Transcriptomic profile of gut T follicular helper cells during persistent HIV infection

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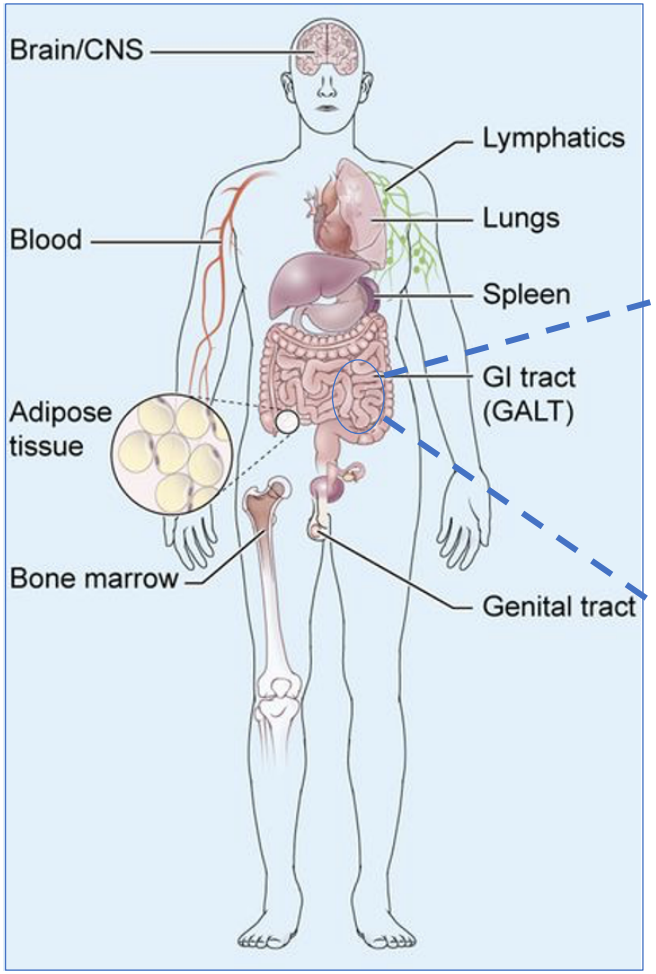
CONFLICTS OF INTEREST

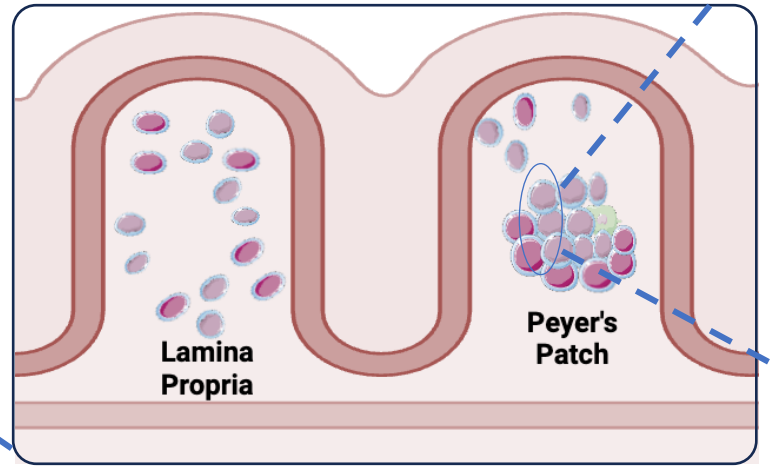
None

Background and Aim of the Study

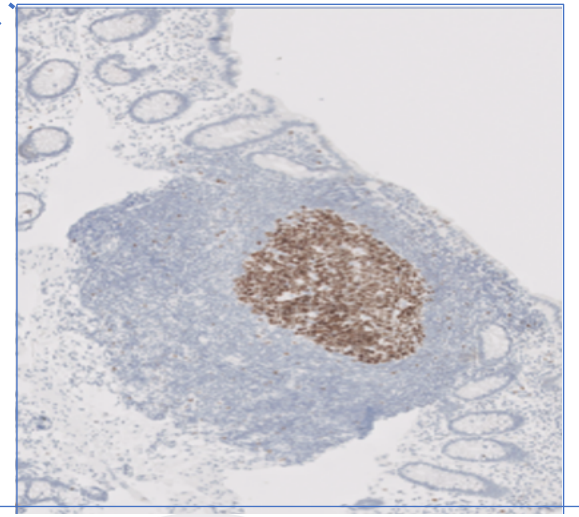
Anatomical reservoir sites

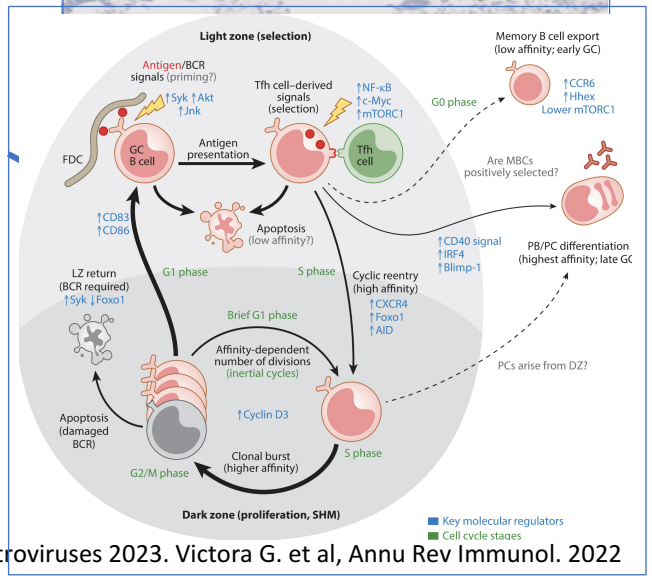


Gut-associated lymphoid tissue



Lymphoid aggregates



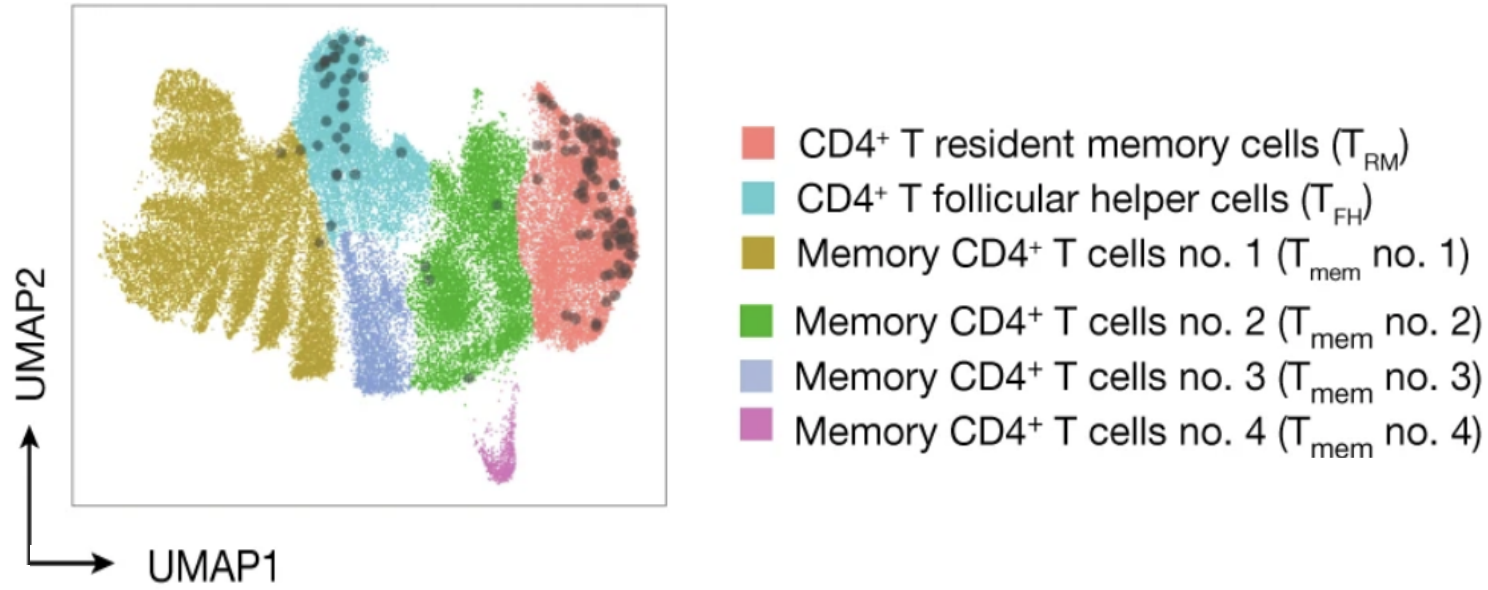


Henderson J.L et al. J Virology 2020, Breitfeld D, et al, JCI 2000. Shulman Z et al Science 2013. Estes JD. et al, Nat Med 2017. Cossarini F. et al, AIDS Res Hum Retroviruses 2023. Victora G. et al, Annu Rev Immunol. 2022

Background and Aim of the Study

TFH cells in People with HIV:

- Highly susceptible to infection
- Relatively higher frequency compared to other $CD4^+$ T cell subsets
- Altered transcriptional profile with increased in pro-inflammatory signatures
- Represent a tissue HIV reservoir



Tenner-Racz K. et al, J Eep Med 1998. Connick E. et al, J Immunol 2007. Kohler SL et al, J Immunol. 2016. Lindqvist M et al, JCI 2012, Colineau L et al, PLoSOne 2015. Sun W et al. Nature 2023

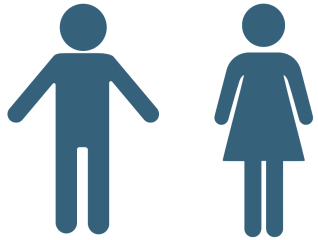
Background and Aim of the Study

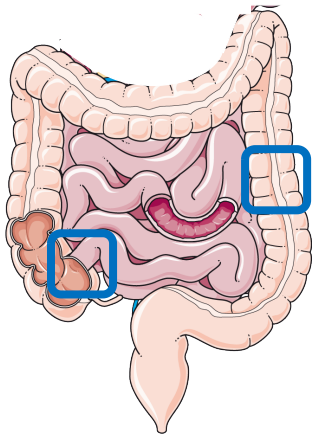
Aims of the study

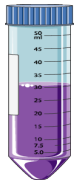
- Identify intestinal tissue cellular compartment where HIV can be identified at a single cell level
- Characterize the transcriptional profile of intestinal TFH cells in people with HIV with suppressed peripheral viral load
- Characterize the signatures associated with HIV persistence in intestinal cells

Methods: sample prep and sc-RNAseq analysis

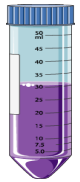
Prospective enrollment of Study participants undergoing ileo-colonoscopy



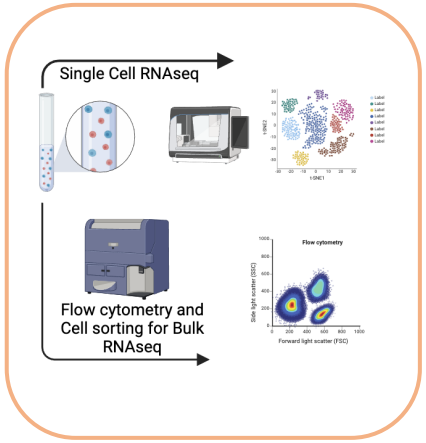




EDTA mediated dissociation of epithelium



Enzymatic digestion of lamina propria



Sc-RNAseq parameters	
Assay	10X Genomics NextGem 3'v3.1
Target cell recovery	8,000
Target depth sequencing per cell	25,000 reads

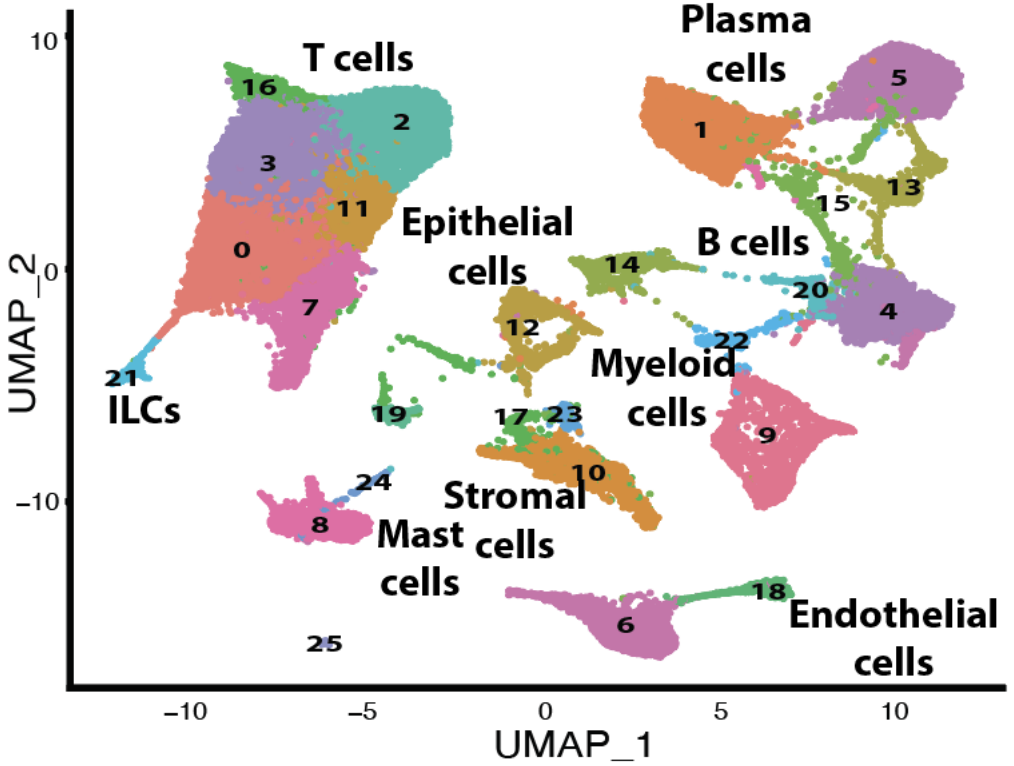
Seurat package analysis parameters	
Total UMI counts threshold	1,000
Mitochondrial genes fraction threshold	10%
Clustering resolution	0.5

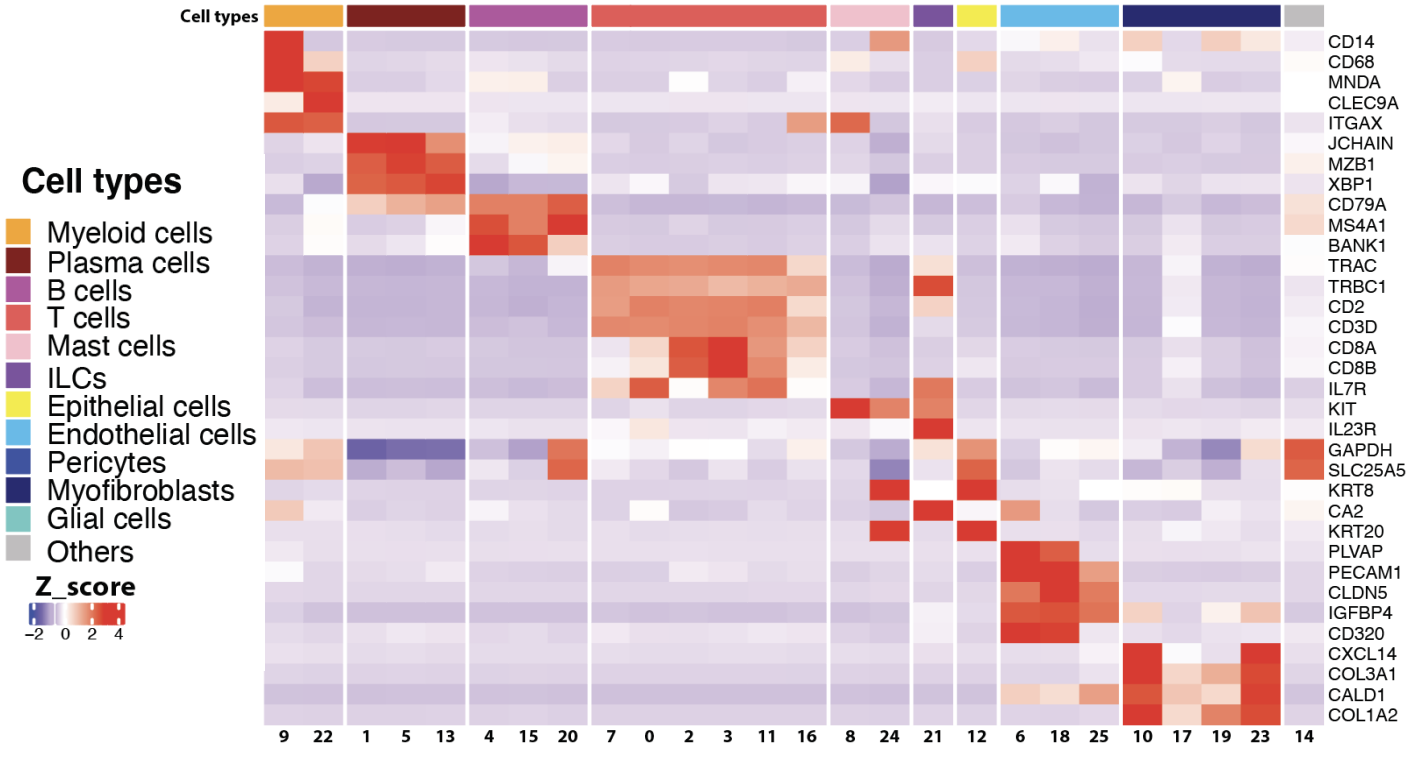
Study participants

Student t-test or Chi-square as appropriate

Identification of immune and non-immune cells via sc-RNAseq

Ileum

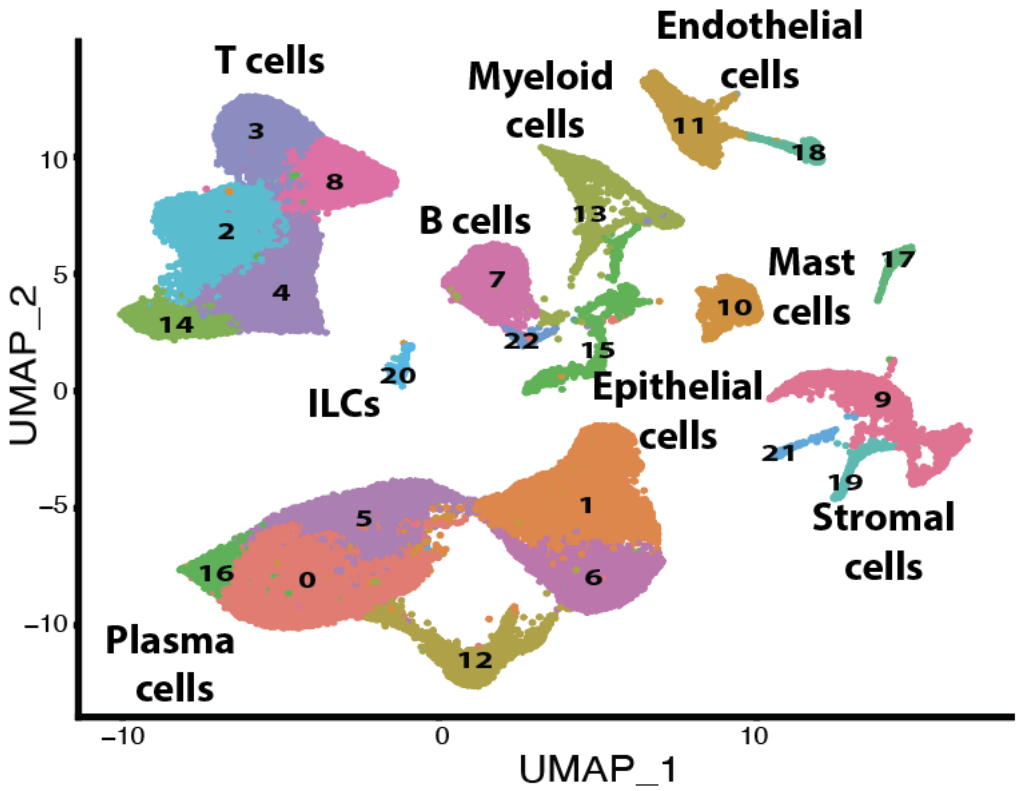


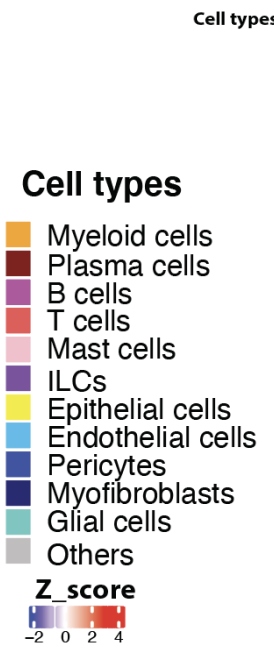


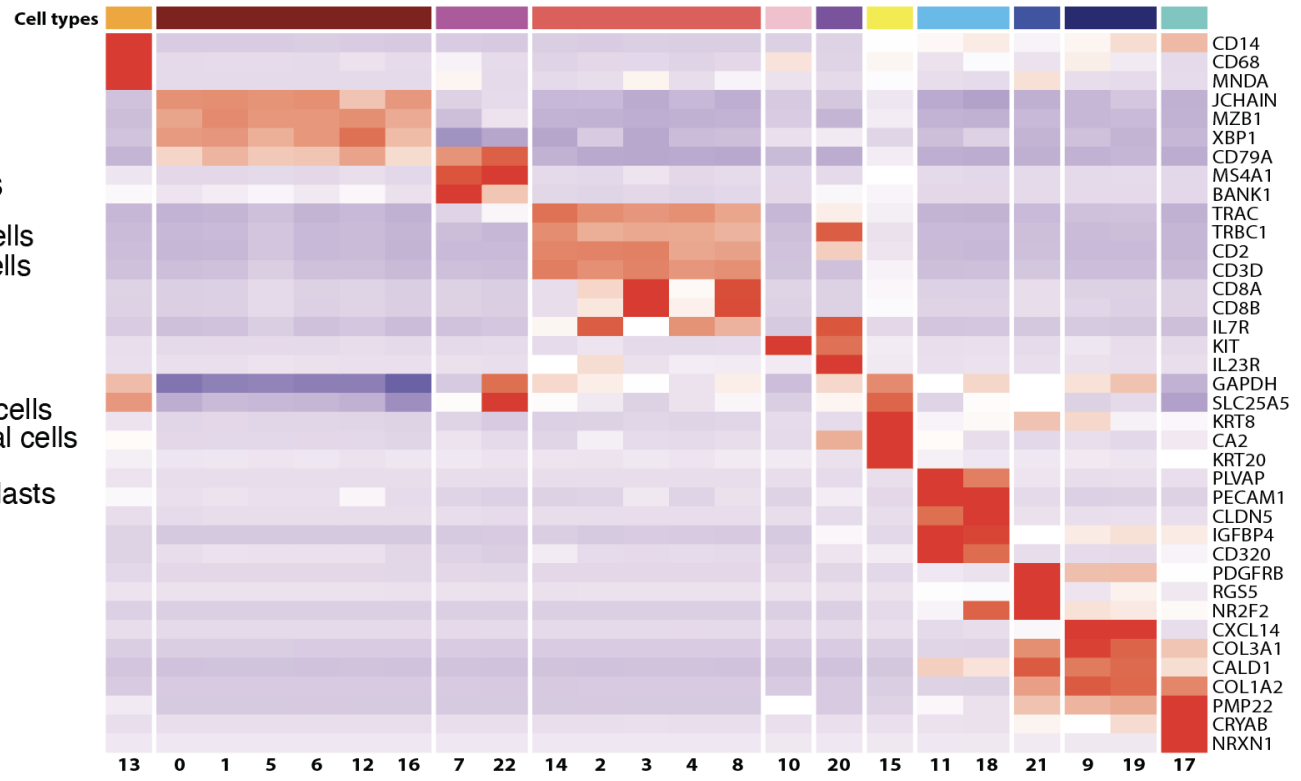
Total #cells analyzed 14,299

Identification of immune and non-immune cells via sc-RNAseq

Colon







Total #cells analyzed 16,610

Identification of T cell subsets via sc-RNAseq

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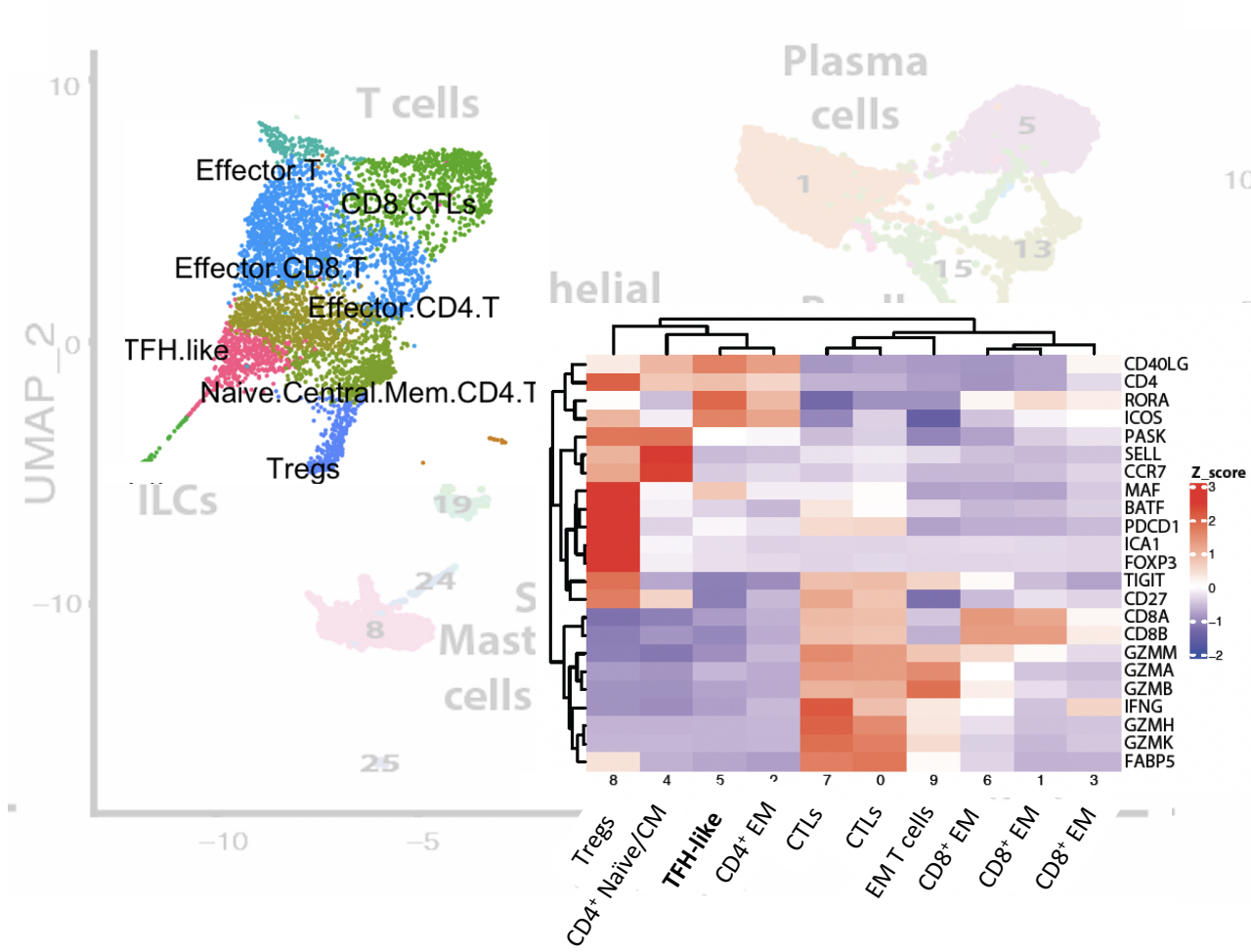

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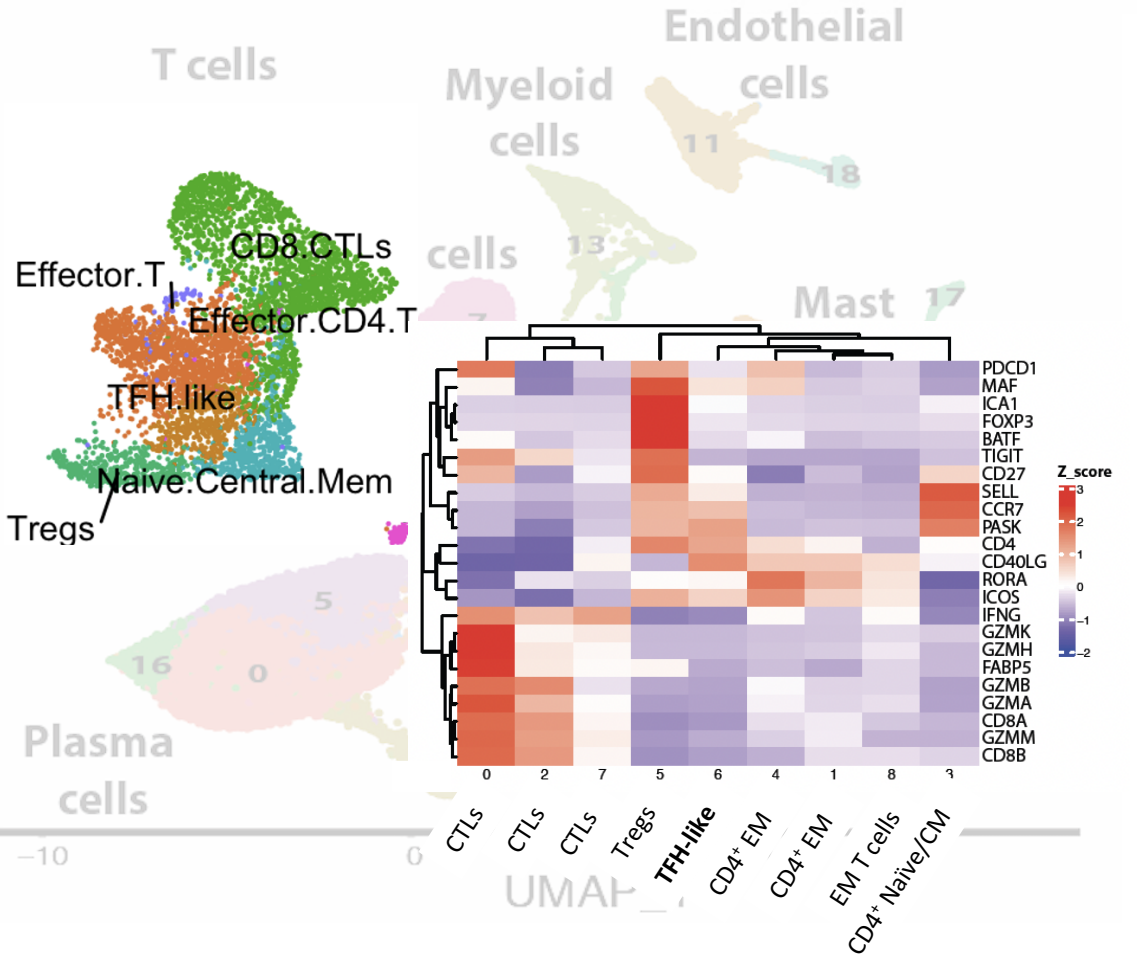
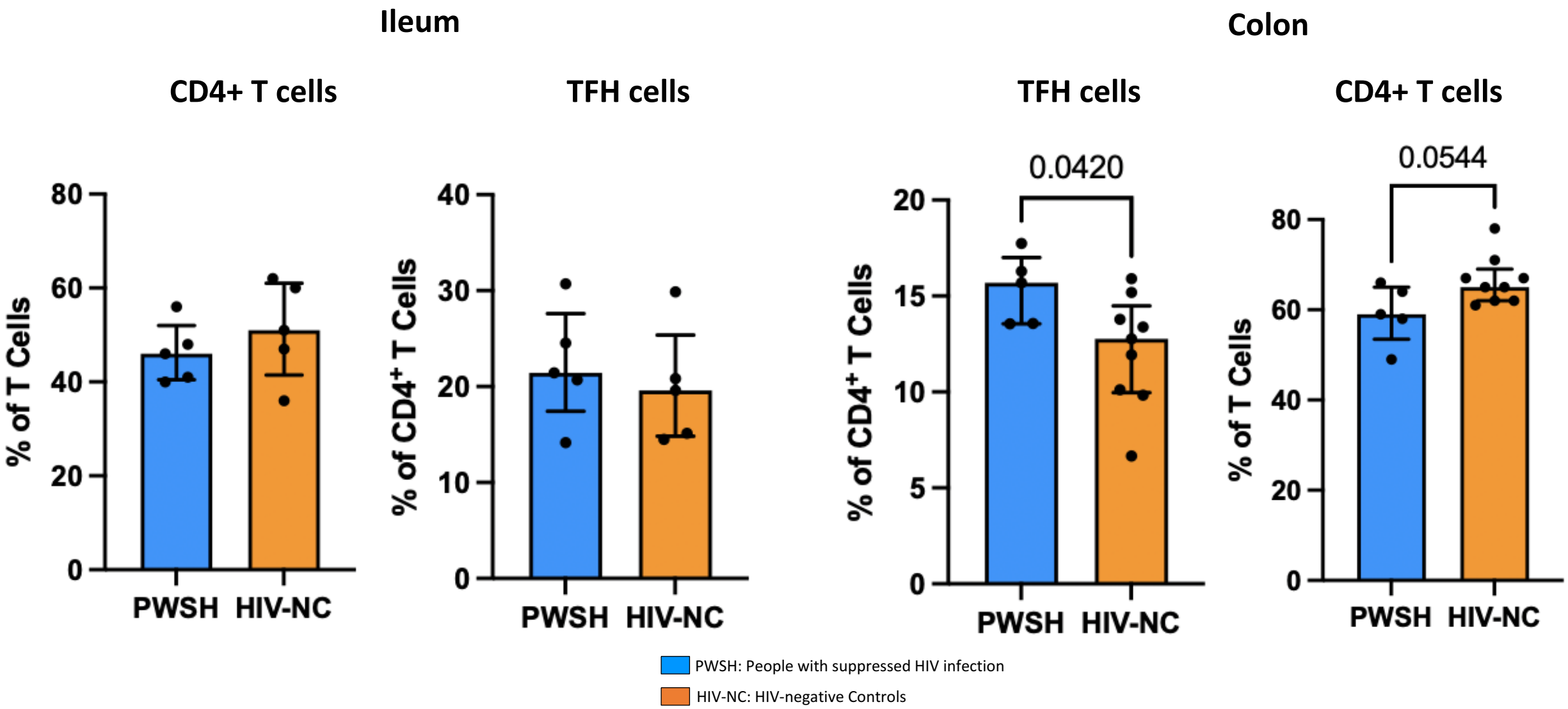
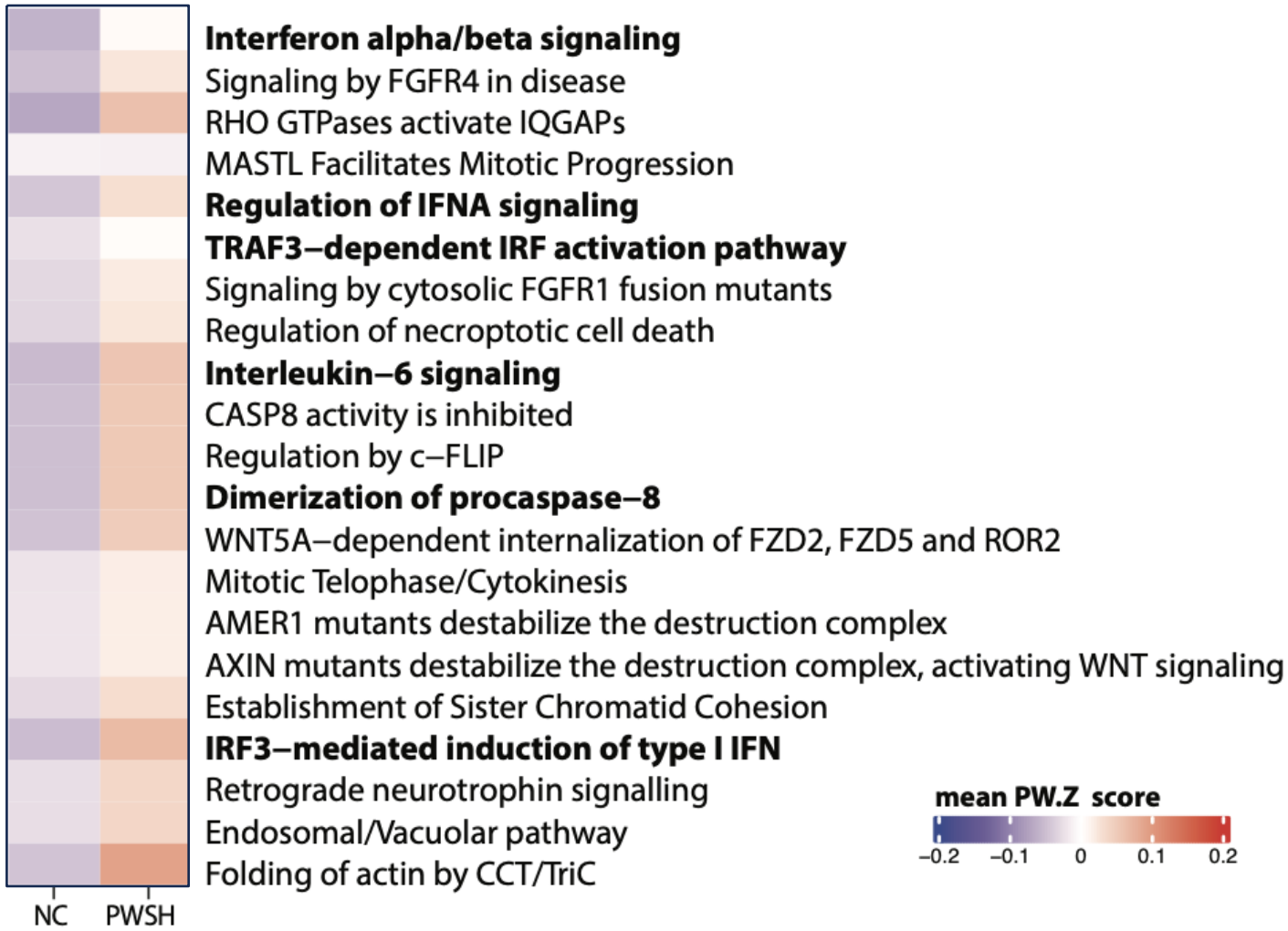


Preserved proportion of TFH cells in PWSH compared to HIV-NC



Increased activation of type-I IFN, IFN-response responses in TFH cells from PWSH



NC: HIV negative controls
PWSH: People with suppressed HIV

TFH cells and Effector Memory cells harbor HIV transcripts in the gut

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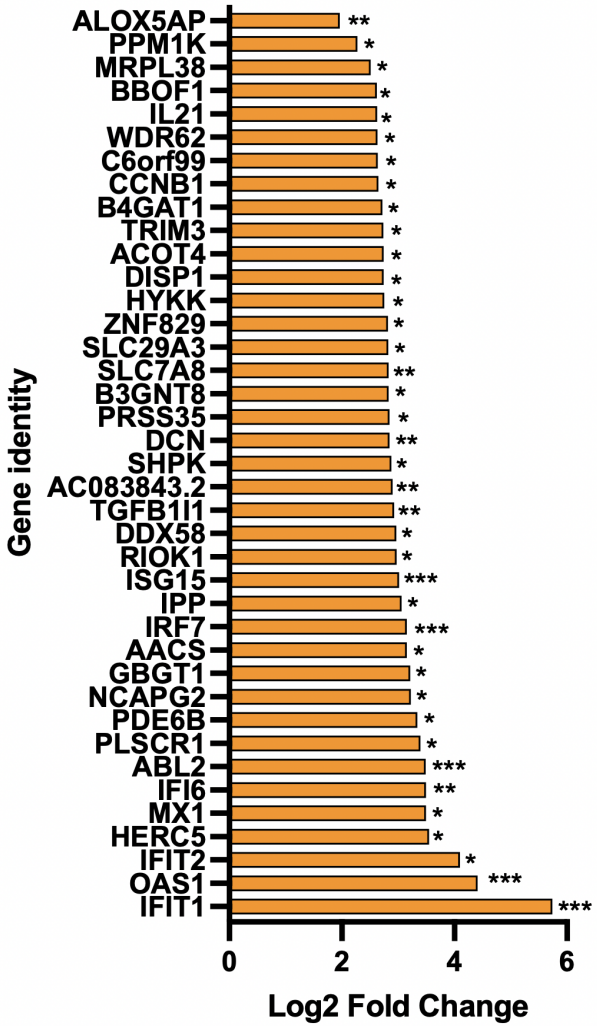
	TFH	CD4⁺ Effector Memory	Tregs	Naïve/Central Memory
HXB2+	1	2	0	0
HXB2-	498	767	335	585

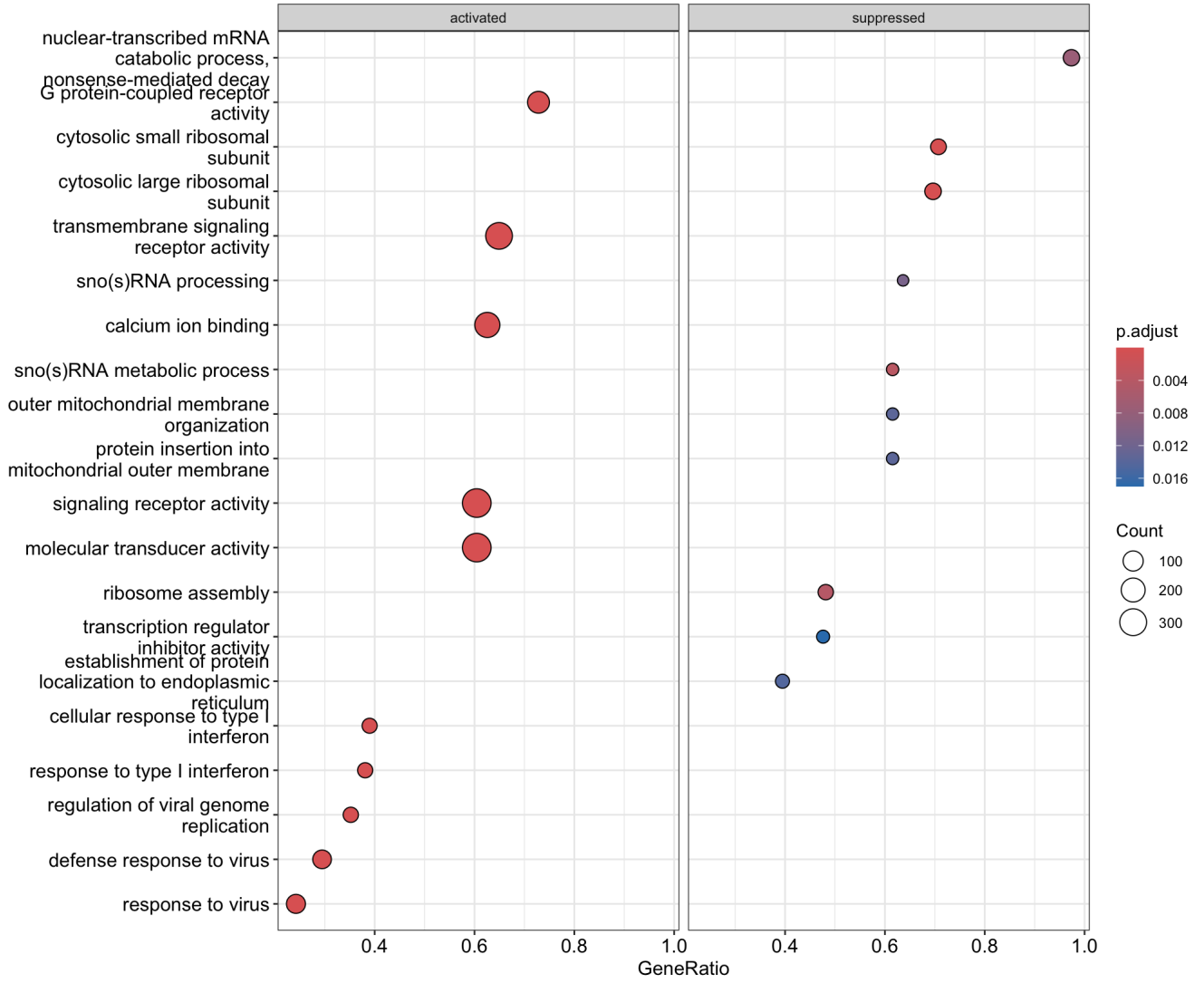
Colon

	TFH	CD4⁺ Effector Memory	Tregs	Naïve/Central Memory
HXB2+	2	2	0	0
HXB2-	437	1394	464	574

Increase in antiviral responses and cellular signaling signatures in cells harboring HIV transcripts



* FDR<10%
** FDR<1%
*** FDR<0.1%

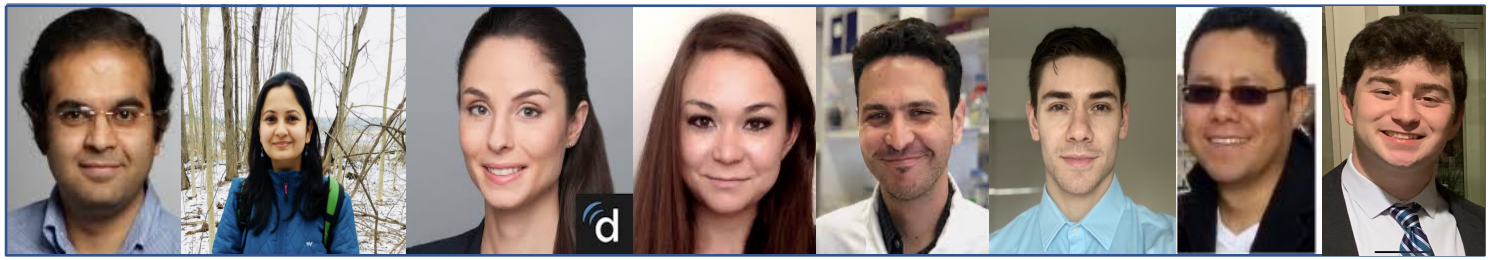


Summary and conclusions

- Amongst intestinal CD4+ T cell subsets, HIV transcripts are primarily found in TFH cells and Effector Memory cells.
- In line with previous data from other tissues, intestinal TFH cells continue to harbor HIV transcripts during virological suppression.
- Despite not being depleted in frequency, Intestinal TFH cells in PWSH show persistently altered transcriptional profile compared to TFH cells from people without HIV.
- HIV+ cells showed upregulated signatures of antiviral response and cell-signaling compared to cells where no HIV transcripts were found.

Initial landscape analysis of intestinal tissue at a single-cell level resolution offers additional insights on the characterization of the intestinal tissue reservoir

Acknowledgements



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