# Oxidative Stress and DNA Metabolism Programs are Upregulated in Latently Infected Cells in GALT



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## BACKGROUND

The gut-associated lymphoid tissue (GALT) harbors a large proportion of the HIV reservoir despite durable plasma viral suppression with antiretroviral therapy (ART). However, the cellular composition of this reservoir and determinants of viral persistence within GALT remain incompletely understood. We aimed to define the cellular identity and transcriptional signatures of transcriptionally active infected cells in two distinct GALT sites.

## METHODS

People with HIV (PWH) on ART with well-controlled HIV undergoing ileo-colonoscopy for colorectal cancer screening were enrolled. Fresh ileal and colonic biopsies were processed by enzymatic and mechanical dissociation to isolate mononuclear cells, followed by single-cell RNA sequencing. Reads were aligned to the human genome and HIV reference (HXB2). Cell clustering was performed using Seurat, annotated via the Pan-Human Azimuth reference, and manually curated. HIV-infected cells were defined as those with ≥2 HIV UMI counts. Only genes with False Discovery Rate<10% were considered differentially expressed between HIV-infected and HIV-uninfected cells.

## RESULTS

**Table 1:** Demographic and HIV-related characteristics of study participants. More than half study participants were African-American, about a third were female. Median duration of virological suppression on ART was 7 years.

| Characteristics | N (%) or Median (IQR) Total N = 30 |
|---|---|
| Female sex | 10 (33%) |
| Race | |
| African-American | 16 (53%) |
| Hispanic-latino/a | 8 (27%) |
| Caucasian | 3 (10%) |
| Mixed race/other/unknown | 3 (10%) |
| Age | 56 (52-65) |
| Duration of viral suppression (years) | 7 (4-11) |
| PWSH with available ileum samples | 13 |
| PWSH with available colon samples | 30 |
| Total cells analyzed | |
| Colon | 146,734 |
| Ileum | 78,922 |

**HIV persists in cytotoxic and memory CD4+ T cells in the gut-associated lymphoid tissue in people on ART with virological suppression. Transcriptionally active infected cells upregulate energy-metabolism and cell-proliferation transcriptional programs**

## RESULTS

**Figure 1:** Uniform Manifold Approximation and Projection (UMAP) identified major **(A and D)** cell population and refined T cell **(B and E)** clusters in Ileum **(left panels)** and Colon **(right panels)**. Cell-type identities were assigned by mapping them to the Pan-Human Azimuth reference atlas. HIV+ cells are identified by HXB2 expression in ileal **(C)** and colonic **(F)** T cell subsets.

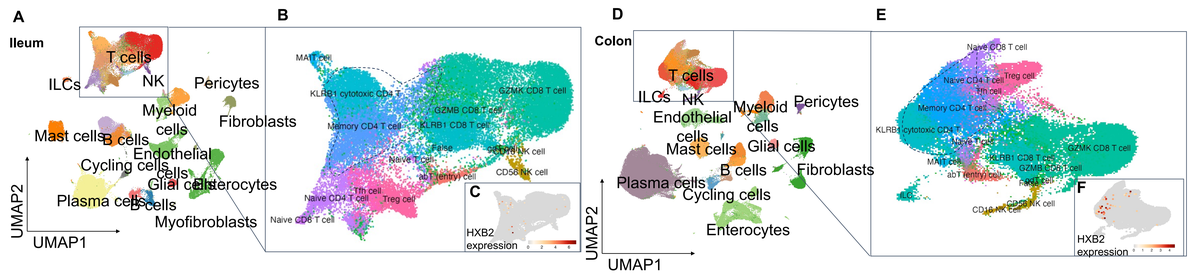
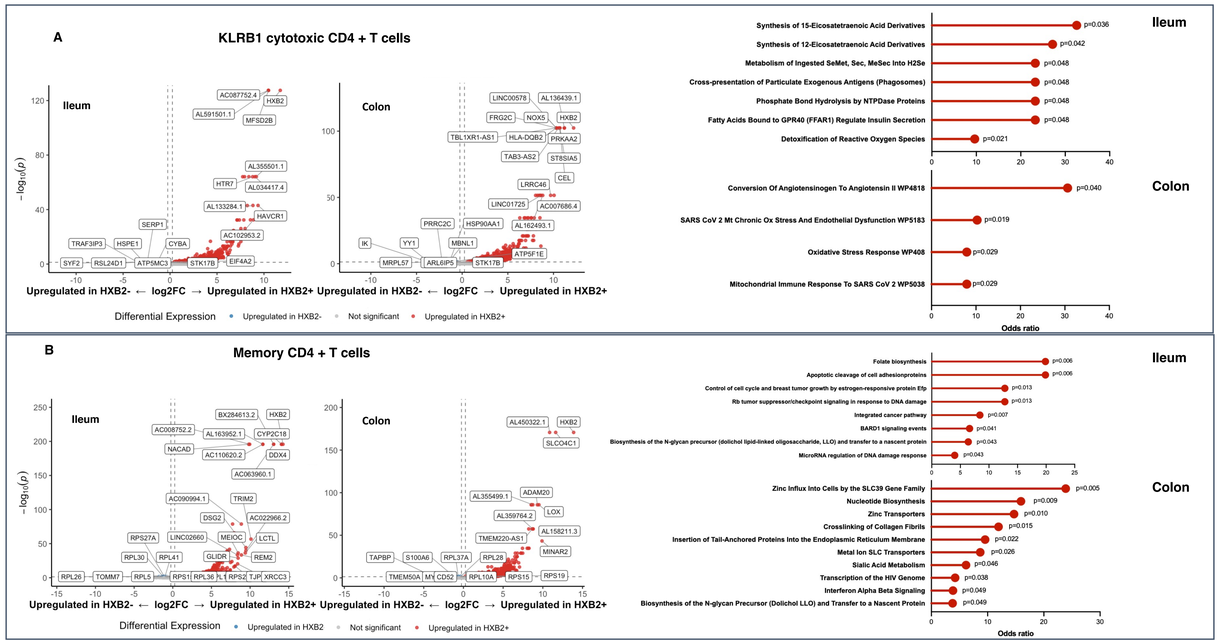



**Figure 2:** Compared to HIV-negative cells, in both Ileum and Colon, HIV+ cytotoxic CD4+ T cells **(A)** showed upregulation of oxidative stress metabolism while HIV+ memory CD4+ T cells **(B)** showed upregulation of cell fate and proliferation. Volcano Plots show Differentially Expressed Genes for each of cytotoxic and memory CD4+ T cells **(left)** and bar plots show significantly upregulated pathways **(right)**




## RESULTS

**Table 2:** Frequency and distribution of HIV+ cells among ileal **(top)** and colonic **(bottom)** CD4+ T cells. Among 14,797 ileal CD4+ T cells, 10 (0.07%) were HIV-infected, and among 19,233 colonic CD4+ T cells, 21 (0.1%) were HIV-infected. Across both sites, HIV-infected cells were enriched within cytotoxic and memory CD4+ T cells.

| Ileum | HIV+ | HIV- | % of cell type |
|---|---|---|---|
| KLRB1$^+$ cytotoxic CD4$^+$ T cells | 7 | 4064 | 0.2 |
| Memory CD4$^+$ T cell | 3 | 5375 | 0.06 |
| Naïve CD4$^+$ T cells | - | 2069 | - |
| TFH cells | - | 1481 | - |
| Tregs | - | 1798 | - |
| Total CD4$^+$ T cells | 10 | 14787 | 0.07 |

| Colon | HIV+ | HIV- | % of cell type |
|---|---|---|---|
| KLRB1$^+$ cytotoxic CD4$^+$ T cells | 10 | 4829 | 0.2 |
| Memory CD4$^+$ T cell | 9 | 7198 | 0.1 |
| Naïve CD4$^+$ T cells | 1 | 3140 | 0.03 |
| TFH cells | 1 | 1428 | 0.07 |
| Tregs | - | 2617 | - |
| Total CD4$^+$ T cells | 21 | 19212 | 0.1 |

## CONCLUSIONS

HIV persists within cytotoxic and memory CD4+ cells in the GALT. Distinct transcriptional programs including DNA repair and oxidative stress pathways, may promote survival of these infected subsets consistently with what has been previously shown in both in actively[1-3] and latently[4-5] infected cells. These findings highlighting potential targets to disrupt intestinal HIV reservoirs.

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## ADDITIONAL KEY INFORMATION

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**References**

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## PLAIN LANGUAGE SUMMARY

HIV can be found in cytotoxic and memory lymphocytes in the intestinal tissue of people with HIV taking effective antiretroviral therapy. Intestinal lymphocytes that contain HIV show evidence of increased oxygen consumption activity and cell proliferation.