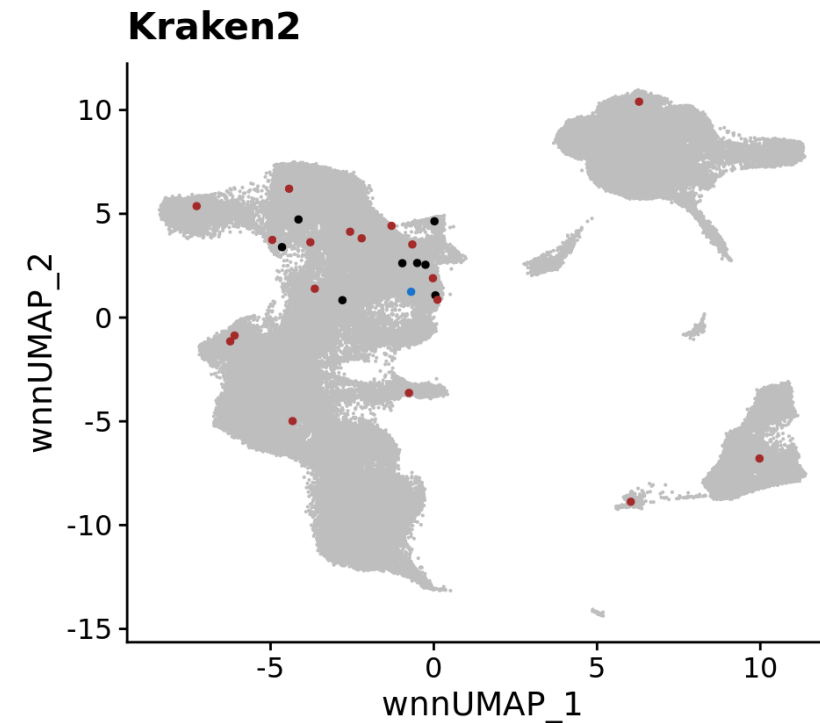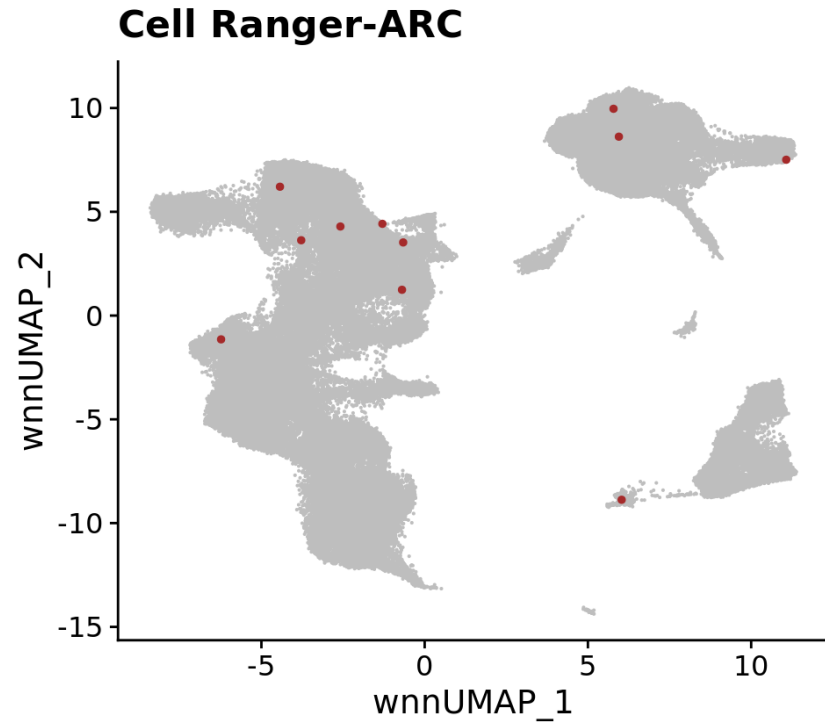
**Kraken2**

- Non-HIV
- ATAC
- GEX
- ATAC+GEX

**11 HIV+ cells**

| | Pre-HIV | Post-HIV | Post-ART |
|---|---|---|---|
| HIV RNA+ | 1 | 10 | 0 |

Kraken2 tool is reliable and detects more HIV+ cells than a classical alignment-based method

**27 HIV+ cells**

| | Pre-HIV | Post-HIV | Post-ART |
|---|---|---|---|
| HIV DNA+ | 0 | 7 | 1 |
| HIV RNA+DNA+ | 0 | 1 | 0 |
| HIV RNA+ | 0 | 16 | 2 |
| Total HIV+ cells | | 24 | 3 |
| Expected HIV+ cells (Bulk Total HIV DNA) | | 41 | 5 |

# Identification of HIV DNA+ and RNA+ cells
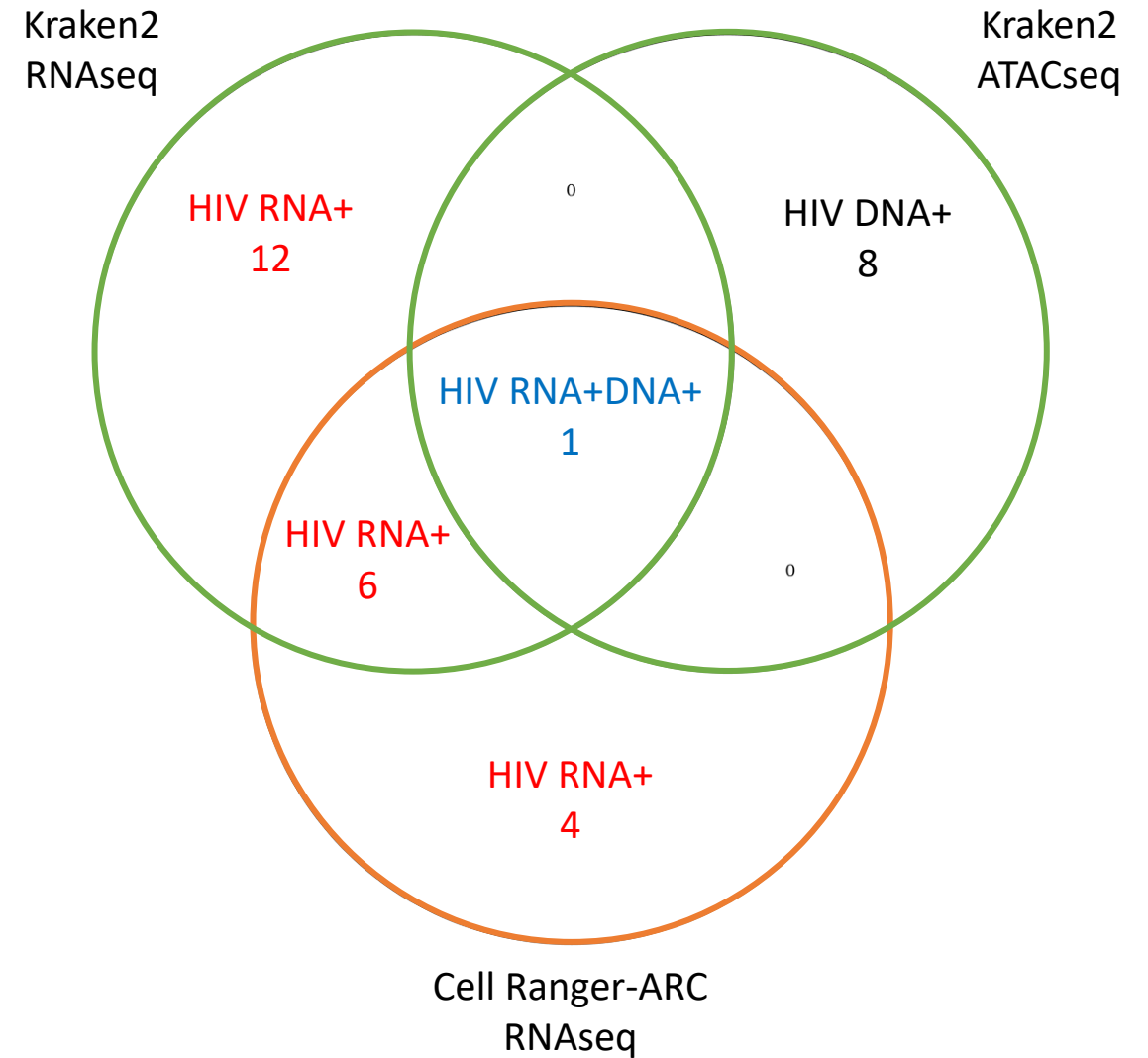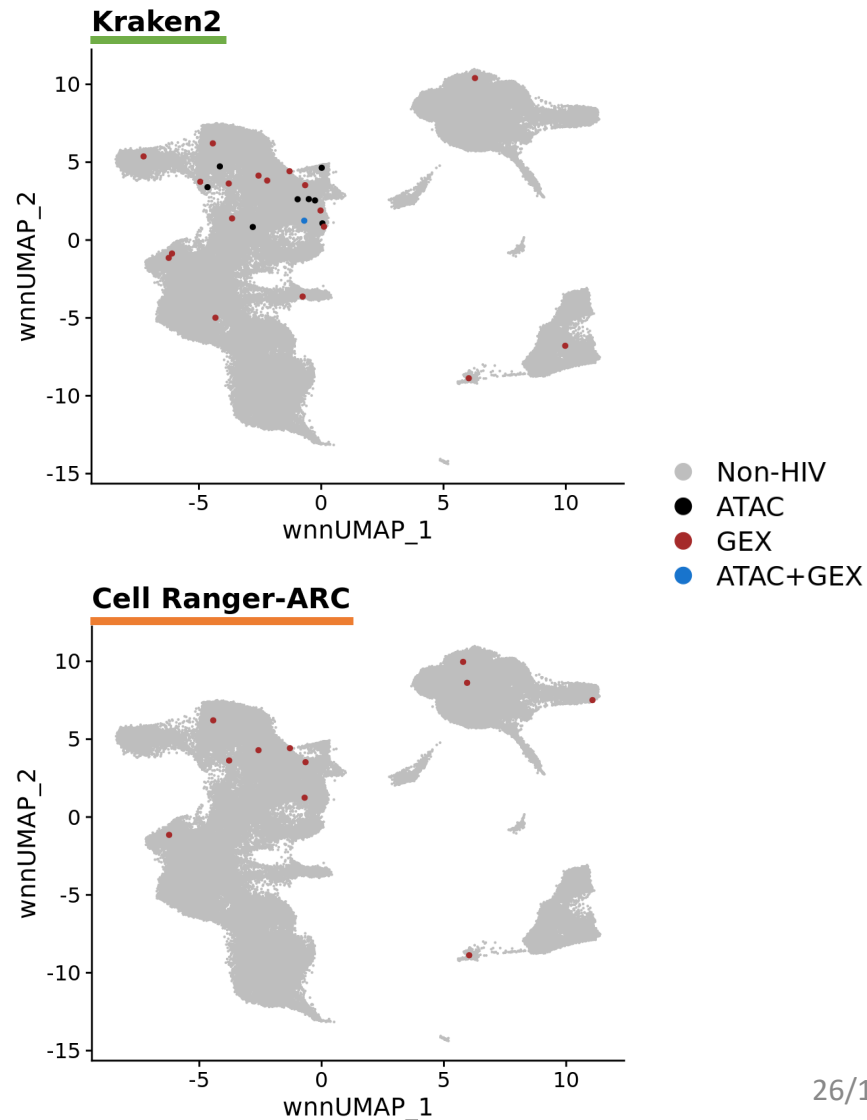
# Heterogeneous detection of HIV DNA+ and RNA+ cells
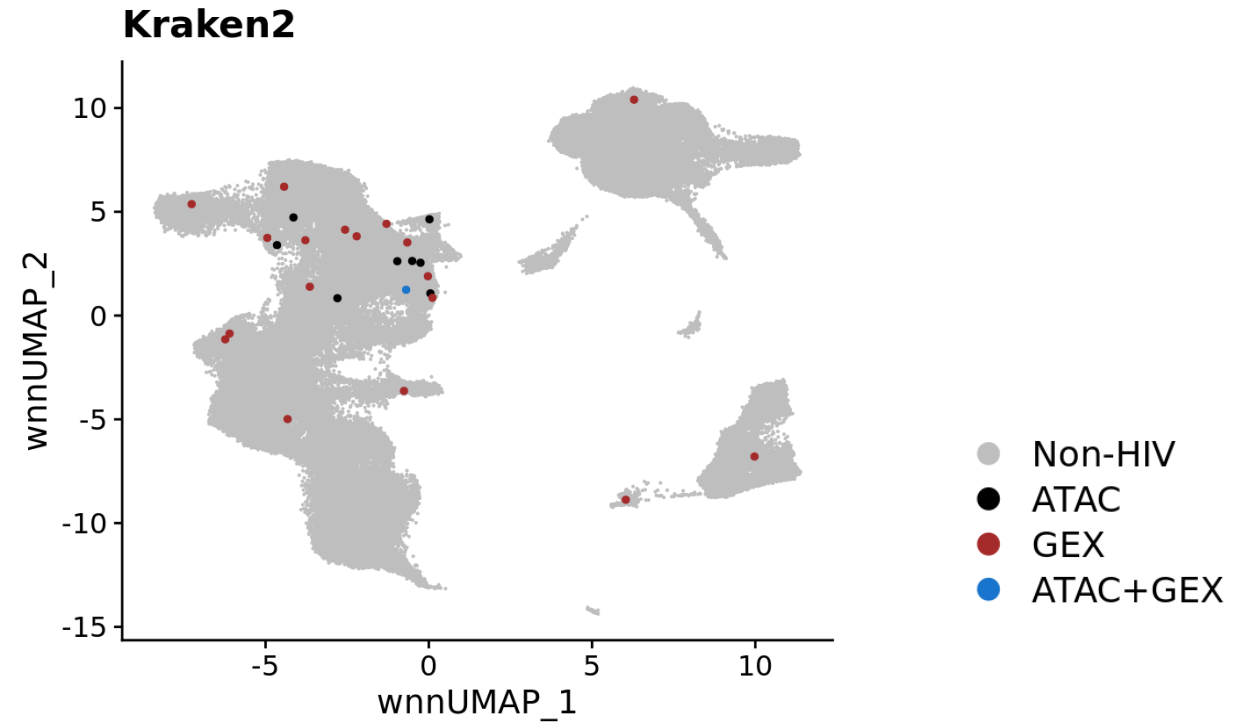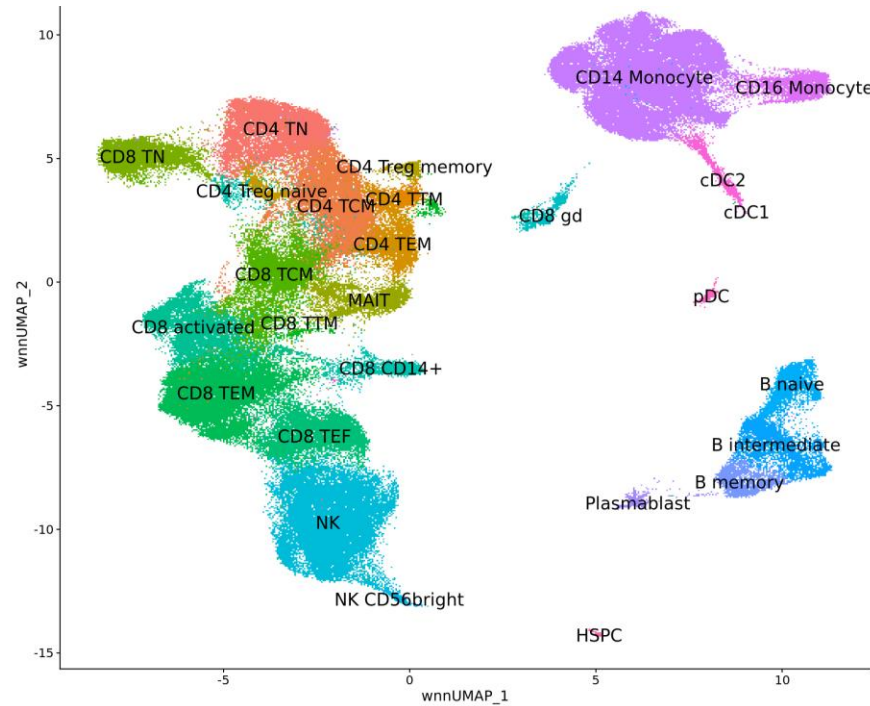


All HIV DNA+ (including one HIV DNA+RNA+) cells were CD4 T cells

CD8 and B HIV RNA+ cells were only detected in viremia (post-HIV)

Monocyte HIV RNA+ cell was detected post-ART

**27 HIV+ cells**

| | B | CD4 T | CD8 T | Mono |
|---|---|---|---|---|
| 8 HIV DNA+ | 0 | 8 | 0 | 0 |
| 1 HIV RNA+DNA+ | 0 | 1 | 0 | 0 |
| 18 HIV RNA+ | 2 | 9 | 6 | 1 |

# Conclusions

- 10X single-cell multiome identifies cell changes in PBMC after HIV infection and ART administration
  - Increase of activated and γδ CD8 T cells after infection
  - Decrease of these activated and γδ CD8 T, and an increase of naïve B cells after 1 year under suppressive ART

- 10X single-cell multiome identifies transcriptomic and epigenetic changes after HIV infection and ART administration
  - Transcriptomic and epigenetic changes are not completely reversed after 1 year under suppressive ART

- Kraken2 tool is reliable and detects more HIV+ cells than the alignment-based method CellRanger-ARC
  - Heterogeneous detection of HIV DNA+ and HIV RNA+ cells

# Future directions

- Comparison of epigenetic and transcriptomic signatures between HIV- and HIV+ CD4 T cells

- Epigenetic regulators and motifs linked to differentially expressed genes

- Finding potential mechanistic understanding and/or therapeutic interventions associated with HIV infection
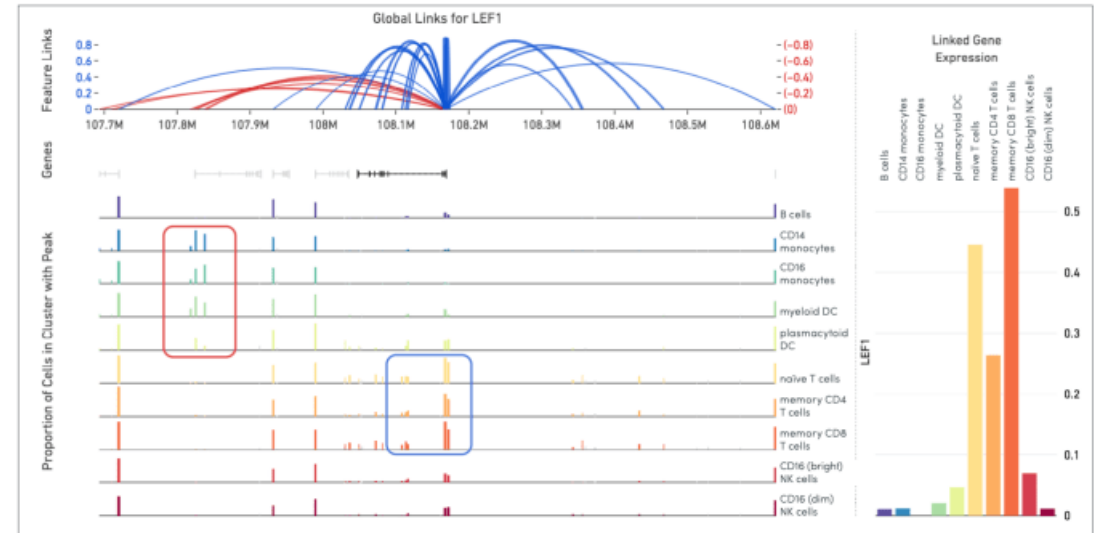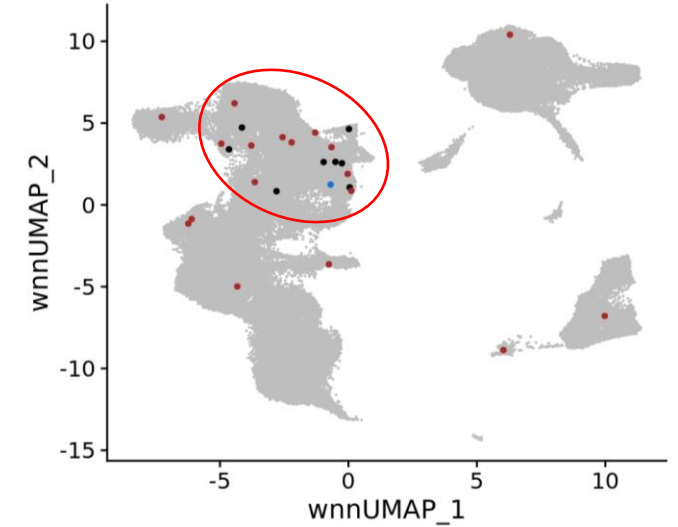
# Acknowledgements

## All the participants!

**GREC**
Javier Martinez-Picado
Lidia Garrido-Sanz
Víctor Urrea Gales
Ángel Bayon Gil
Maria C. Puertas
Judith Dalmau
Rest of the group

**TIV**
Christian Brander
Beatriz Mothe
Marta Ruiz-Riol

Pep Coll
Michael Meulbroek
Ferran Pujol
Rest of the team

**Applied Metabolomics**
Óscar Pozo Mendoza
Noemí Haro Villaverde

Amalio Telenti
Leah Soriaga
Emily Wong

Holger Heyn
Ester Planas-Rigol
Berta Colom
Juan Luis Melero

# Effects of HIV infection on single cell epigenome and transcriptome

**Sara Morón-López, PhD**

Associate Researcher

Retrovirology and Clinical Studies Group

AIDS Research Institute IrsiCaixa

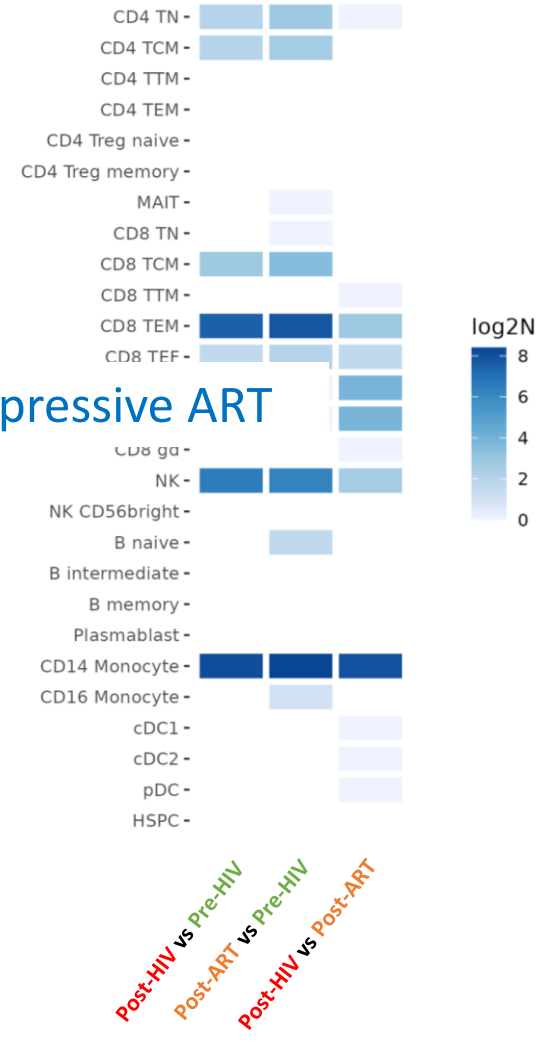# Identification of epigenetic changes after HIV infection and ART



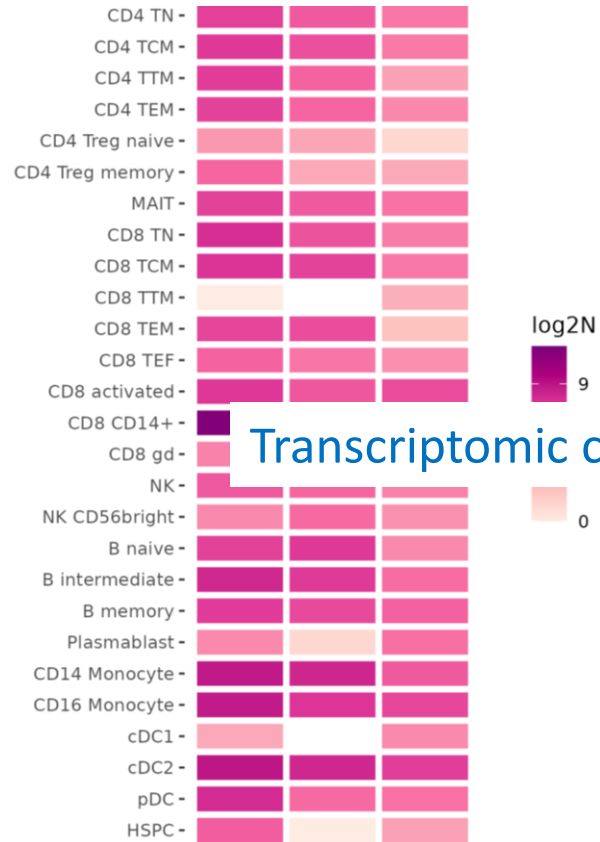ATACseq – DNA accessibility
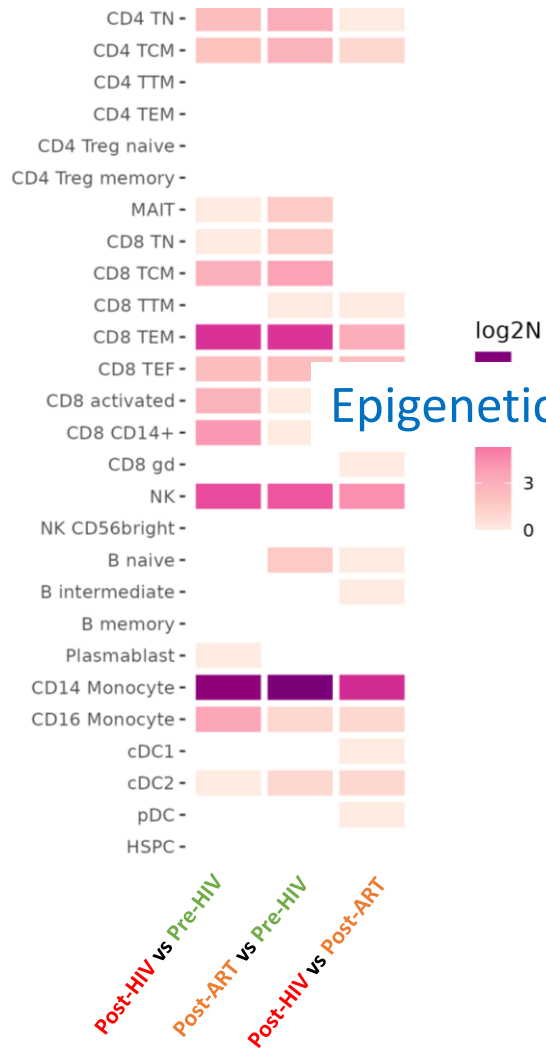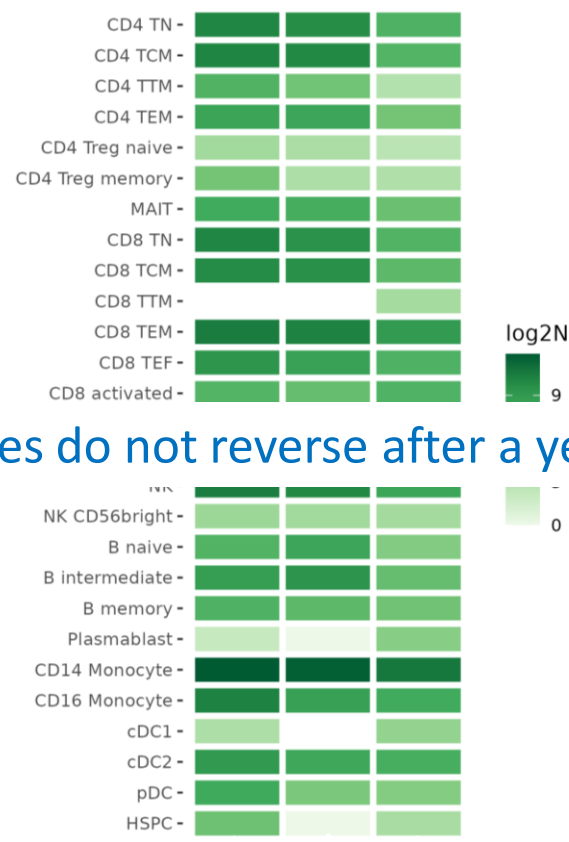
Increased DNA accessibility
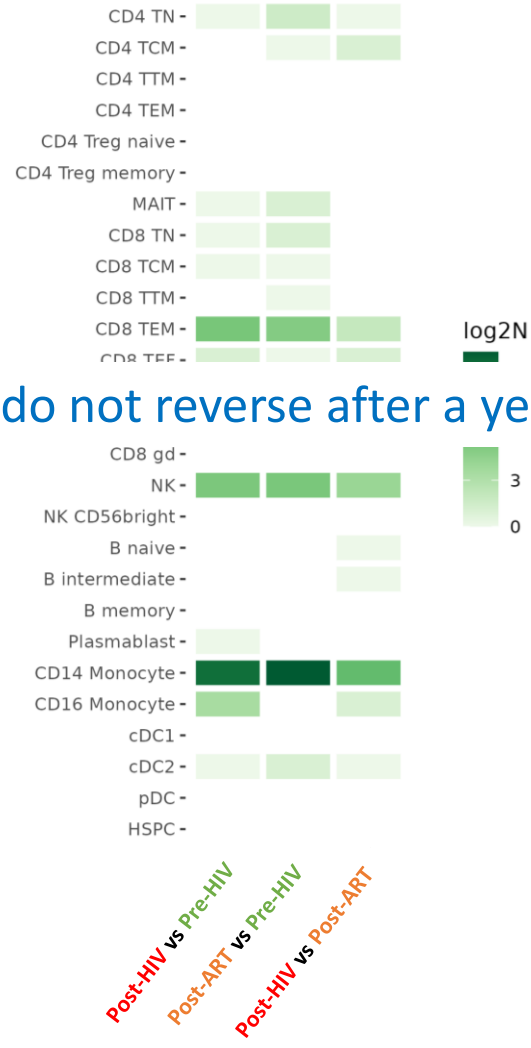
Decreased DNA accessibility

Epigenetic changes do not reverse after a year of suppressive ART

# Identification of transcriptomic changes after HIV infection and ART
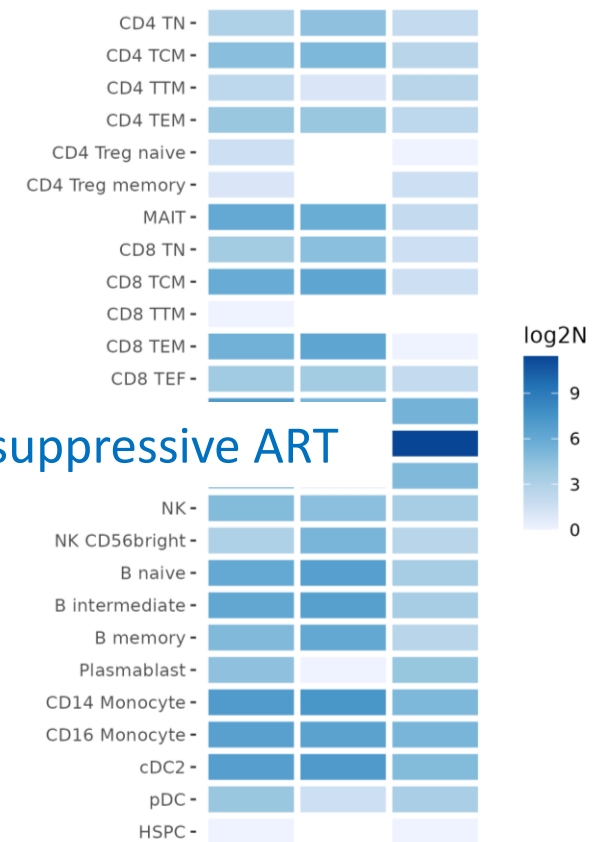


RNAseq – Cellular transcriptome

Increased cellular transcription

Decreased cellular transcription

Transcriptomic changes do not reverse after a year of suppressive ART