Tissue resident memory programs of intestinal CD4+ and CD8+ T cells facilitate HIV-1 persistence

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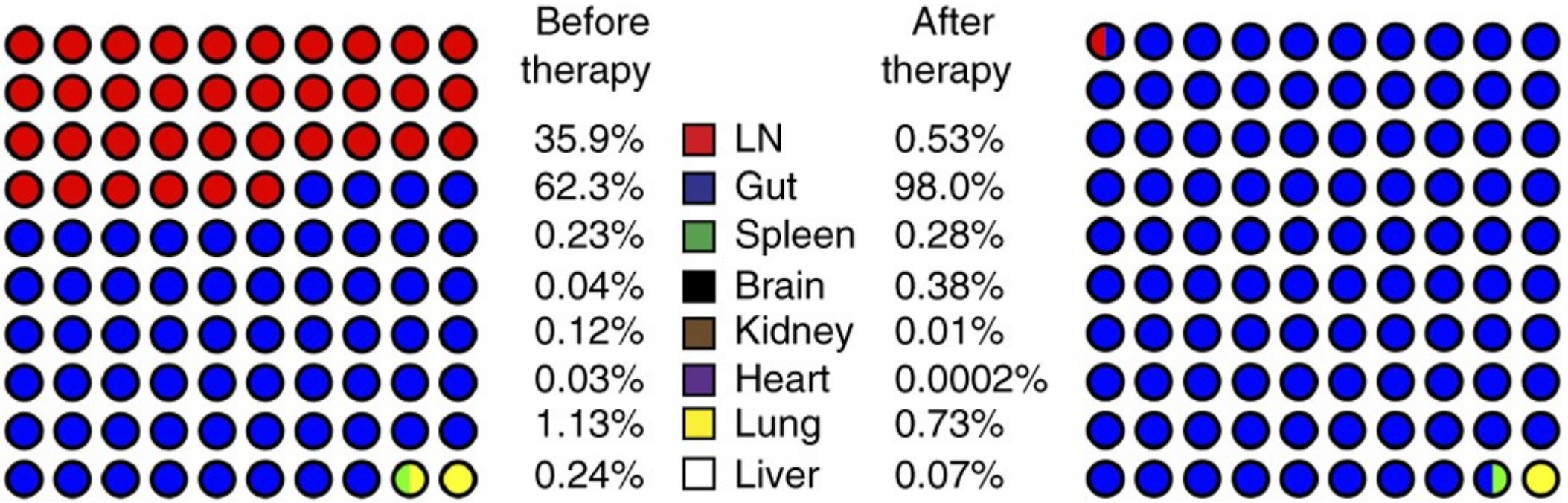
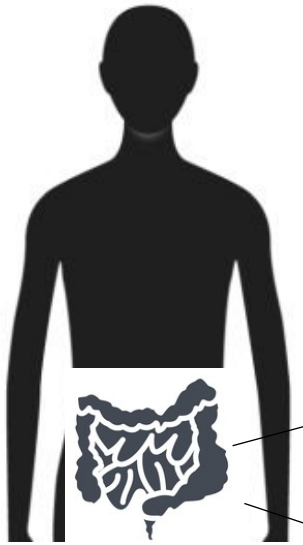
Conflict of interests: None

The gut accounts for 98% of the reservoir after ART



Estes *et al.*, Nature Med 2017; Chun *et al*. J Infect Dis. 2008; Li *et al*. Nature 2005; Yukl *et al*. J Infect Dis. 2010

Understanding HIV persistence in the gut



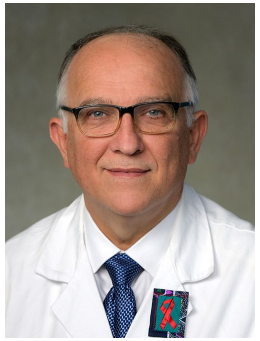
CD3 enriched gut T cells

CD4s: 43,113 CD4+ cells from PLWH
6,092 CD4+ T cells from HIV– donor

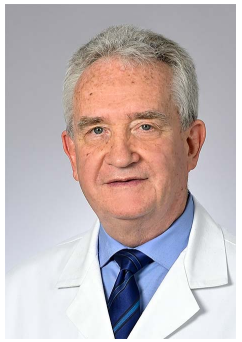
CD8s: 45,475 CD8+ T cells from PLWH
6,064 CD8+ T cells from HIV– donor

Luis Montaner
Wistar

Kenneth Lynn
UPenn



Pablo Tebas
UPenn



Ricardo Morgenstern
UPenn

Liza Konnikova
Yale

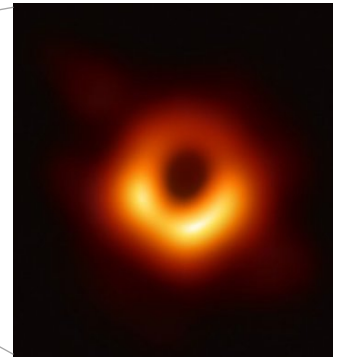
Colon biopsies from:
10 people living with HIV-1 **(PLWH) under ART**
5 HIV– donor

Rectal tissue biopsies were collected by Fiber optically guided flexible sigmoidoscopy abd processed into single-cell suspensions and viably frozen.
Aliquots of >2.5 million cells isolated from gut biopsy were thawed.
After dead cell depletion, T cells were isolated by CD3+ selection kit.

Challenge: Understanding HIV persistence is challenging because of the heterogeneity and rarity of HIV-infected cells

CD4⁺ T cells from people living with HIV

HIV RNA⁺ cell



Heterogeneous

Polarization
Th1
Th2
Th17
Treg

Memory
Naïve
Central memory
Effector memory
Effector

Activation

Exhaustion

Antigen specificity

Proliferation capacity

Cytokine response

Rare

HIV RNA⁺ cells: 1/10^4–10^6 cells (<0.1%)

What drives the survival of HIV-infected cells in the gut?

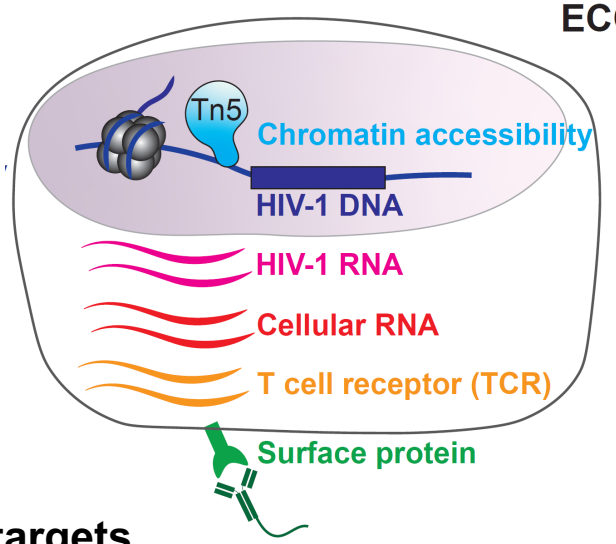
Single-cell multi-omic understanding of HIV-1-infected cells

Central dogma: DNA → RNA → protein

DNA — **Epigenetics**
Transcription factors that regulate gene expression

RNA — **Transcriptome**
Differentiation, immune responses, activation, exhaustion

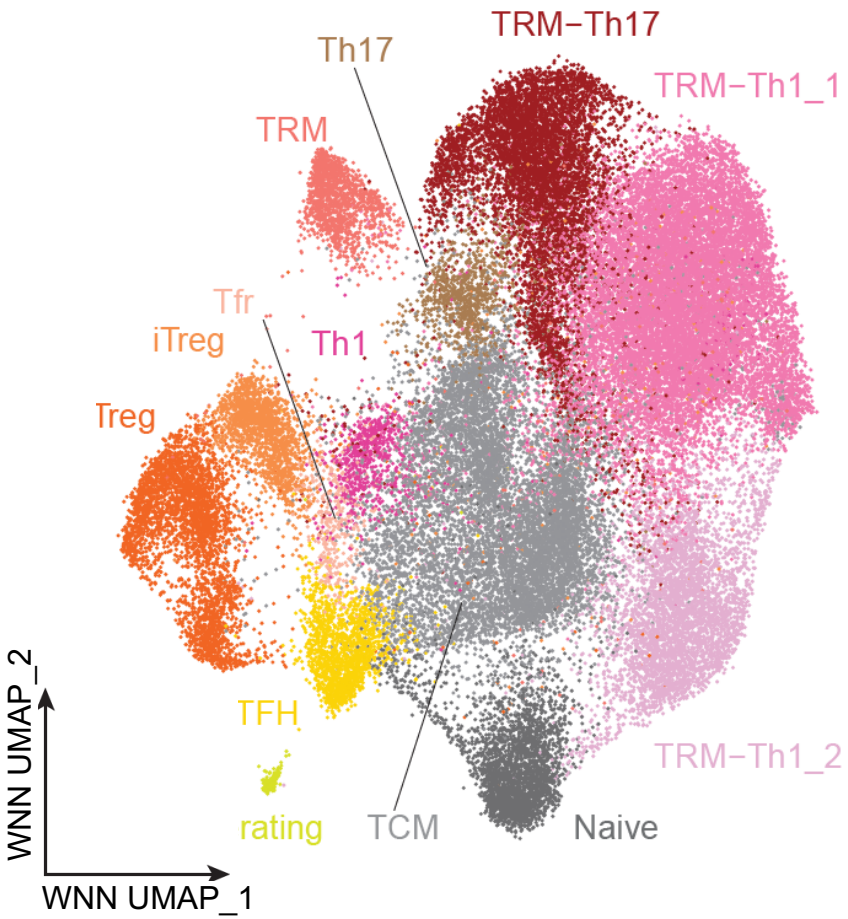
Protein — **Protein expression**
Markers and therapeutic targets



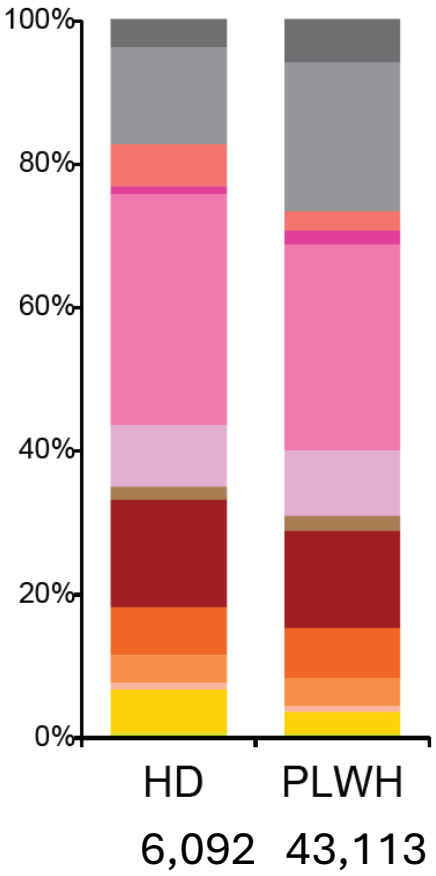
	ECCITEseq	FINDseq	PhePseq	ASAPseq	DOGMAseq	DOGMAseq & TREKseq
Chromatin accessibility				✓	✓	✓
HIV-1 DNA		✓	✓ Intactness Targeted integration site	✓	✓	✓
HIV-1 RNA	✓	✓ Bulk			✓	✓
Cellular RNA	✓	✓ Bulk			✓	✓
T cell receptor (TCR)	✓					✓
Surface protein	✓		✓	✓	✓	✓
	Collora *et al.*, Immunity 2022	Clark *et al.*, Nature 2023	Sun *et al.*, Nature 2023	Wu *et al.*, Nature Immunol 2022	Wei *et al.*, Immunity 2023	This study

Review by Wong, Wei, and Ho, Current Opinion HIV AIDS in press

More than half of gut CD4+ T cells are TRM



- CD4 naive
- CD4 TCM
- CD4 TRM
- CD4 Th1
- CD4 TRM−Th1_1
- CD4 TRM−Th1_2
- CD4 Th17
- CD4 TRM−Th17
- CD4 Treg
- CD4 iTreg
- CD4 Tfr
- CD4 TFH
- CD4 proliferating

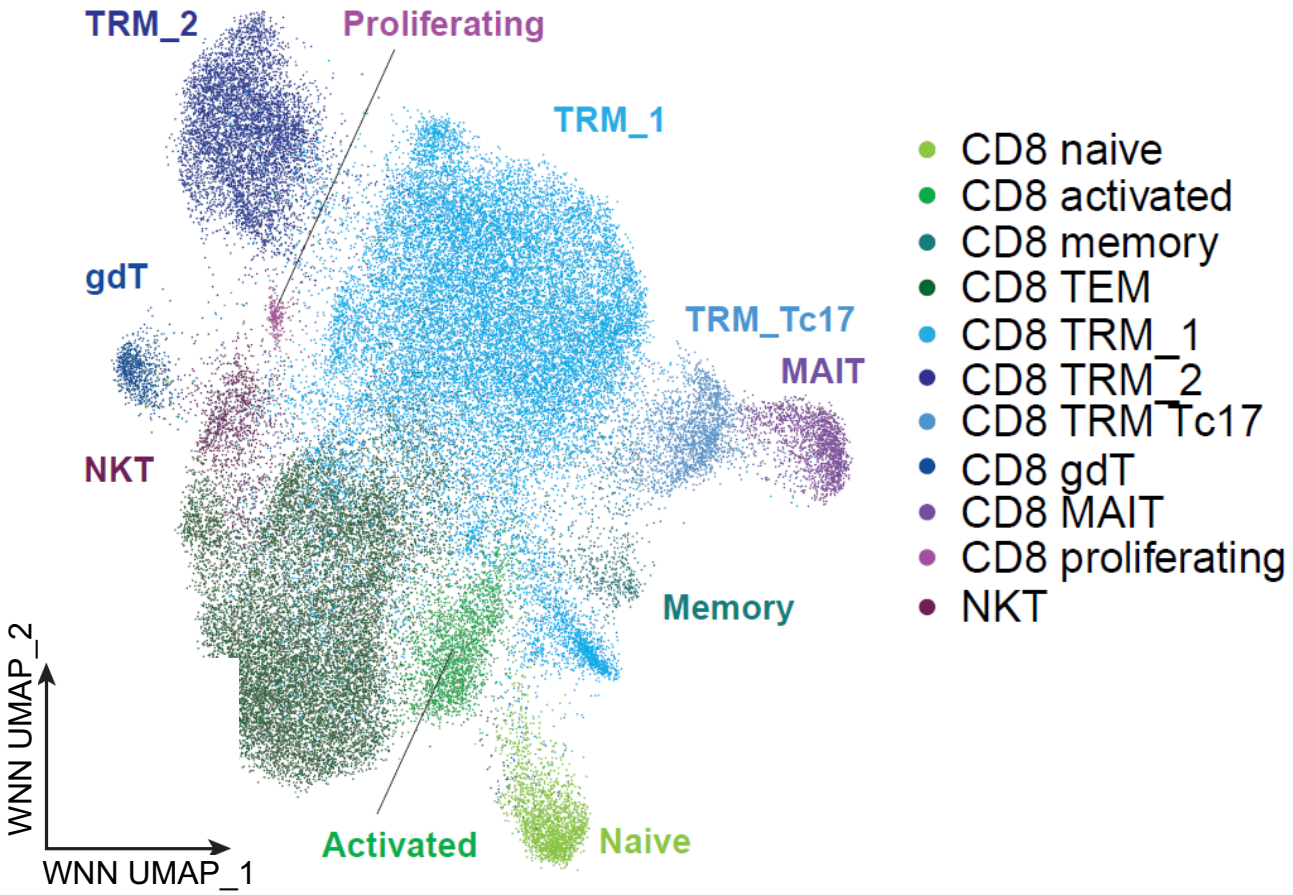


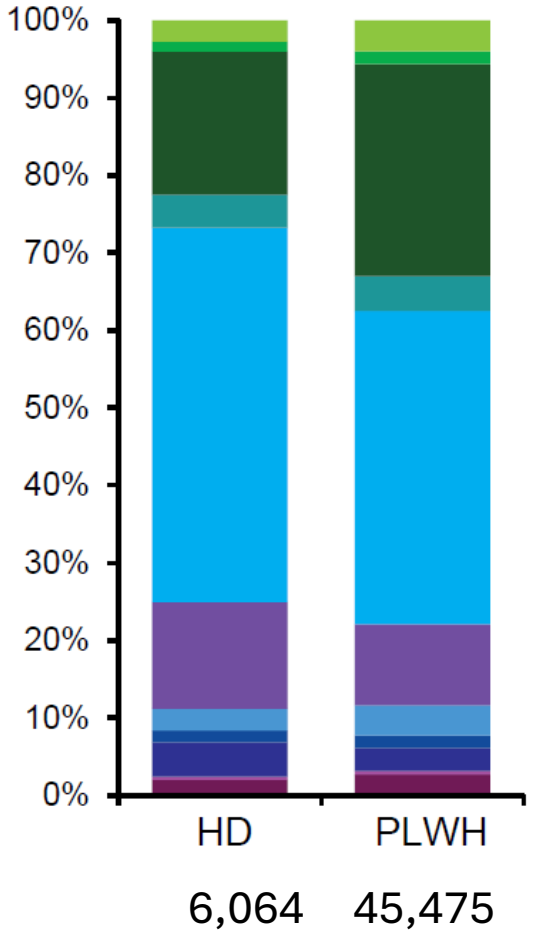
PLWH: 54.17% TRM

HD: 61.64% TRM

43,113 CD4+ T cells from 10 PLWH, 6,092 CD4+ T cells from 5 HIV- individuals.
All dots (cells) have integrated single cell ATAC-seq, RNA-seq, and protein expression profile in the same cell
QC: remove ATACseq ≤ 200 unique ATAC fragments, nucleosome signal strength ≥ 1, TSS enrichment score ≤ 2, and ATAC UMI counts ≤ 350 or ≥ 20,000
QC: remove RNAseq ≥ 25% mitochondrial gene content, ≤ 200 genes, and RNA UMI counts ≤ 500 or ≥ 25,000
Doublet removal: hashtag, SNP, scDblFinder; **Batch effect correction**: reciprocal LSI for ATAC and Harmony for RNA; **Cluster determination**: Clustree
Integration of ATAC + RNA: Weighted nearest neighbor (WNN)

More than half of gut CD8+ T cells are TRM





PLWH: 54.79% TRM

HD: 64.89% TRM

45,475 CD8+ T cells from 10 PLWH, 6,064 CD8+ T cells from 5 HIV- individuals.
All dots (cells) have integrated single cell ATAC-seq, RNA-seq, and protein expression profile in the same cell
QC: remove ATACseq ≤ 200 unique ATAC fragments, nucleosome signal strength ≥ 1, TSS enrichment score ≤ 2, and ATAC UMI counts ≤ 350 or ≥ 20,000
QC: remove RNAseq ≥ 25% mitochondrial gene content, ≤ 200 genes, and RNA UMI counts ≤ 500 or ≥ 25,000
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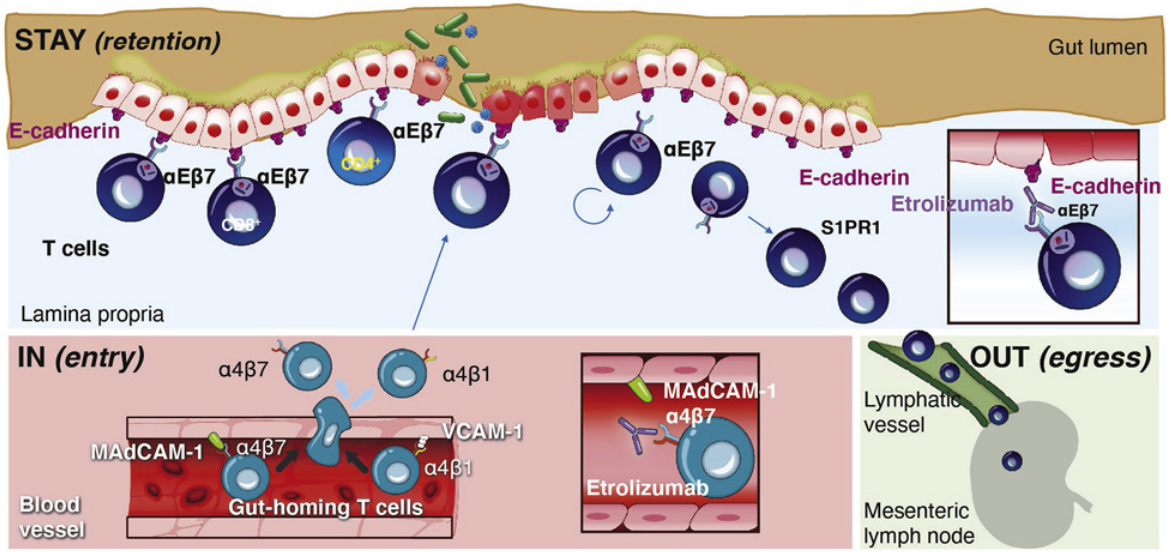
Integration of ATAC + RNA: Weighted nearest neighbor (WNN)

Tissue resident memory T cells remain in the gut

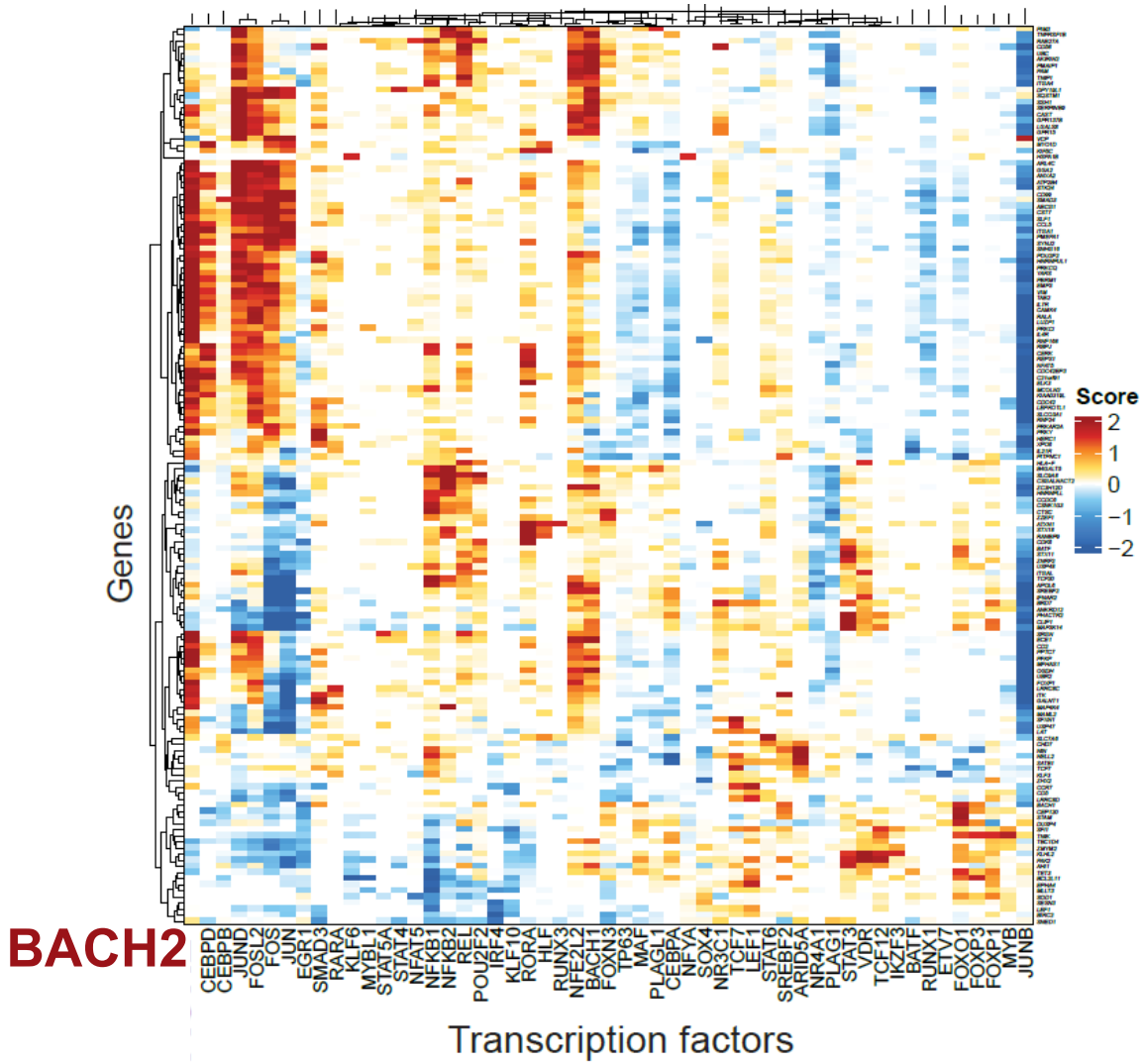


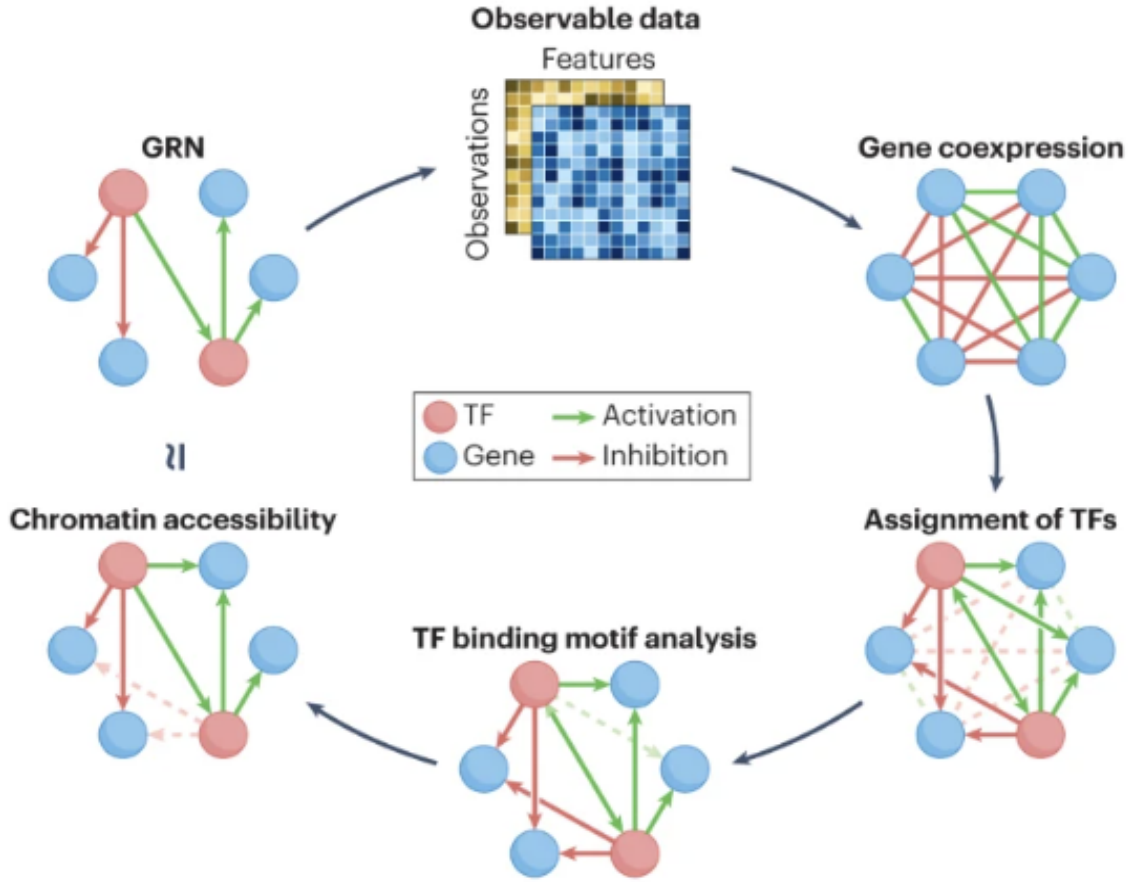
Tissue resident marker: CD49a, CD103, CD69

Collagen Laminin	Epithelium E-cadherin	Egress signal S1PR1
▮	▮	⊘
Integrin α1β1	**Integrin αEβ7**	**CD69**
CD49a **= *ITGA1*** **= Integrin α1**	**CD103** **= *ITGAE*** **= Integrin αE**	

Dai *et al.*, Cell Reports Medicine 2021

Unbiased identification of immune programs dominating gut CD4s: Gene regulatory network analysis (GRN)

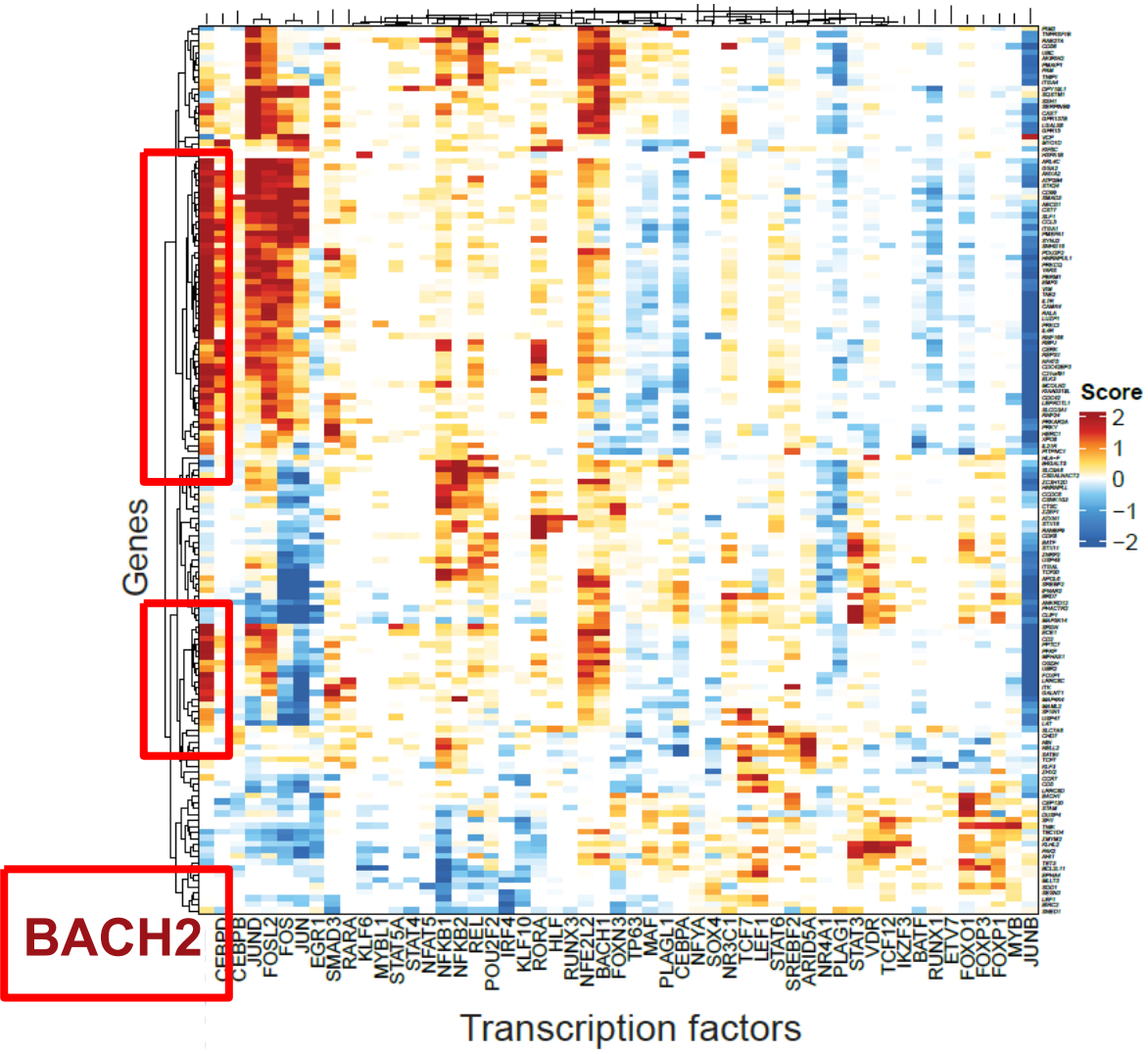


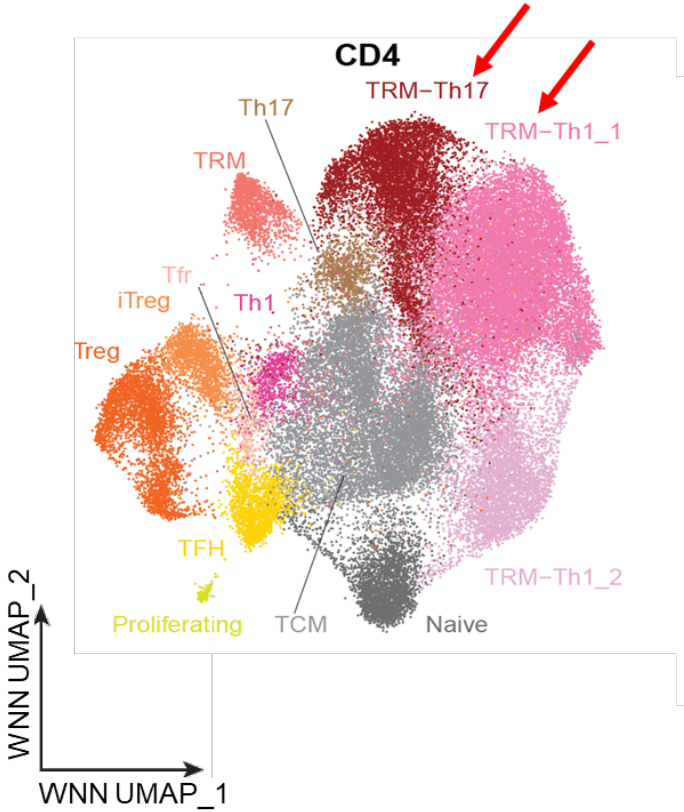


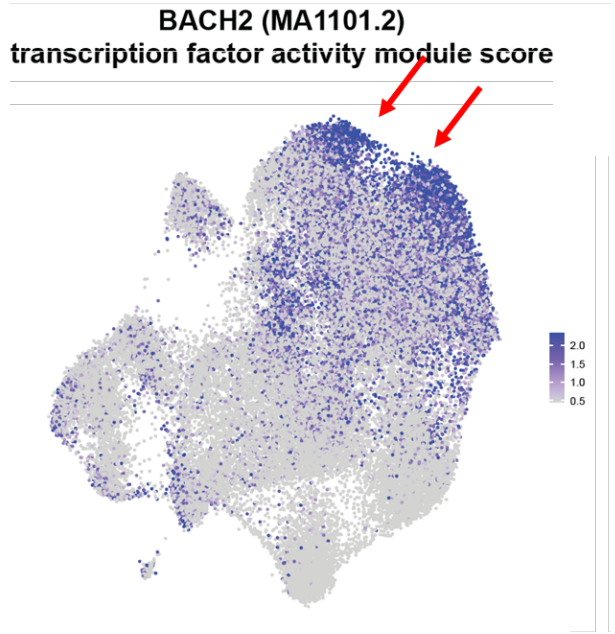
Badia-i-Mompel *et al.*, Nat Rev Genet 2023

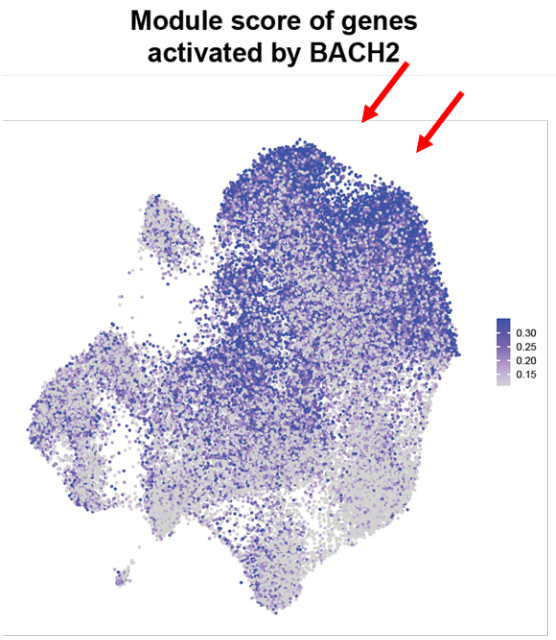
Gene regulatory network was constructed by linking gene-TSS peak and gene-TF associations using FigR, for all domains of regulatory chromatin identified by cisTopic.

Unbiased identification of immune programs dominating gut CD4s: Gene regulatory network analysis (GRN)



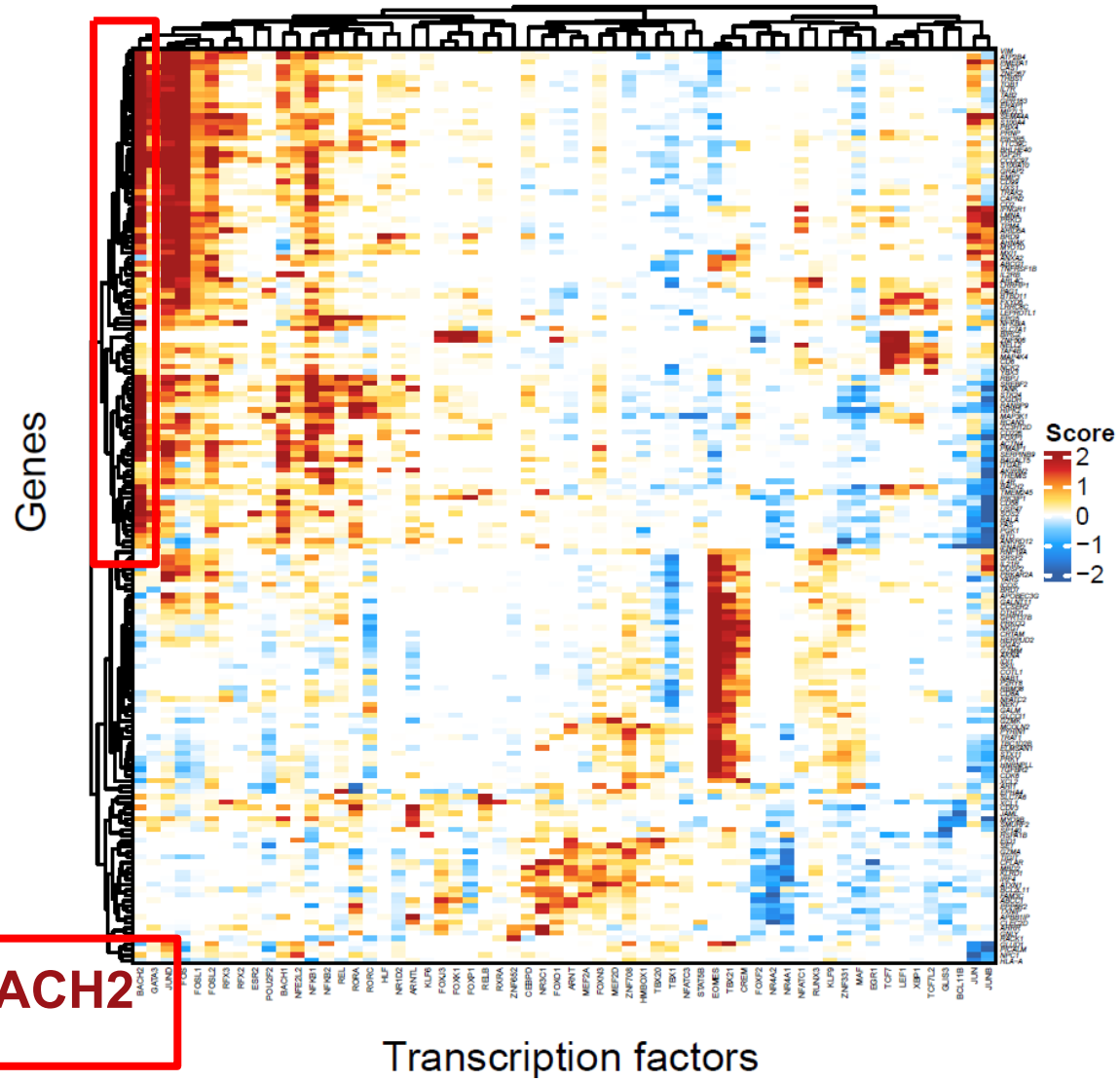


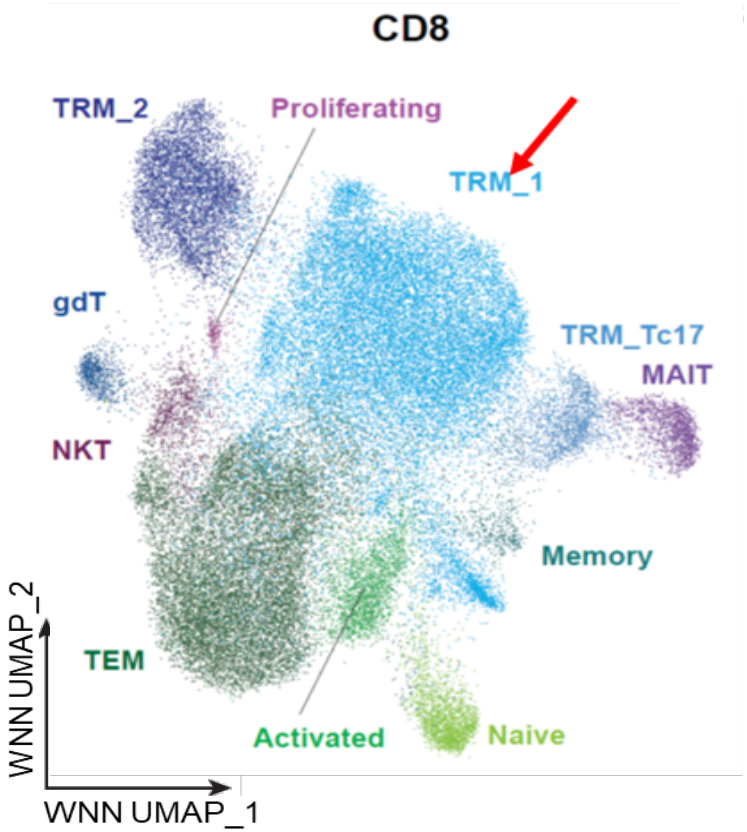


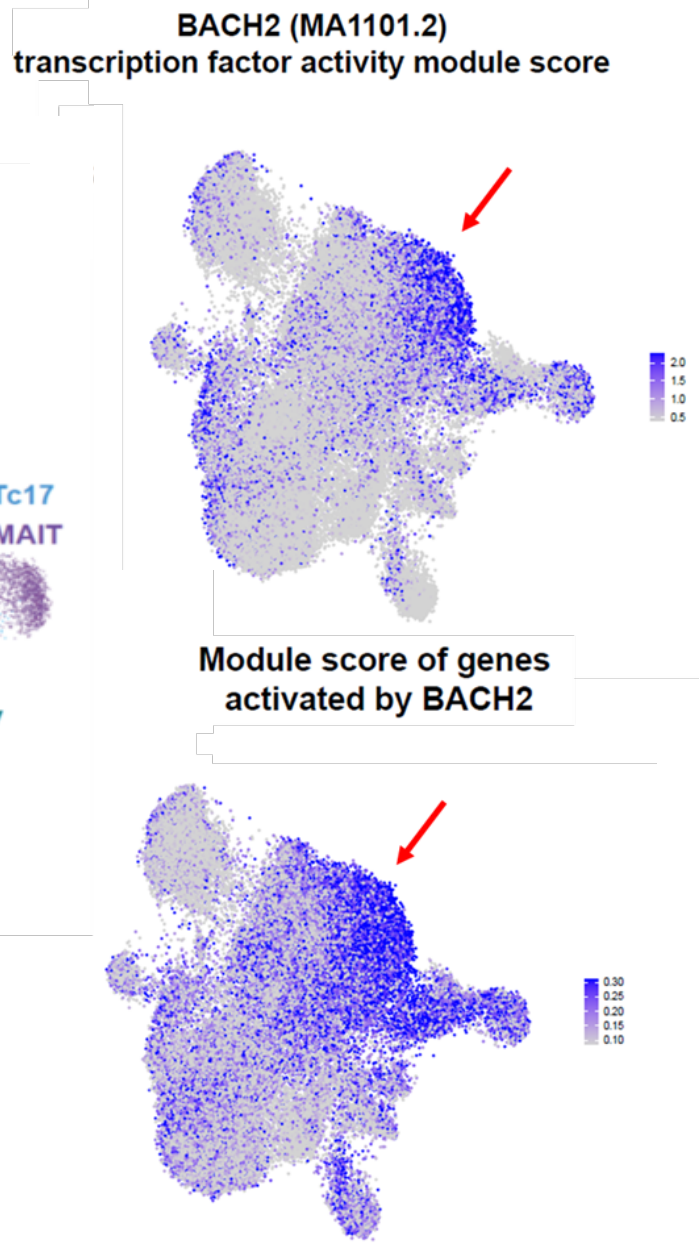


Example BACH2 target genes
Cytokine receptors: *IL7R, IL1R1, Il2RB, IL12RB2, Il21R, IL23R, IL4R, IL6R*
Chemokine receptors: *CCR7, CCR5, CXCR3, CXCR6*
Tissue retention: *ITGAE, ITGA1, CD69*

Unbiased identification of immune programs dominating gut CD8s: Gene regulatory network analysis (GRN)



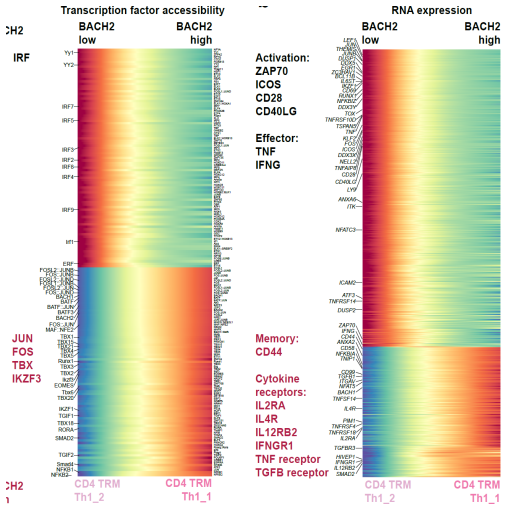




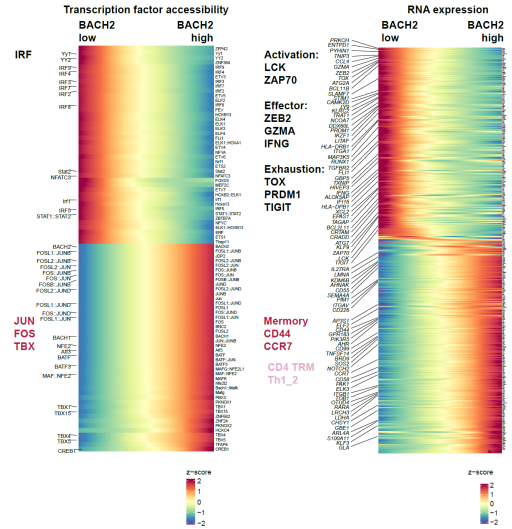
Gene regulatory network was constructed by linking gene-TSS peak and gene-TF associations using FigR, for all domains of regulatory chromatin identified by cisTopic.

BACH2 drives long-lived memory and restrains effector function in CD8+ T cells

CD4+ TRM

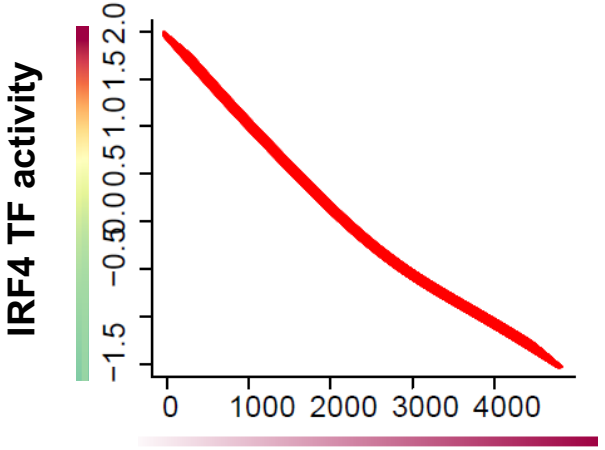


CD8+ TRM

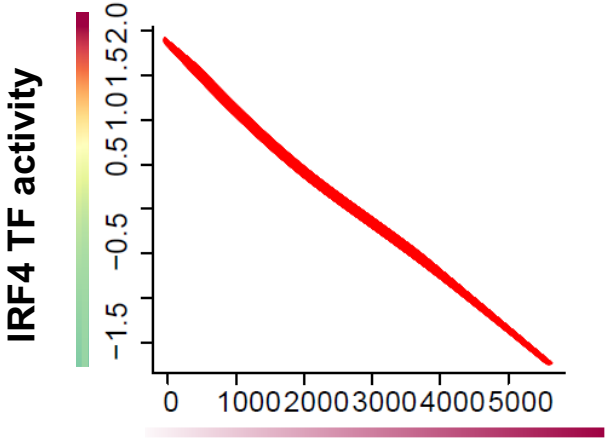


Transcription factor activity

IRF4

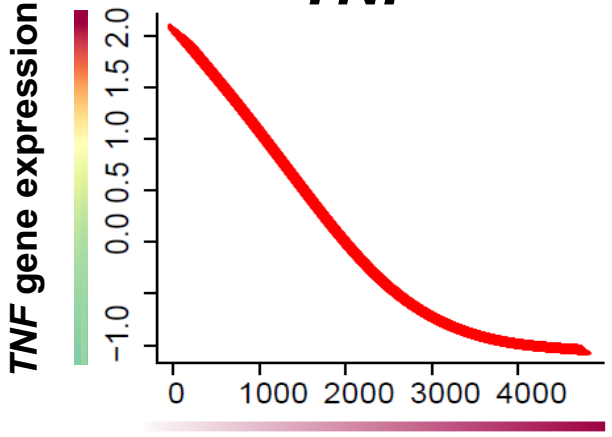


IRF4

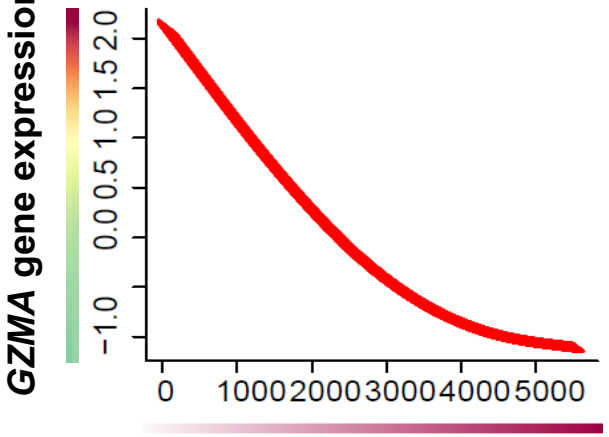


Gene expression

TNF



GZMA



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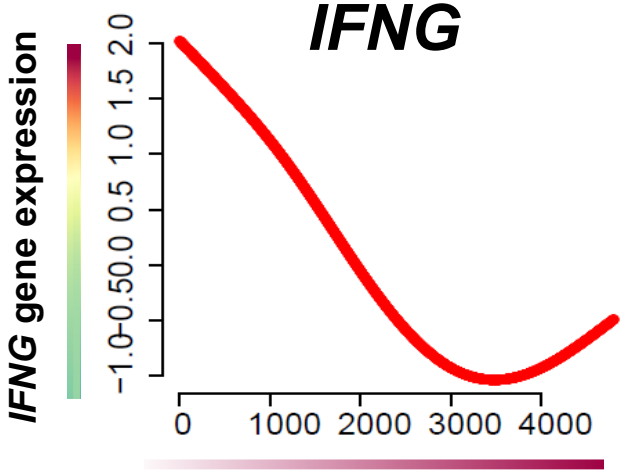

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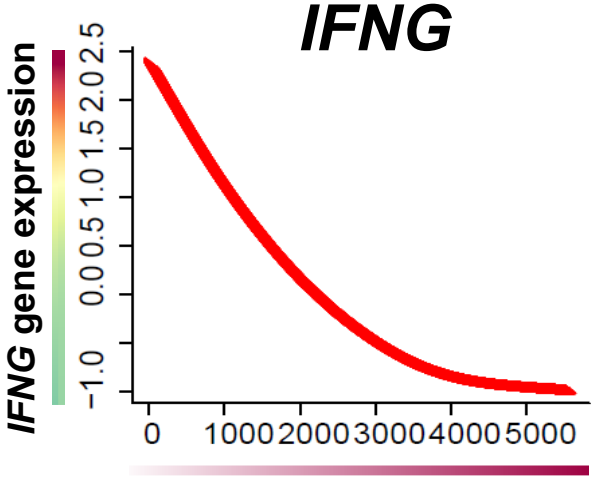
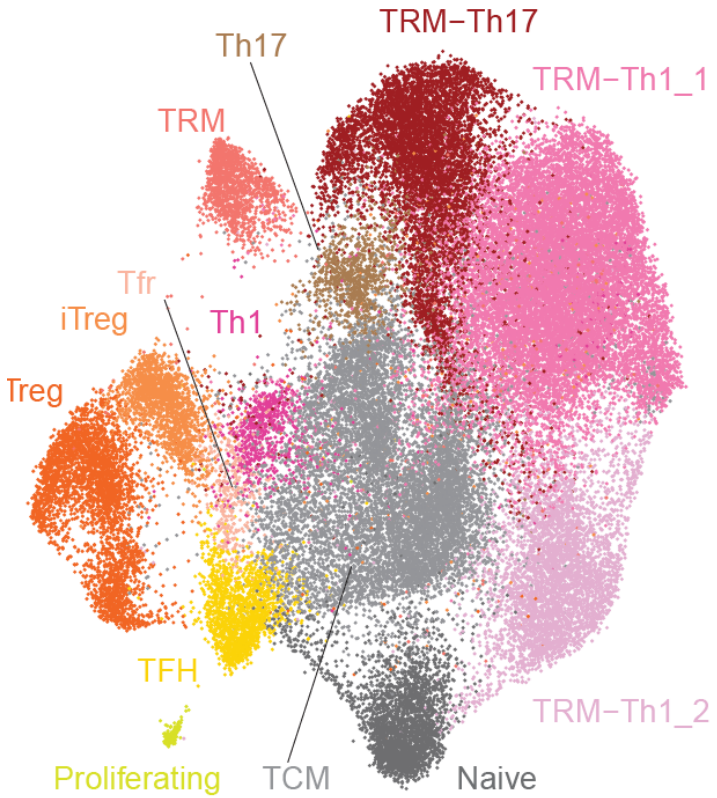
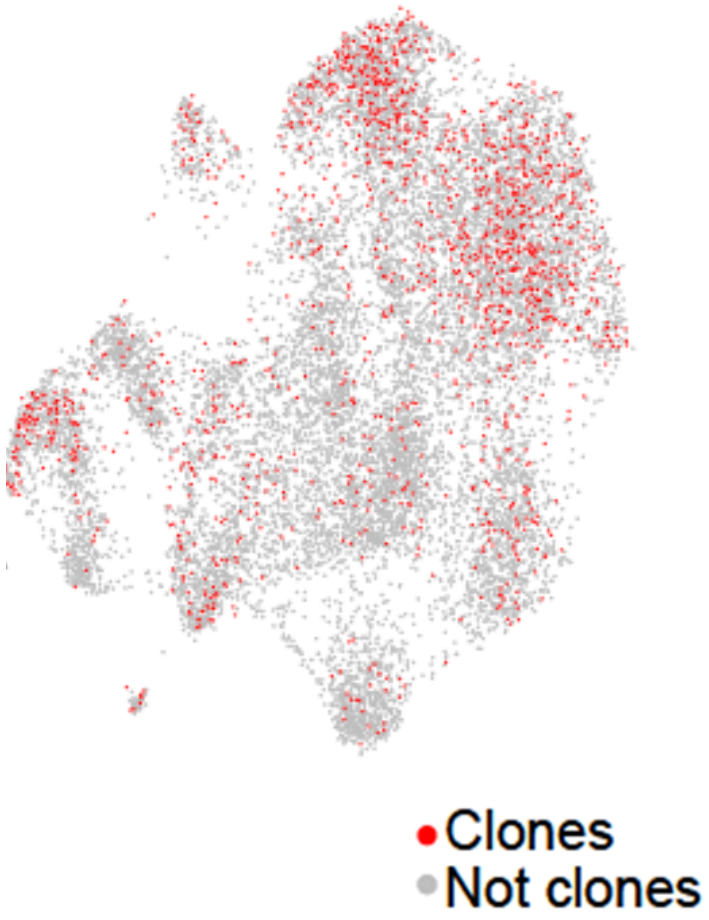
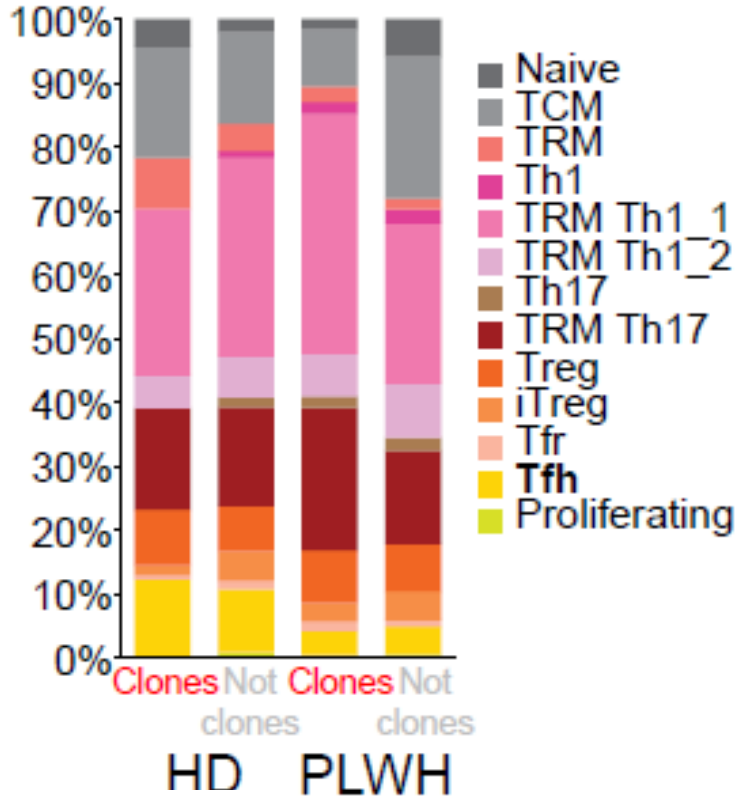


T cell clones and antigen specificity

Capturing T cell receptor (TCR) in DOGMA-seq identified CD4+ T cell clones in the gut

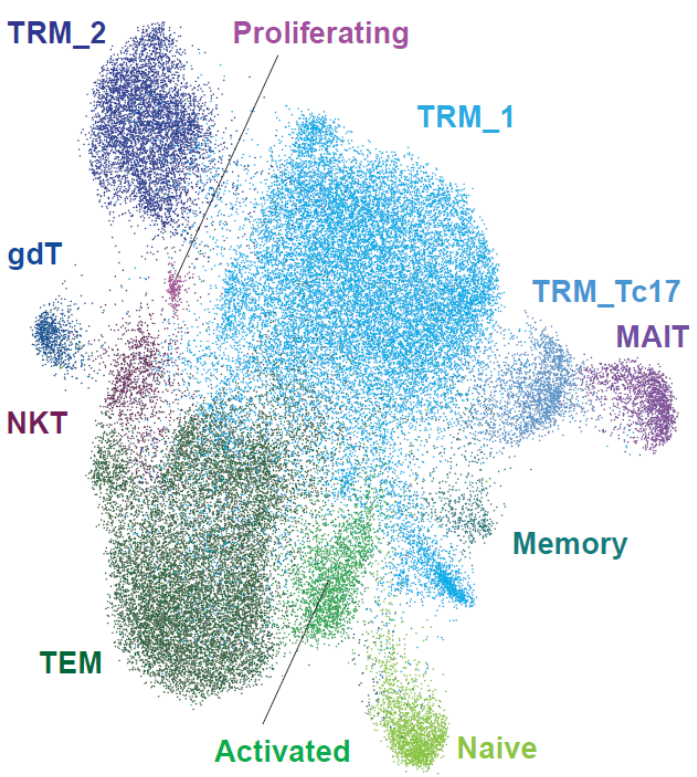


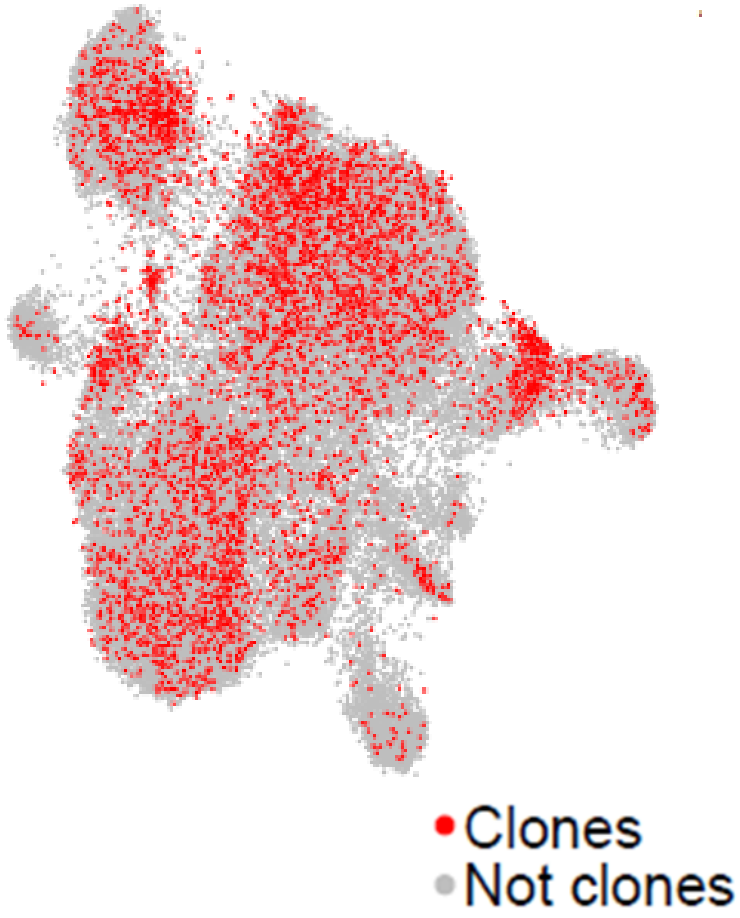


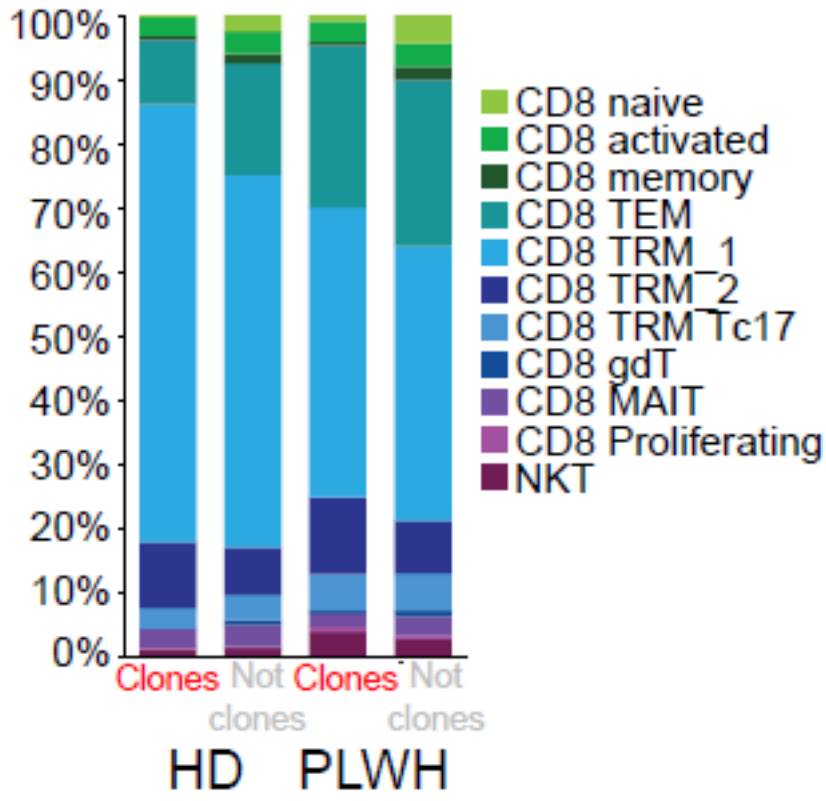


13,012 TCR-captured cells from 43,113 CD4+ T cells in 10 PLWH, including 1,485 cells in clones.
sequences were annotated with IgBLAST and assigned if a cell barcode is associated with ≥ 5 UMI with identifical, productive, TCRβ CDR3 sequences.
Unique clones were determined when at-least 2 cells from the same participant shared the same productive CDR3β junction sequence.

Capturing T cell receptor (TCR) in DOGMA-seq identified CD8+ T cell clones in the gut

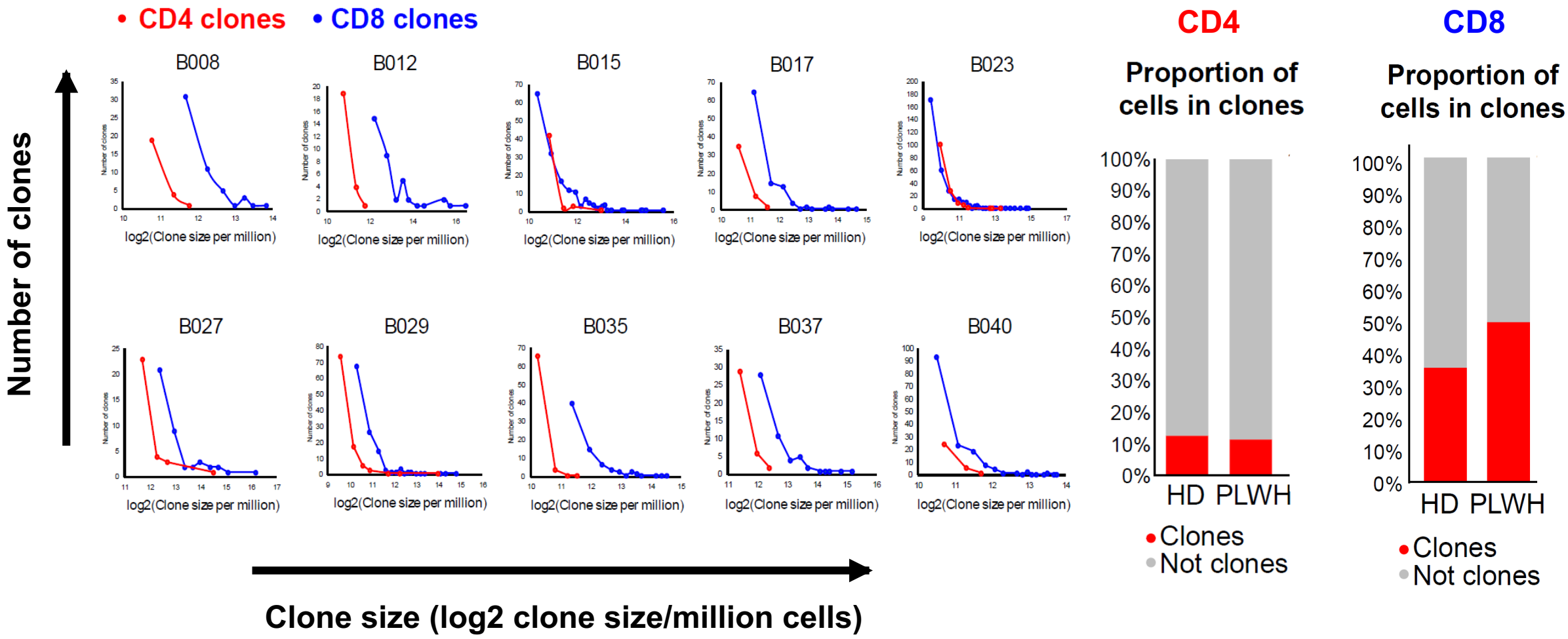






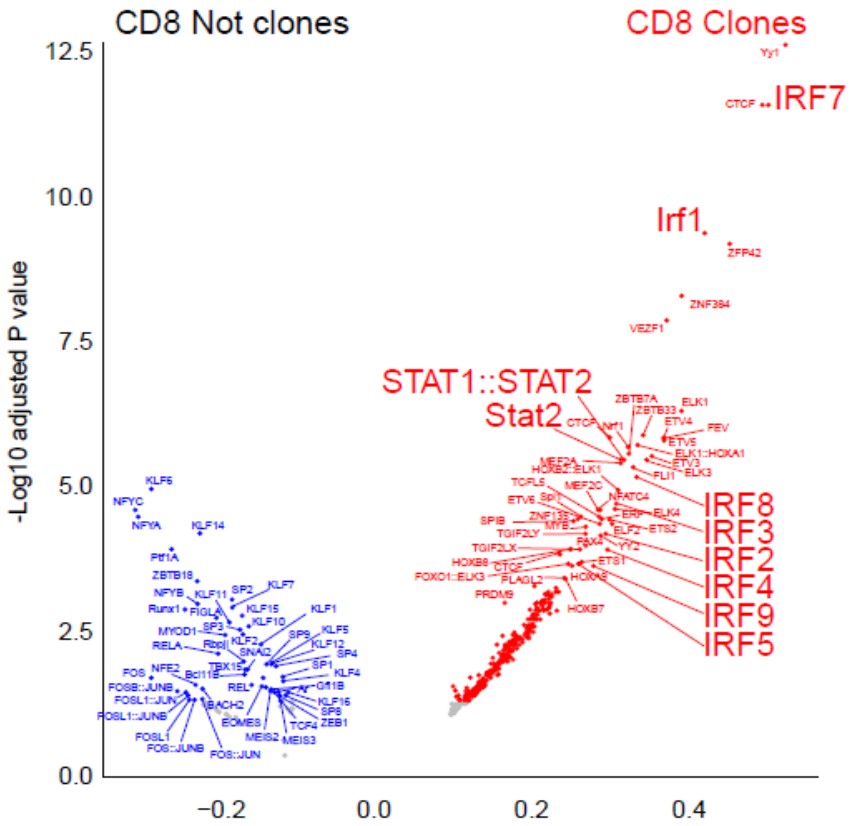
10,868 TCR-captured cells from 45,475 CD8+ T cells in 10 PLWH, including 5,510 cells in clones.
TCR sequences were annotated with IgBLAST and assigned if a cell barcode is associated with ≥ 5 UMI with identical, productive, TCRβ CDR3 sequences.
Unique clones were determined when at-least 2 cells from the same participant shared the same productive CDR3β junction sequence.

CD8+ T cells proliferates much better than CD4+ T cells (larger in T cell clone size)

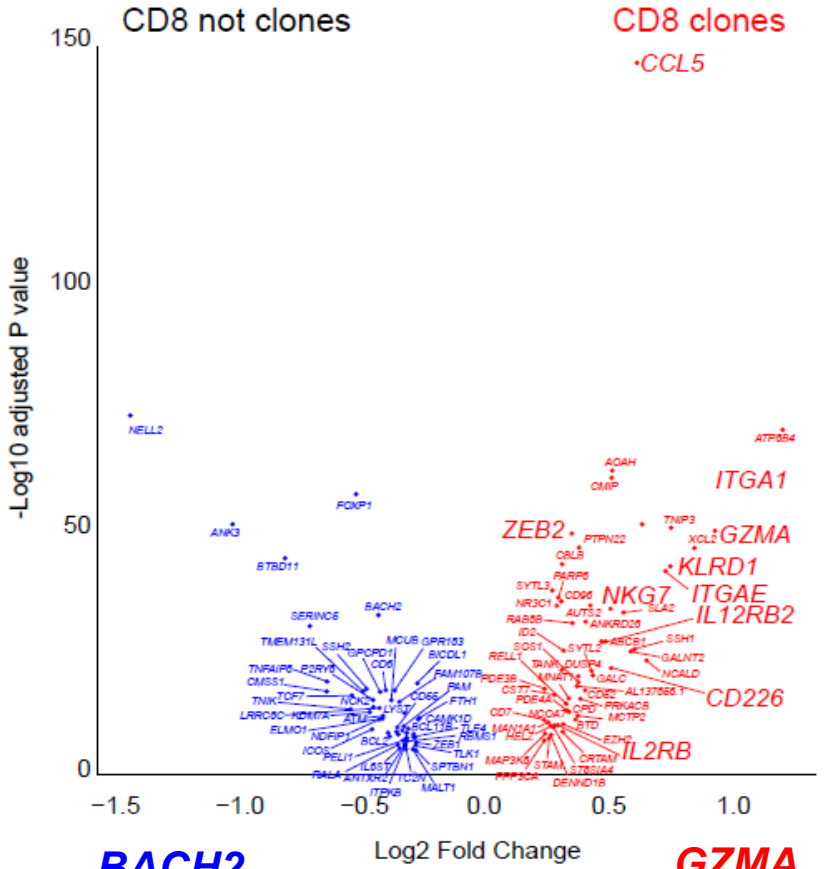


Proliferation of effector CD8+ T cells is driven by interferon regulatory factors

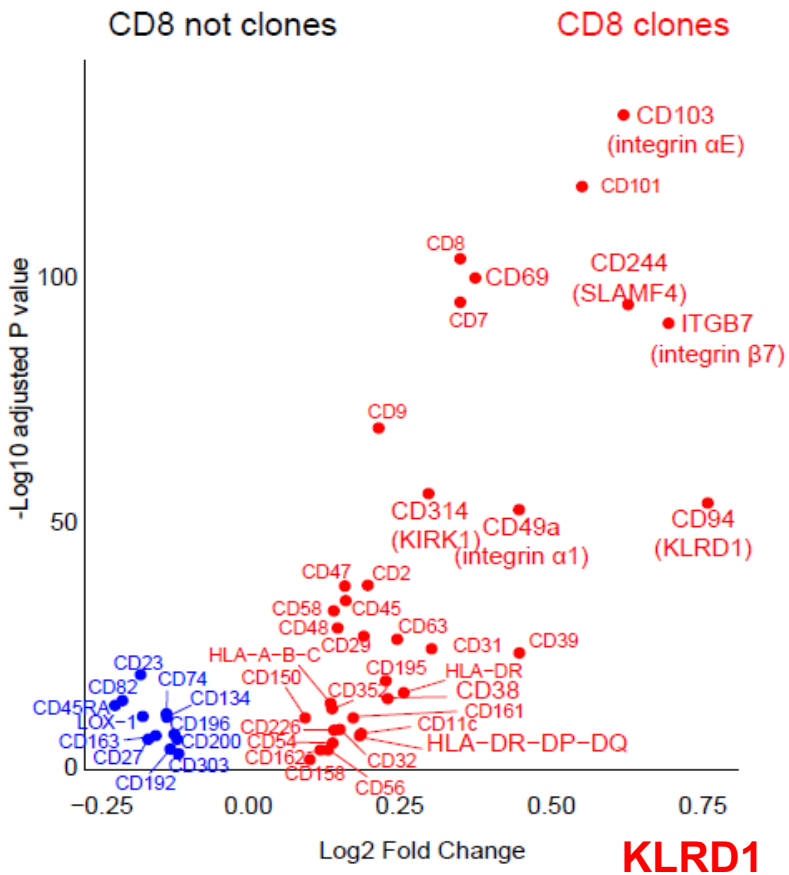
Differential transcription factor accessibilitv (ATAC-seq)



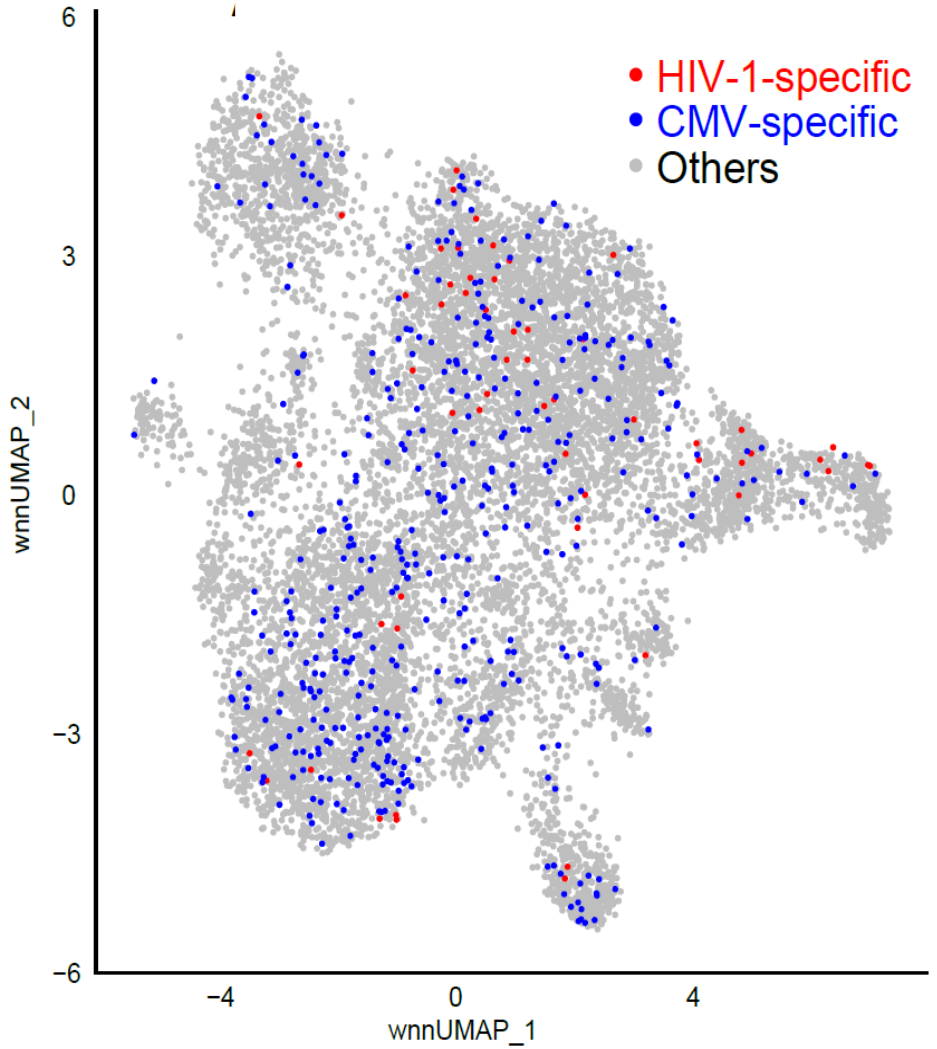
Differential gene transcription (RNA-seq)

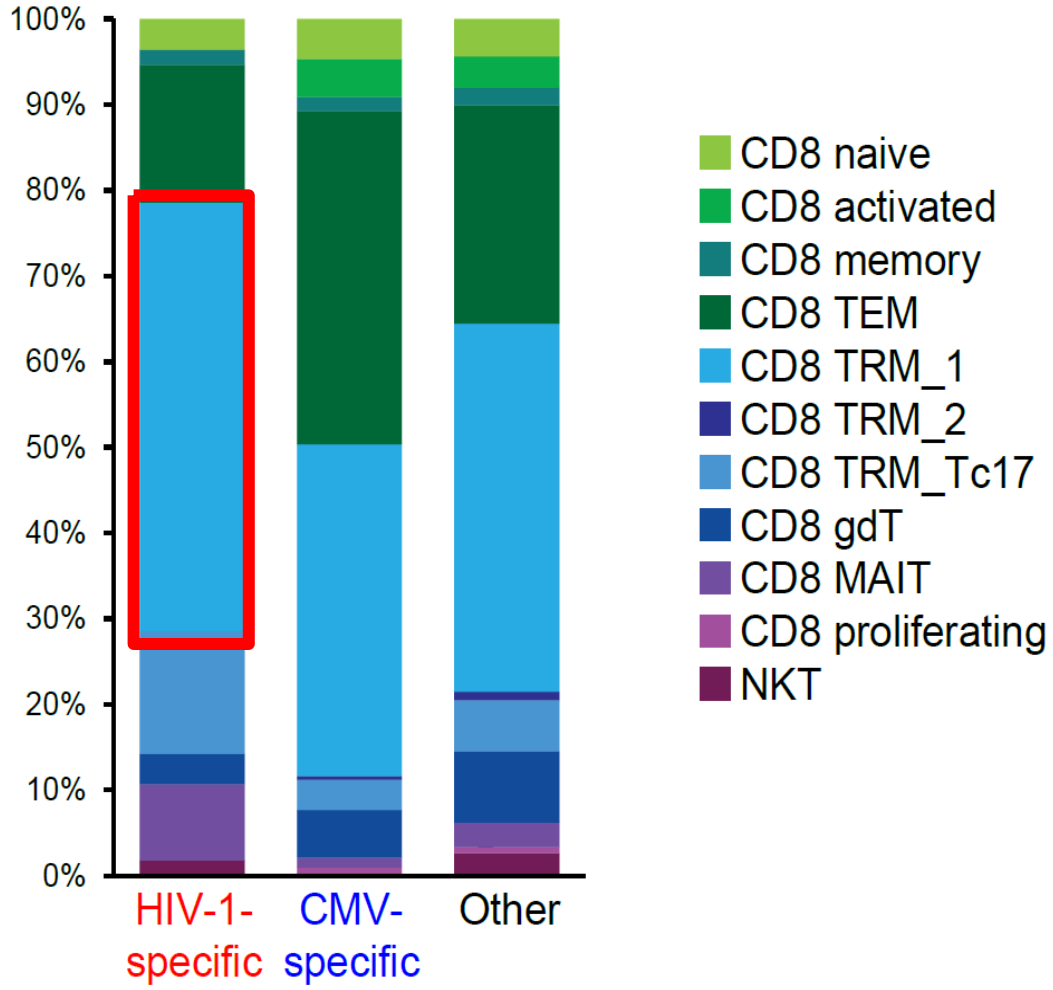


Differential protein expression (CITE-seq)



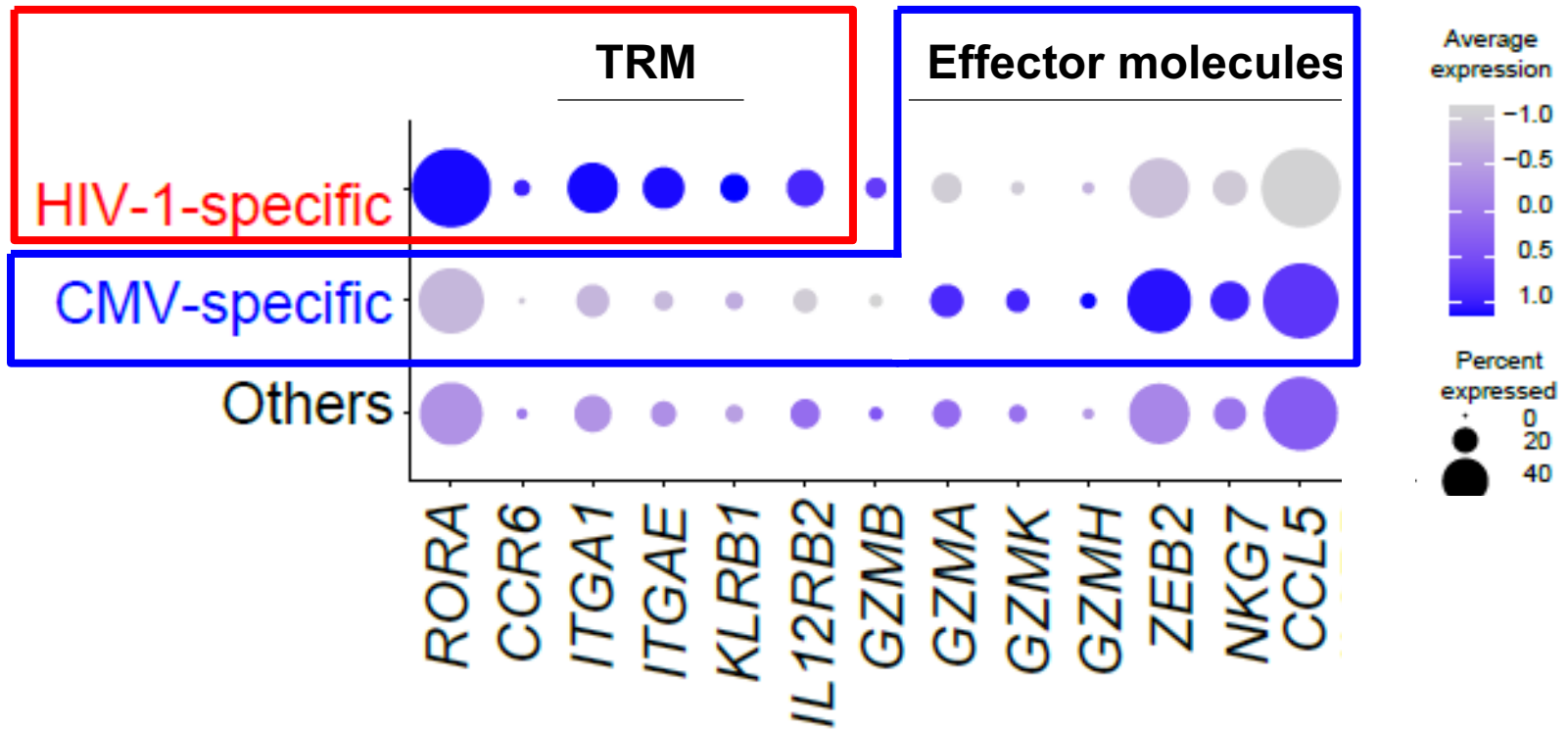
Capturing T cell receptor (TCR) in DOGMA-seq identified HIV-1-specific and CMV-specific CD8+ T cells in the gut





10,868 TCR-mapped CD8+ T cells in PLWH, including 56 HIV-specific and 429 CMV-specific cells. 67.86% of HIV-specific CD8+ T cells are TRMs. Antigen specificity was determined by matching CDR3β junction amino acids against McPAS-TCR database of TCRs with known antigen specificity, following criteria described in Meysman *et al*. Bioinformatics 2019.

HIV-1-specific CD8+ T cells in the gut lack effector function



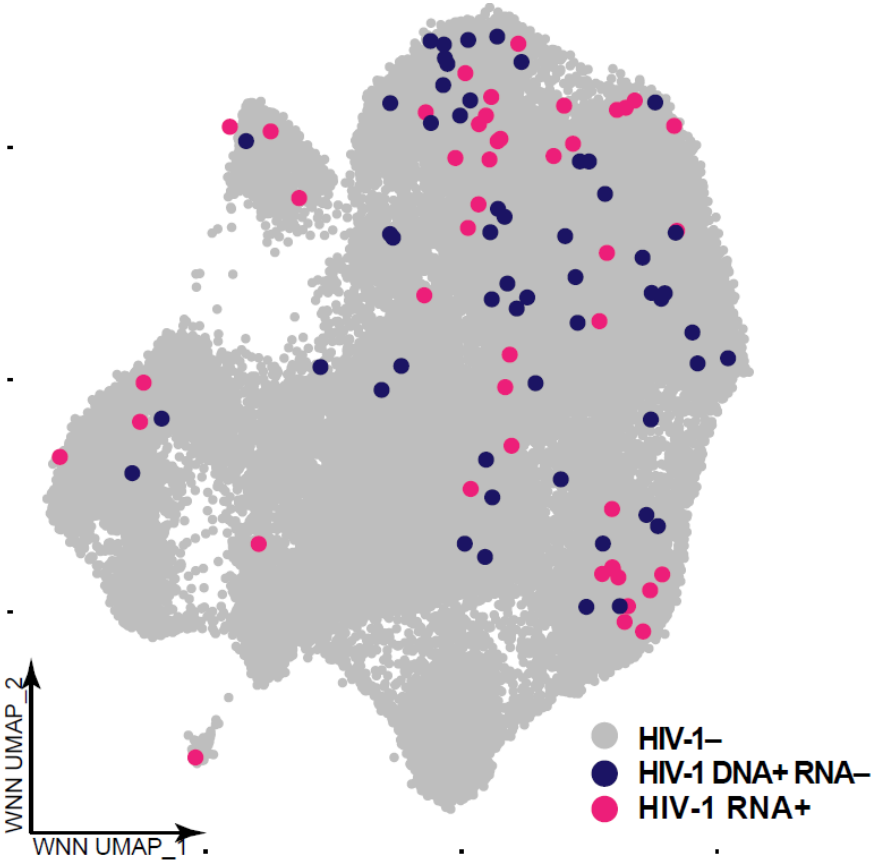
BACH2 drives long-lived memory gut TRM cells

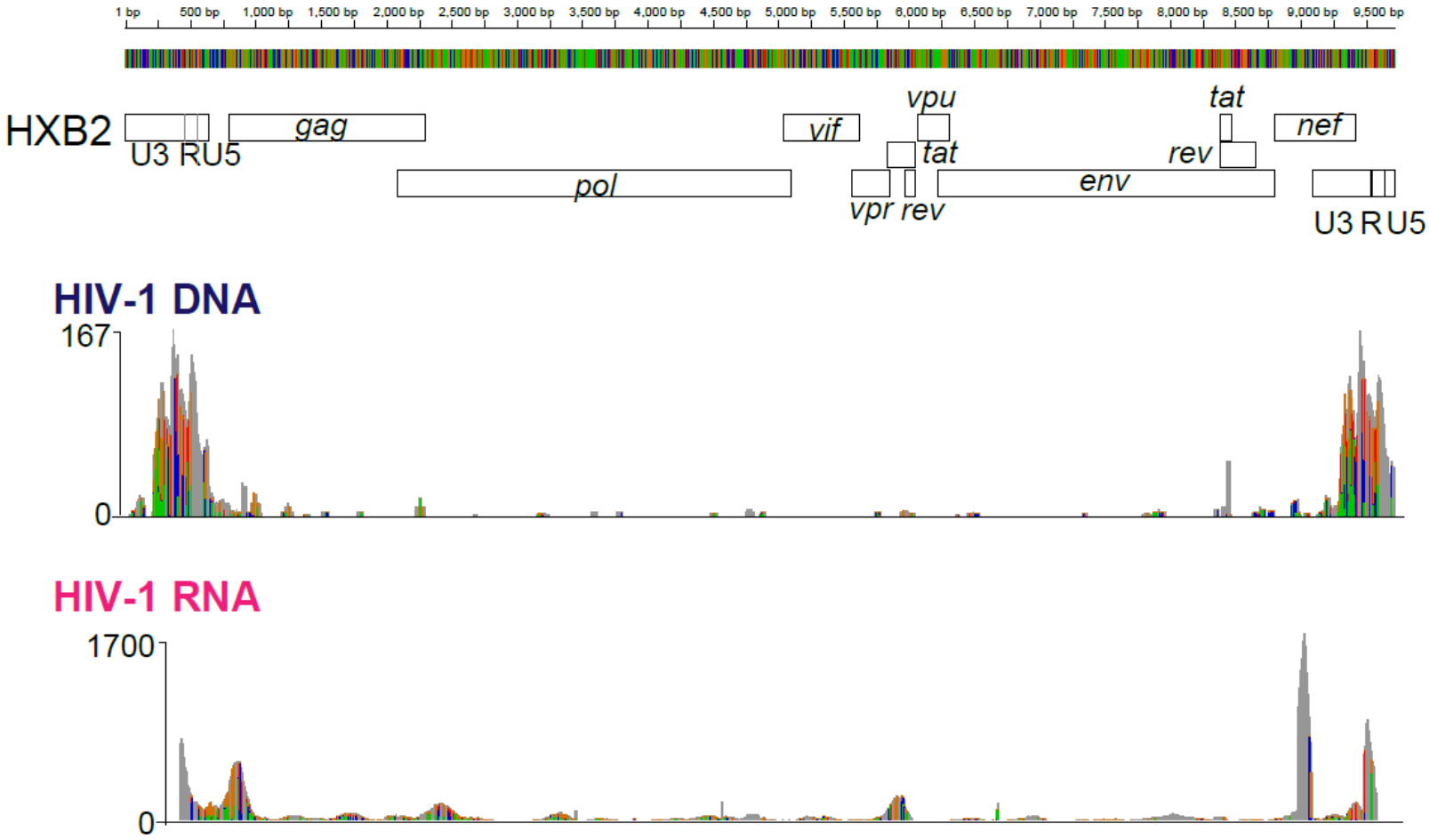
IRFs
Effector function
Short lived
Clonal proliferation

BACH2
Tissue residency
Long-term survival
Low effector function

Where does HIV hide in the gut?

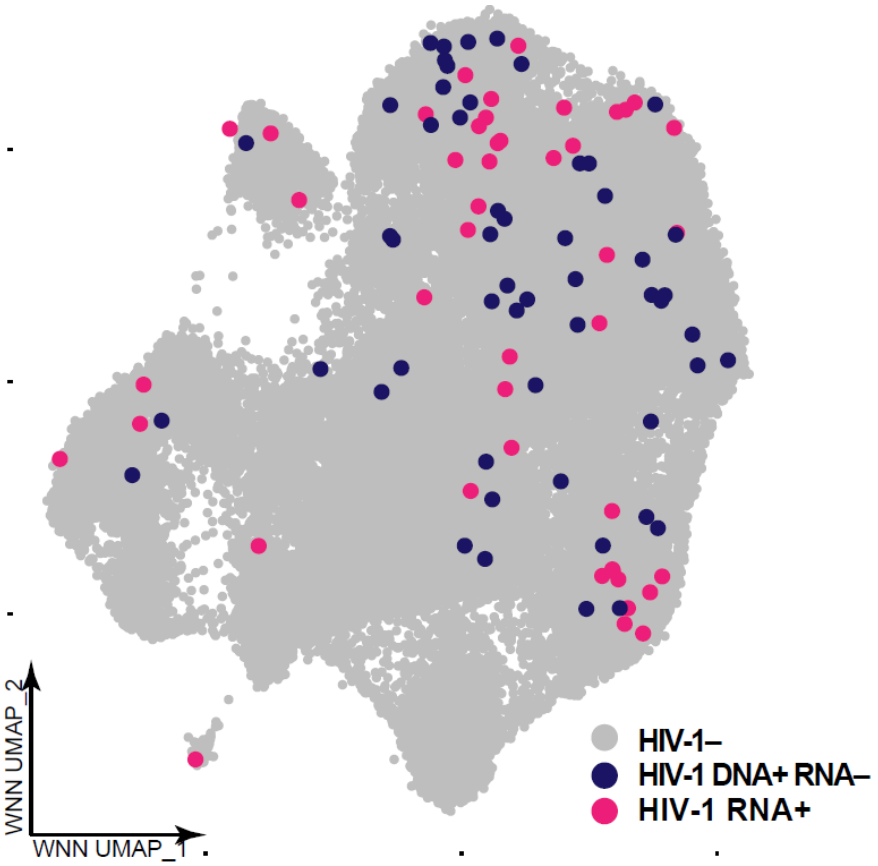
HIV-1-infected cells reside in gut CD4+ TRM

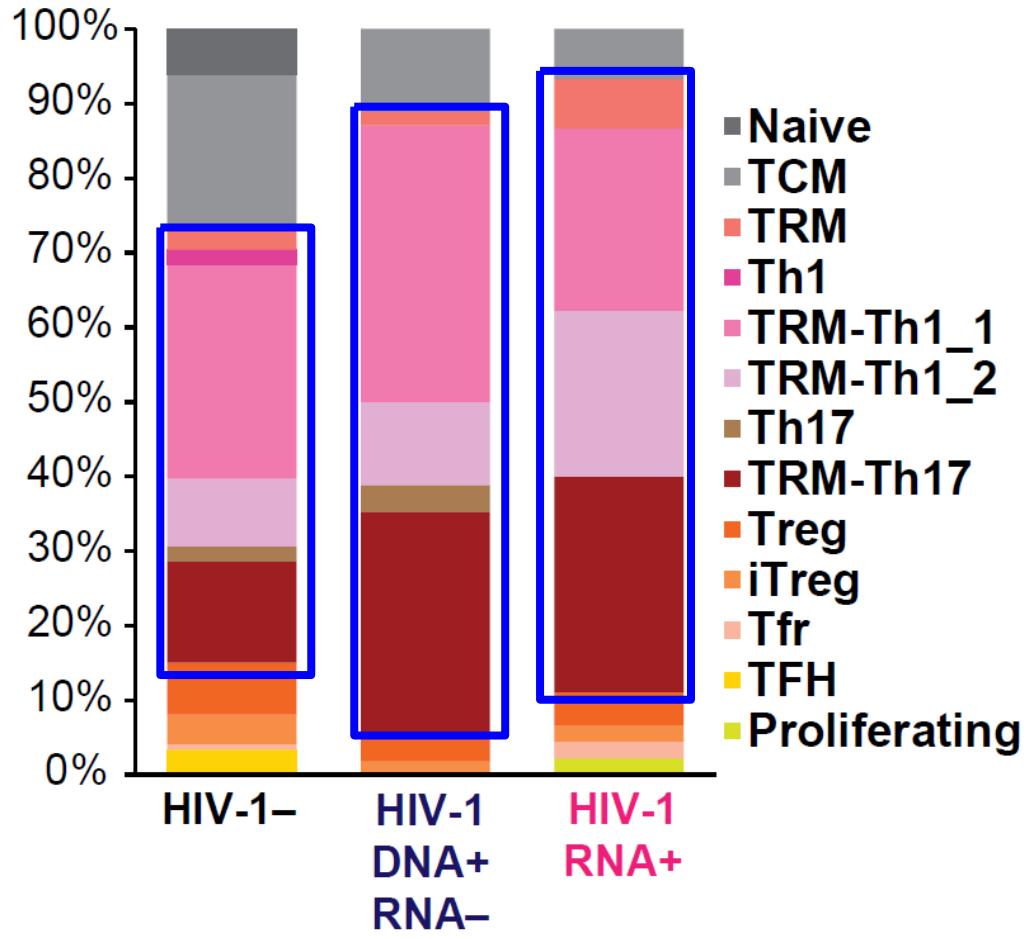




43,113 CD4+ T cells in 10 PLWH under ART, including 99 HIV-1+ cells.
80.81% (80/99) of HIV-1-infected cells are found in TRM.
75% (60/80) of HIV-1-infected TRM are BACH2 high cells.

HIV-1-infected cells reside in gut CD4+ TRM





43,113 CD4+ T cells in 10 PLWH under ART, including 99 HIV-1+ cells.
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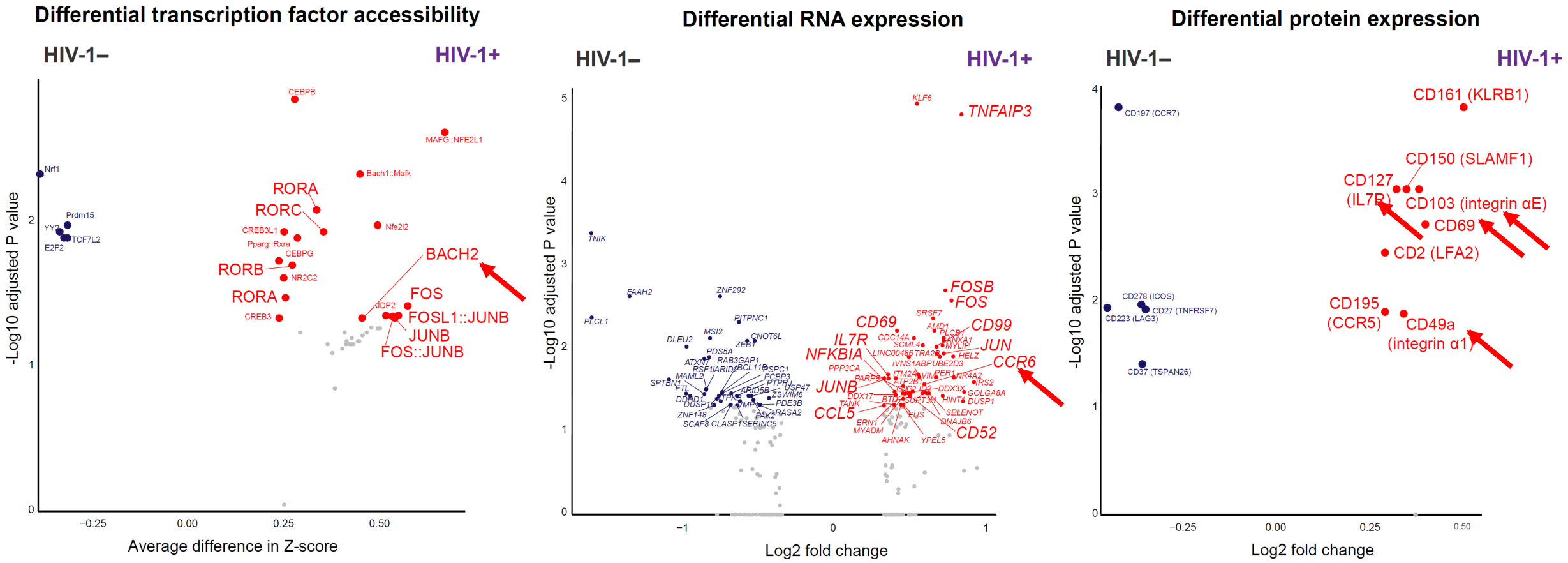
75% (60/80) of HIV-1-infected TRM are BACH2 high cells.

HIV-1-infected cells are shaped by BACH2 and exhibit TRM phenotype



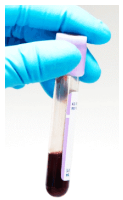
43,113 CD4+ T cells in 10 PLWH under ART, including 99 HIV-1+ cells
Transcription factor accessibility was measured by ChromVAR based on transcription factor reference motif dataset JASPER 2022
Bootstrapping: the sample size of HIV-1– cells were downsized to the sample size of HIV-1+ cells for comparison and repeated with replacement for 1,000 times
P values were calculated by Cauchy combination test.

HIV in gut CCR6+CD4+ T cells: Waleche *et al.* Retrovirology 2016; Gosselin *et al*. AIDS 2016; Planas *et al*. JCI Insight 2017

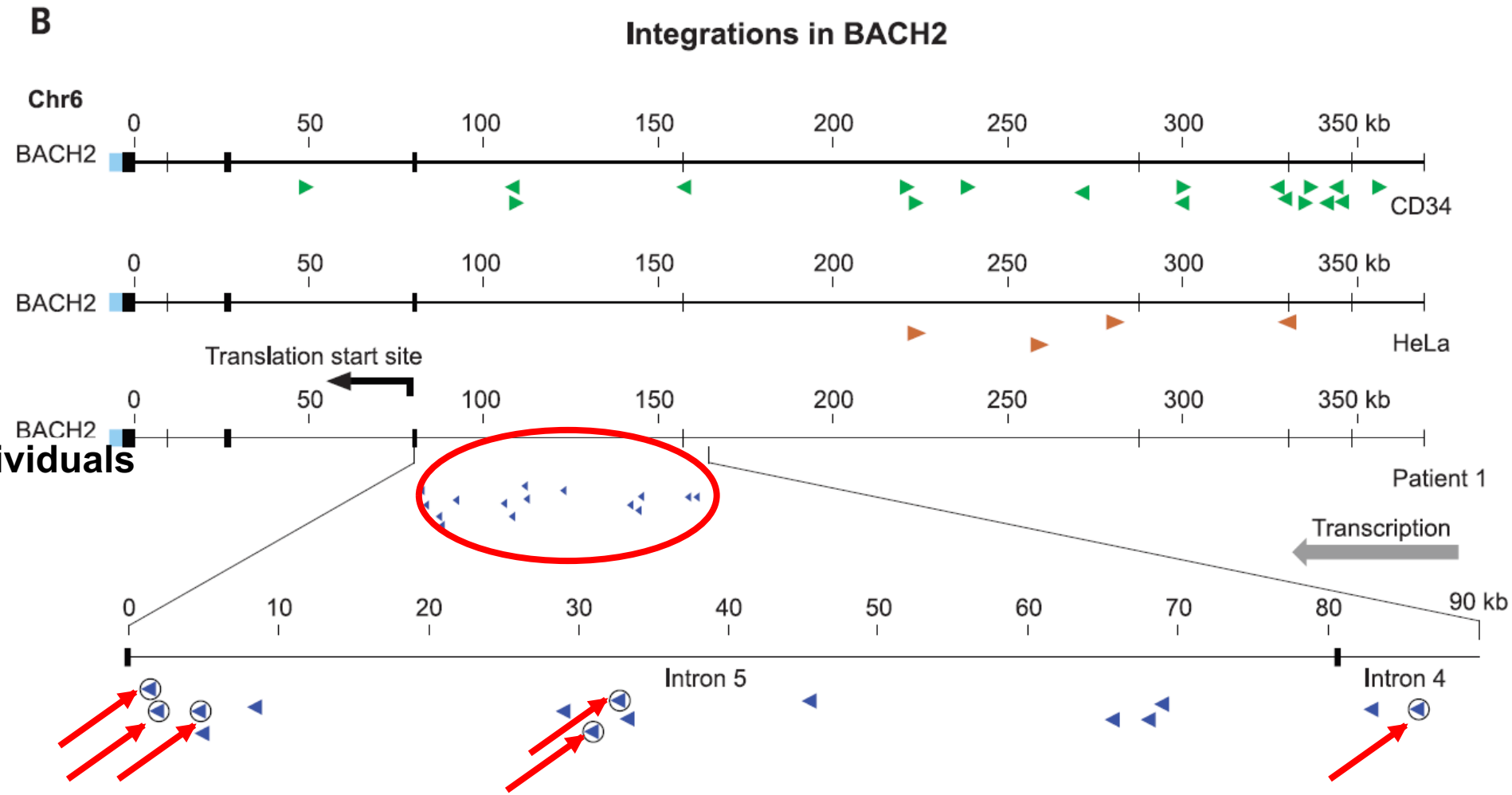
HIV-1 integration into BACH2 drives clonal expansion of the infected cell

***In vitro* infection**

***In vitro* infection**



***In vivo*:**
cells from HIV-1⁺ individuals on ART



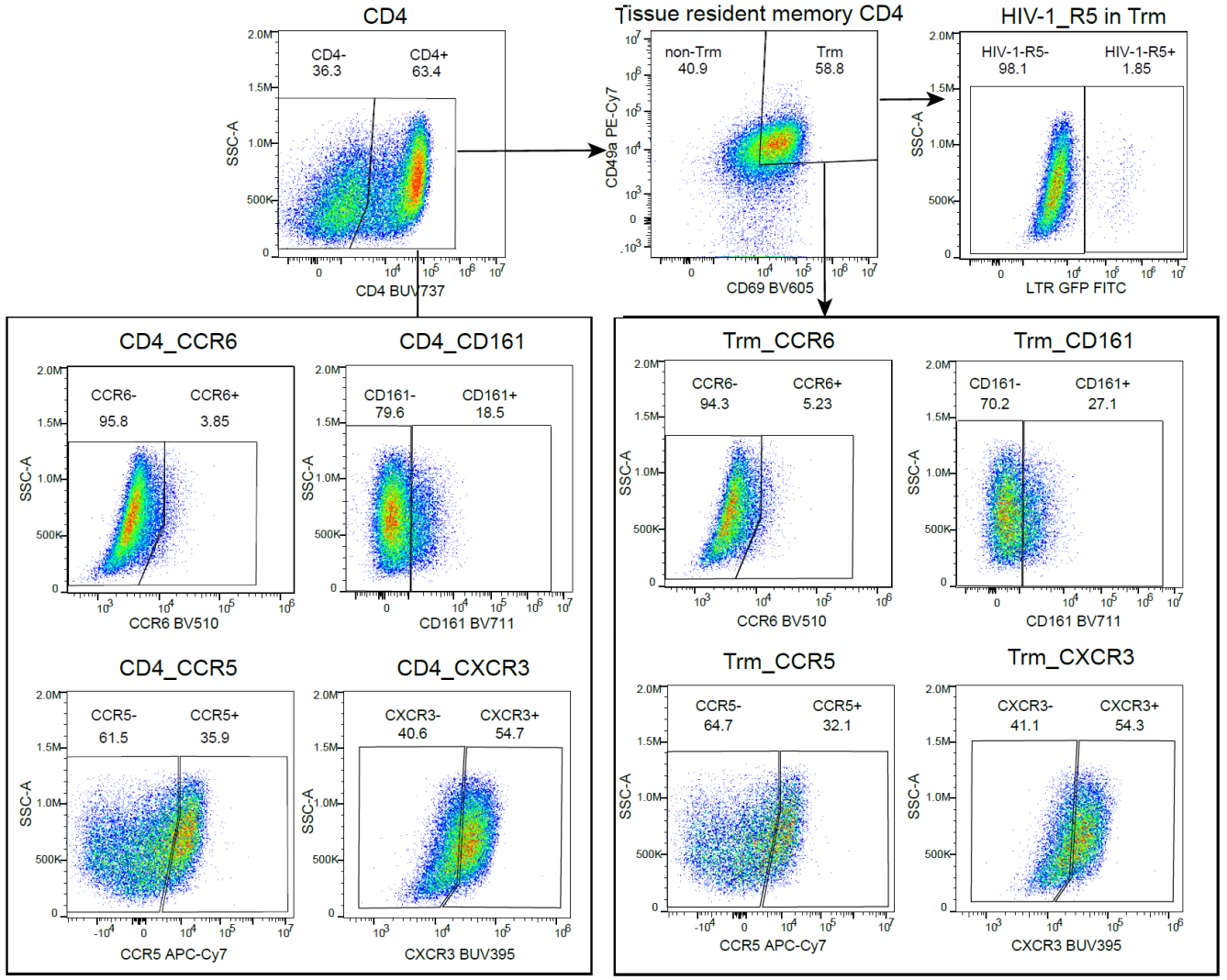
Maldarelli *et al.*, Science 2014; Wagner *et al.*, Science 2014

Validation: does HIV-1 preferentially infect or preferentially persist in gut TRM

Gut from HIV negative adult

Infect with HIV-1-GKO pseudotyped with JR-FL (R5 Env)

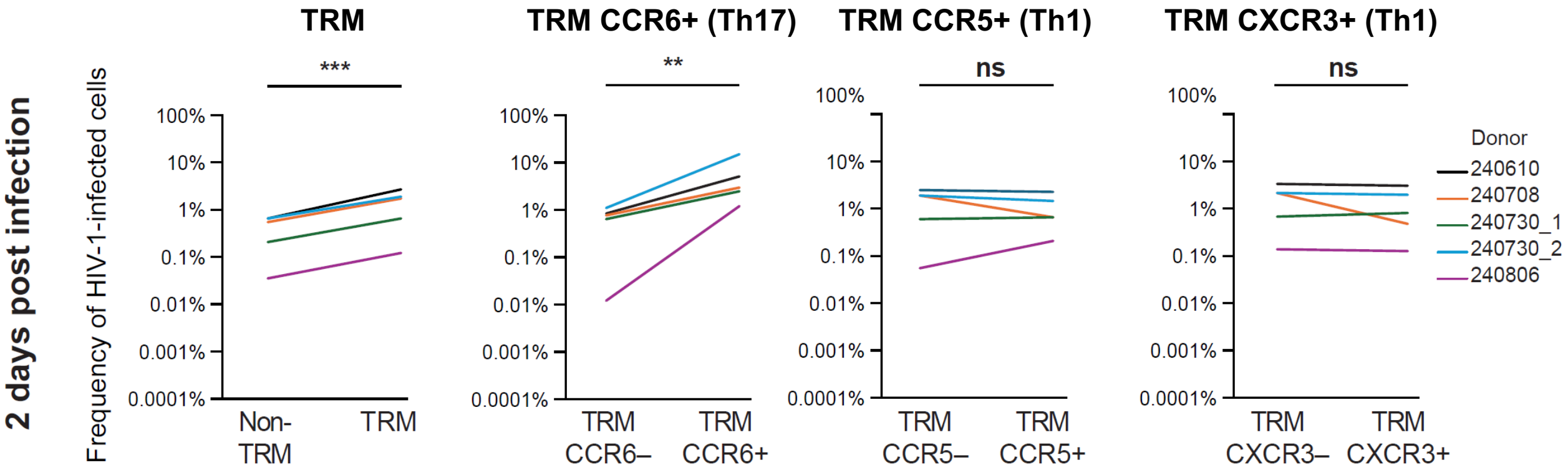
TRM: CD49a+ CD69+
Th1: CXCR3+ CCR5+
Th17: CCR6+



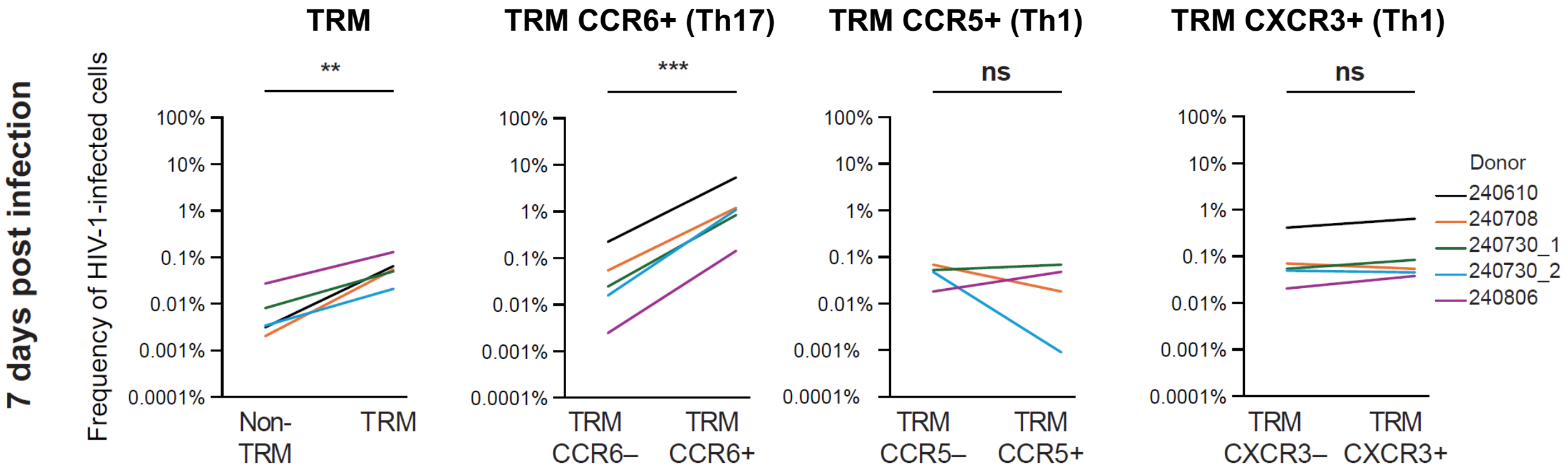
Katherine Ma

Th1: CXCR3+CCR6−; Th17: CCR4+CCR6+ (Becattini *et al.* Science 2015); CD4+ TRM: CD49a+CD69+ (Mackay *et al*. Nature Immuol. 2013)

HIV-1 preferentially infects gut CD4+ TRM (2 days post infection)
Particularly CCR6+ TRM (TRM-Th17), but not CCR5+ TRM

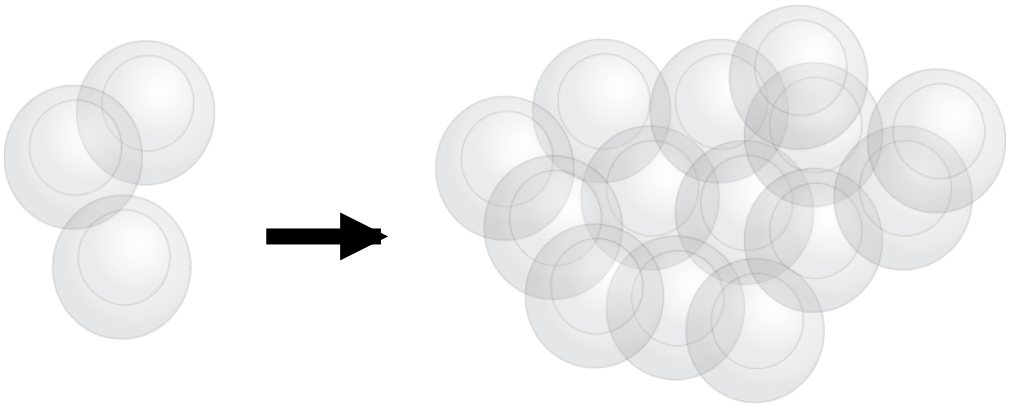


HIV-1 preferentially persists in gut CD4+ TRM (7 days post infection)
Particularly CCR6+ TRM (TRM-Th17), but not CCR5+ TRM



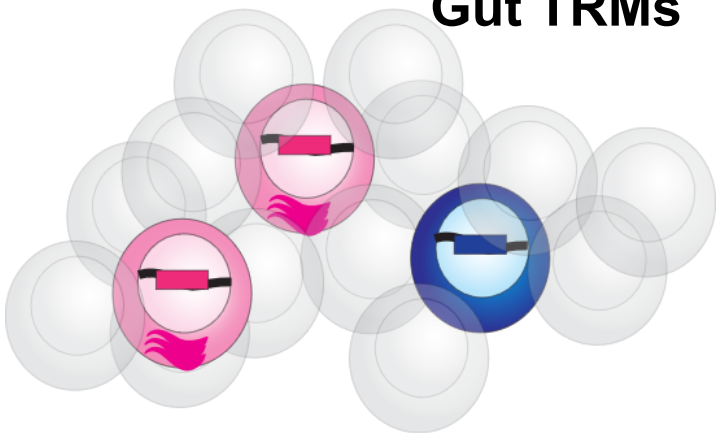
BACH2 promotes tissue resident program and facilitate HIV-1 persistence

IRFs
Effector function
Short lived
Clonal proliferation



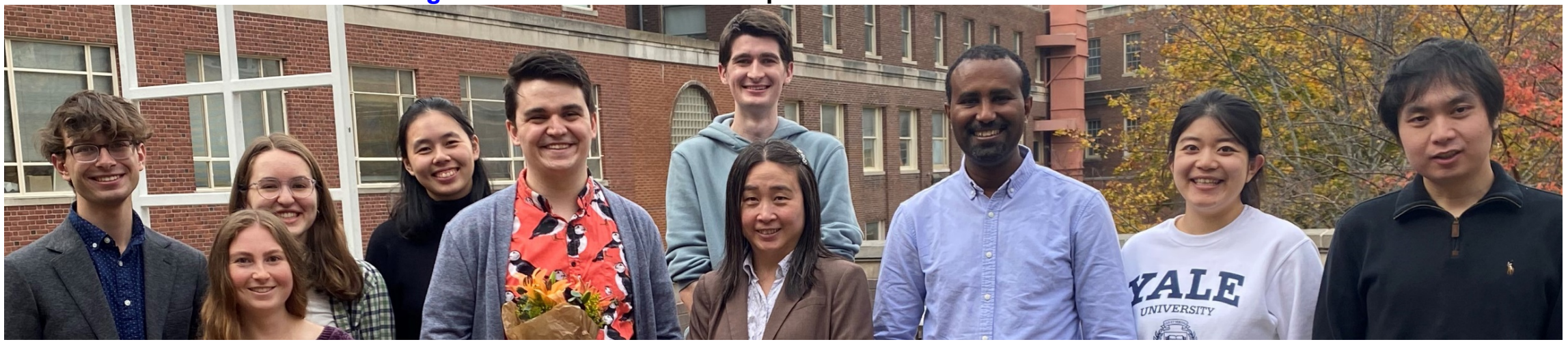
BACH2
Tissue residency
Long-term survival
Low effector function

Gut TRMs



Low effector function in HIV-1-specific CD8+ TRM
Long-term survival of HIV-1-infected CD4+ TRM

Acknowledgements



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U Colorado
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NIH
HIV Reagents Program

All study participants

BEAT-HIV DELANEY COLLABORATORY

REACH For the Cure
Research Enterprise to Advance a Cure for HIV

GILEAD | Research Scholars

Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV (CHEETAH)

ACSR AIDS and Cancer Specimen Resource

NSERC CRSNG

SCORCH

amfAR MAKING AIDS HISTORY

NIH National Institute of Allergy and Infectious Diseases

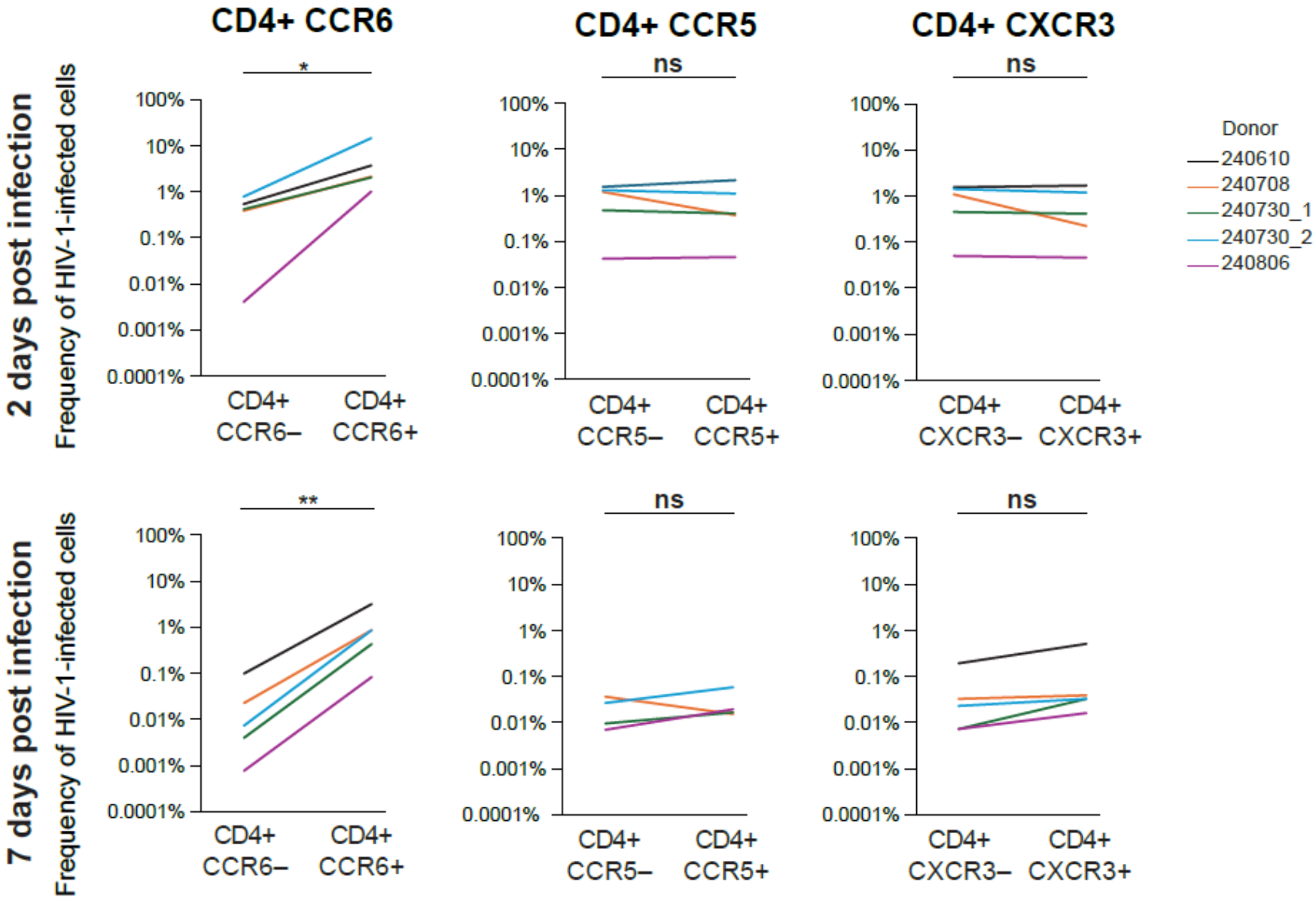
NIH National Institute on Drug Abuse

Clinical demographics

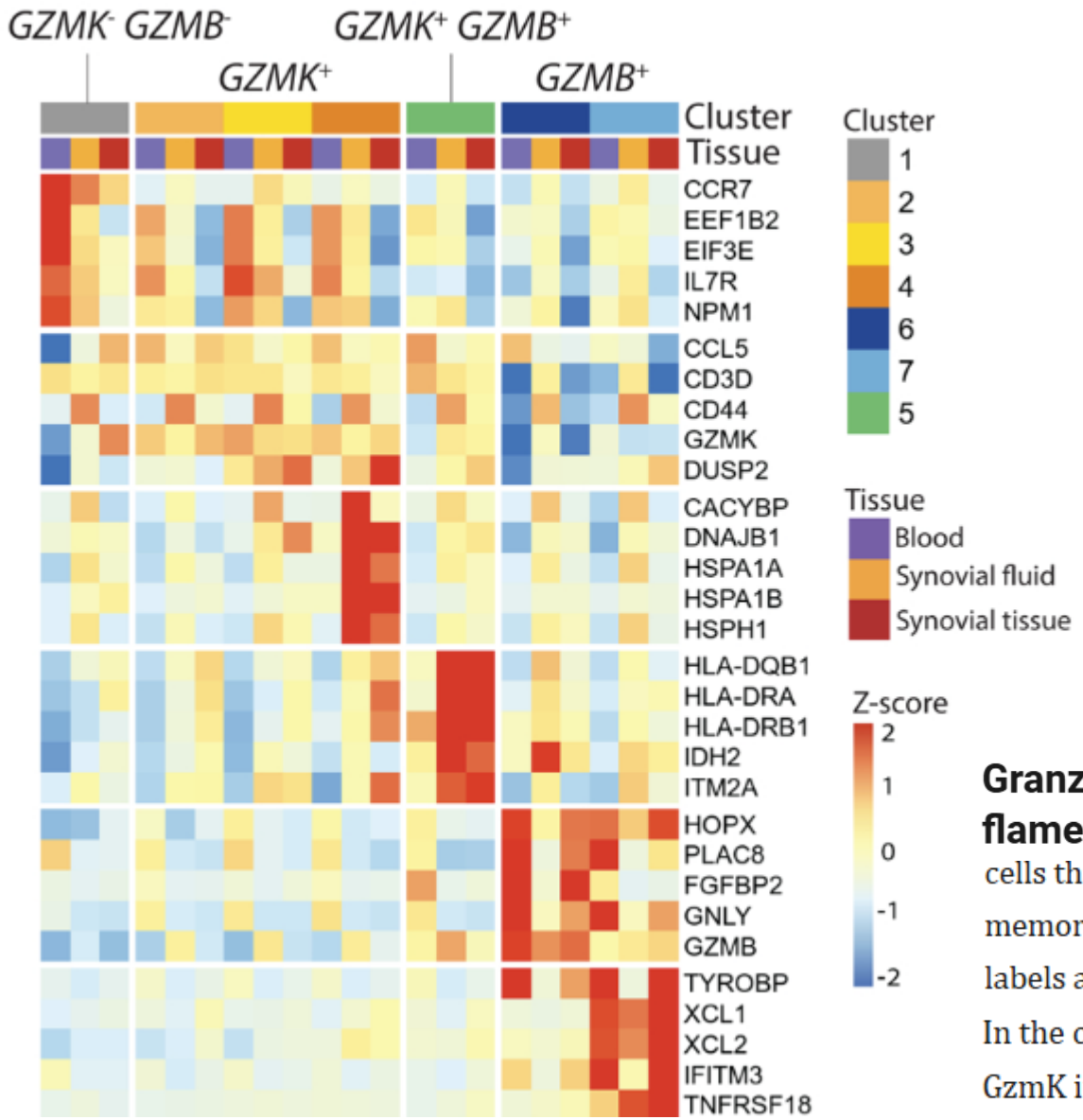
Participant	Age	Sex	Race	Ethnicity	ART	Duration of ART (months)	CD4 count (cells/μl)	HIV viral load (copies/ml)
People living with HIV (PLWH)								
008	48	Male	White	Not Hispanic	FTC/RPV/TDF	48.8	1247	<20
012	47	Female	Black	Not Hispanic	EVG/c/FTC/FTC/TDF	26.3	731	<20
015	58	Male	White	Not Hispanic	DOL/ABC/3TC	8.6	457	<20
017	49	Male	Black	Not Hispanic	FTC/RPV/TDF	20.9	531	<20
023	45	Male	Black	Not Hispanic	FTC/PRV/TDF	50.8	1087	<20
027	41	Male	White	Not Hispanic	EFV/FTC/TDF	86.8	584	<20
029	28	Male	Black	Not Hispanic	EFV/FTC/TDF	50.1	1146	<20
035	37	Male	Other	Hispanic	EFV/FTC/TDF	74.4	732	<20
037	32	Male	Black	Not Hispanic	FTC/RPV/TDF	25.0	884	<20
040	41	Male	White	Not Hispanic	FTC/RPV/TAF	2.4	538	178
People living without HIV (HD)								
351	37	Male	White	Hispanic				
357	51	Male	Black	Hispanic				
360	38	Male	Asian	Hispanic				
361	27	Female	White	Hispanic				
363	25	Female	White	Hispanic				

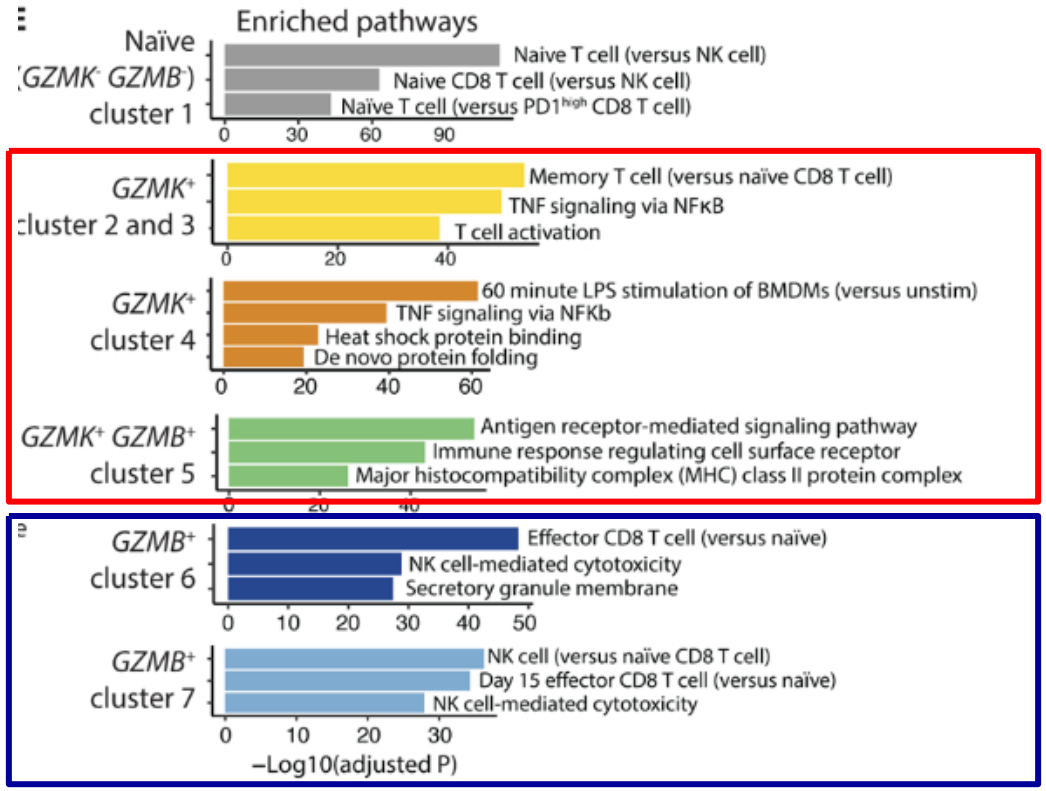
3TC, lamivudine; ABC, abacavir; /c, cobicistat; DOL, dolutegravir; EFV, efavirenz; EVG, elvitegravir; FTC, emtricitabine; RPV, rilpivirine; TAF, tenofovir alafenamide; TDF, tenofovir

HIV-1 preferentially infects and persists in gut CCR6+CD4+ T cells



Tissue CD8+ T express Granzyme A, B, and K





Granzyme K+ CD8 T cells form a core population in inflamed human tissue

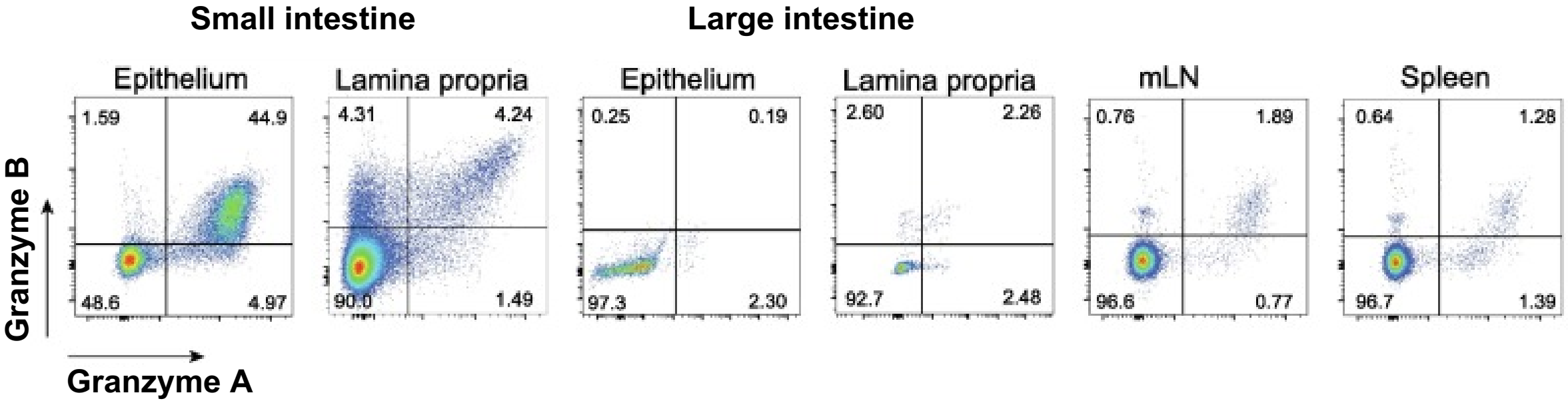
cells that express GzmK alone or alongside GzmB should not automatically be labeled as memory cells or cytotoxic cells in single-cell RNA-seq data, as our findings indicate that such labels are not accurate descriptors of these human tissue-associated CD8 T cells

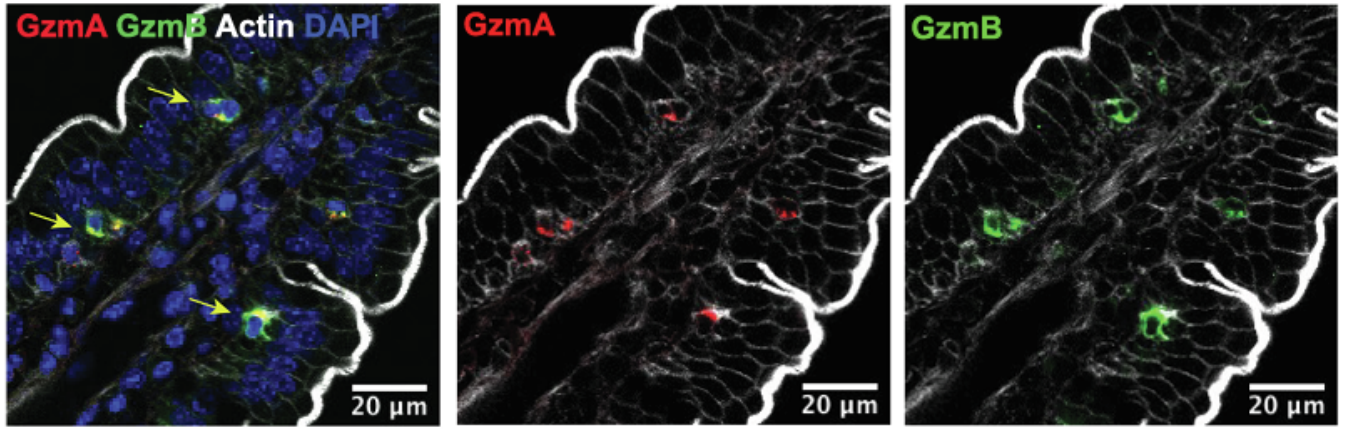
In the context of inflamed RA synovium, GzmK itself acted like a key inflan

GzmK induced synovial fibroblasts to activate pro-inflammatory pathways, including

Jonsson *et al.*, Sci Transl Med 2023

IEL Granzyme A, not granzyme B, controls *Salmonella* infection in the gut



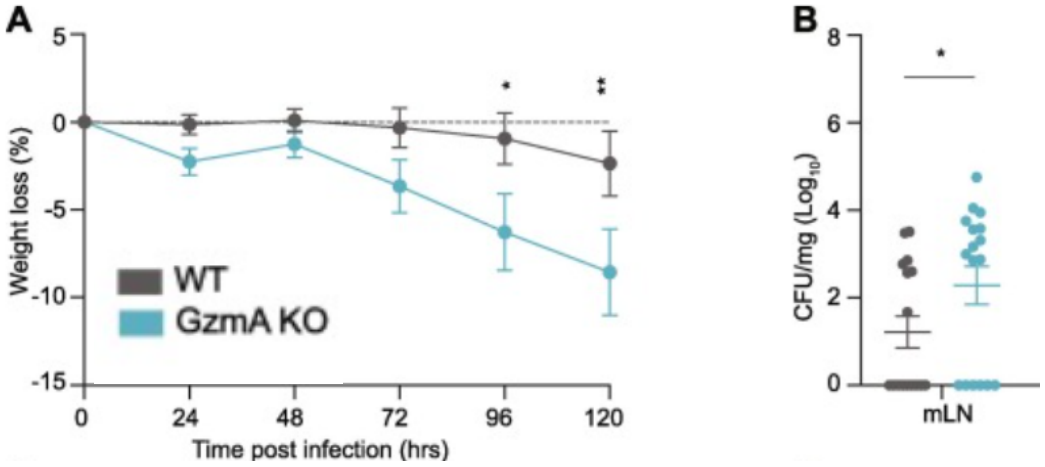


IEL constitutively express high levels of both GzmA and GzmB, with ~20 million molecules of GzmA and ~5 million molecules of GzmB per cell.[18]

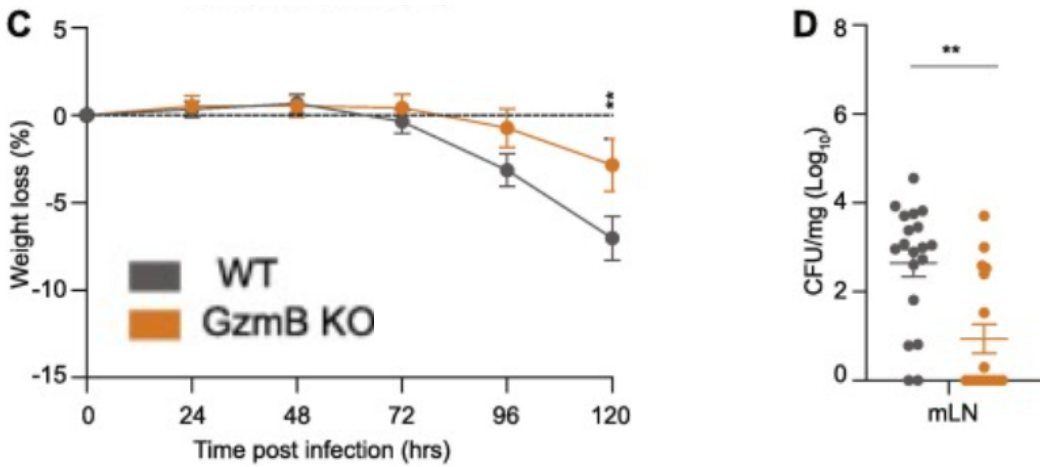
Chawla *et al.*, Mucosal Immunology 2024

IEL Granzyme A, not granzyme B, controls *Salmonella* infection in the gut

Granzyme A KO mice have increased *Salmonella* growth after oral challenge



Granzyme B KO does not affect *Salmonella* growth



IEL utilize granzymes to kill infected epithelial cells independent of perforin

We next explored the mechanisms by which GzmA/B protect the intestinal epithelium. Gzms can cleave extracellular matrix proteins and epithelial cell junction proteins[30–32], and loss of this activity may affect the intestinal barrier, thus increasing bacterial translocation. However, we did not find any difference in intestinal permeability to a small molecule, FITC-Dextran, in GzmA/B dKO mice compared to WT mice

Chawla *et al.*, Mucosal Immunology 2024

BACH2 is a transcription factor that determines T cell fate

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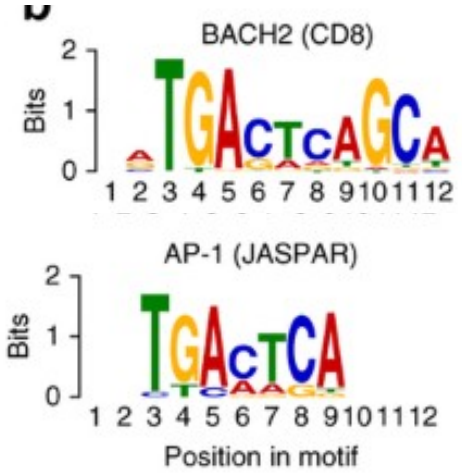
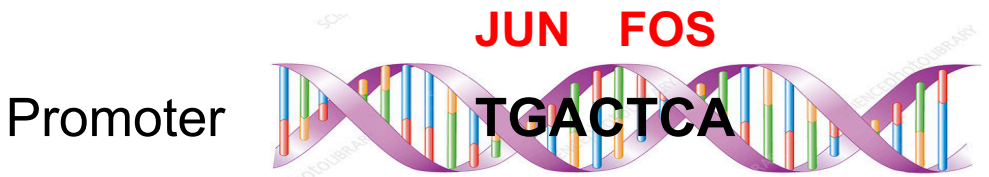
Article | Published: 09 May 2016

BACH2 regulates CD8⁺ T cell differentiation by controlling access of AP-1 factors to enhancers

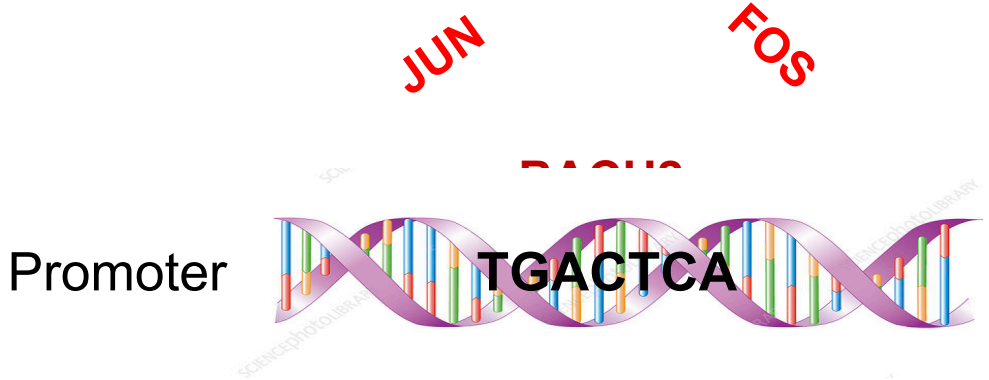
Rahul Roychoudhuri, David Clever, Peng Li, Yoshiyuki Wakabayashi, Kylie M Quinn, Christopher A Klebanoff, Yun Ji, Madhusudhanan Sukumar, Robert L Eil, Zhiya Yu, Rosanne Spolski, Douglas C Palmer, Jenny H Pan, Shashank J Patel, Derek C Macallan, Giulia Fabozzi, Han-Yu Shih, Yuka Kanno, Akihiko Muto, Jun Zhu, Luca Gattinoni, John J O'Shea, Klaus Okkenhaug, Kazuhiko Igarashi, ... Nicholas P Restifo + Show authors

Nature Immunology **17**, 851–860 (2016) | Cite this article





TCR-driven effector programs



Restrains differentiation of short-lived effector cells

Limits TCR-driven effector programs

Maintains long-lived memory

BACH2 directs activated T cells from death to long-lived memory and survival

nature immunology

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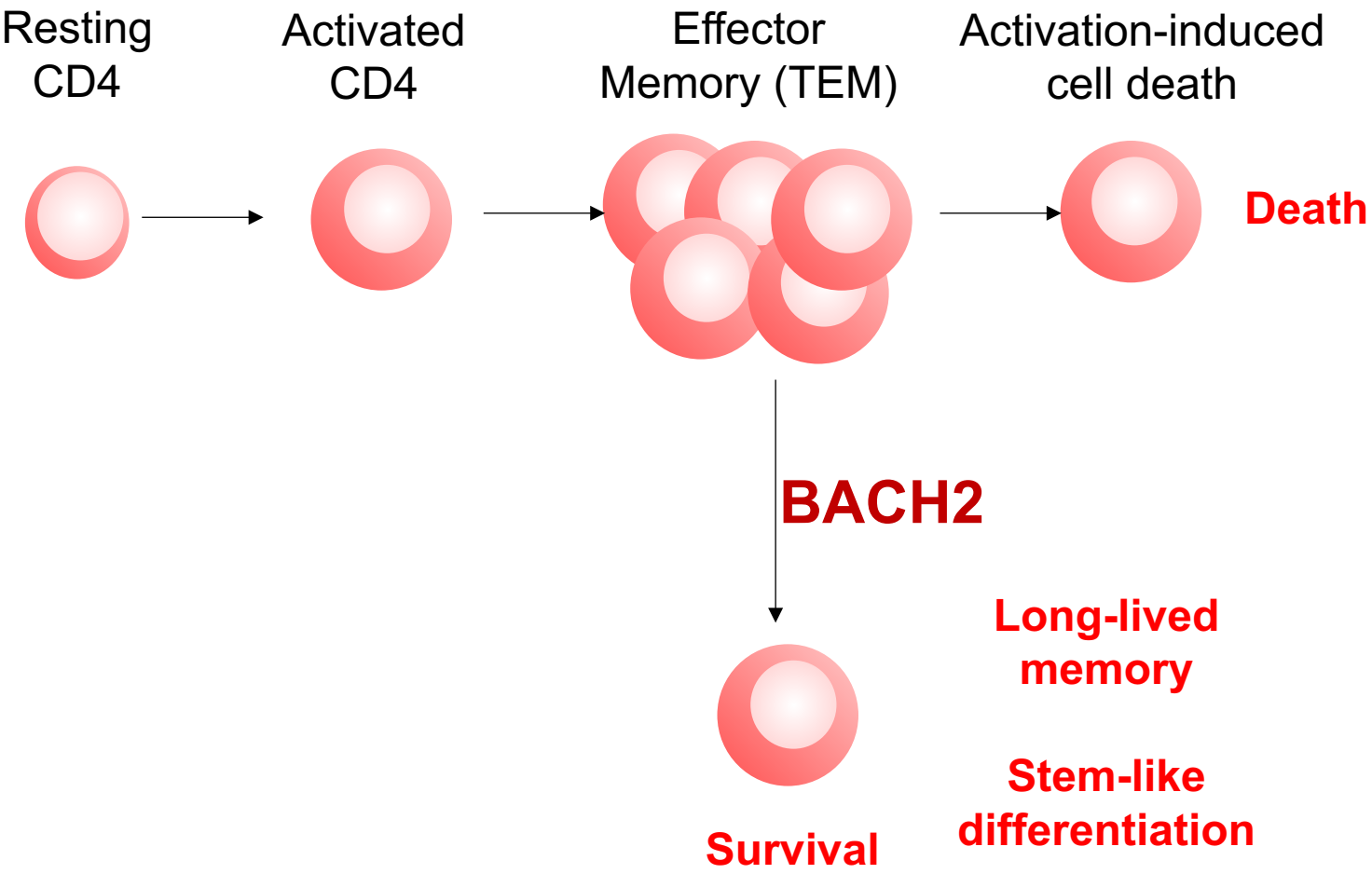
Article | Published: 11 February 2021

BACH2 enforces the transcriptional and epigenetic programs of stem-like CD8$^+$ T cells

Chen Yao ✉, Guohua Lou, Hong-Wei Sun, Ziang Zhu, Yi Sun, Zeyu Chen, Daniel Chauss, E. Ashley Moseman, Jun Cheng, Marc A. D'Antonio, Wangke Shi, Junwei Shi, Kohei Kometani, Tomohiro Kurosaki, E. John Wherry, Behdad Afzali, Luca Gattinoni, Yuwen Zhu, Dorian B. McGavern, John J. O'Shea, Pamela L. Schwartzberg & Tuoqi Wu ✉

Nature Immunology **22**, 370–380 (2021) | Cite this article



Yao *et al.*, Nature Immunology 2021

BACH2 drives tissue resident memory programs in lung and skin (but not the gut) in LCMV-infected mice

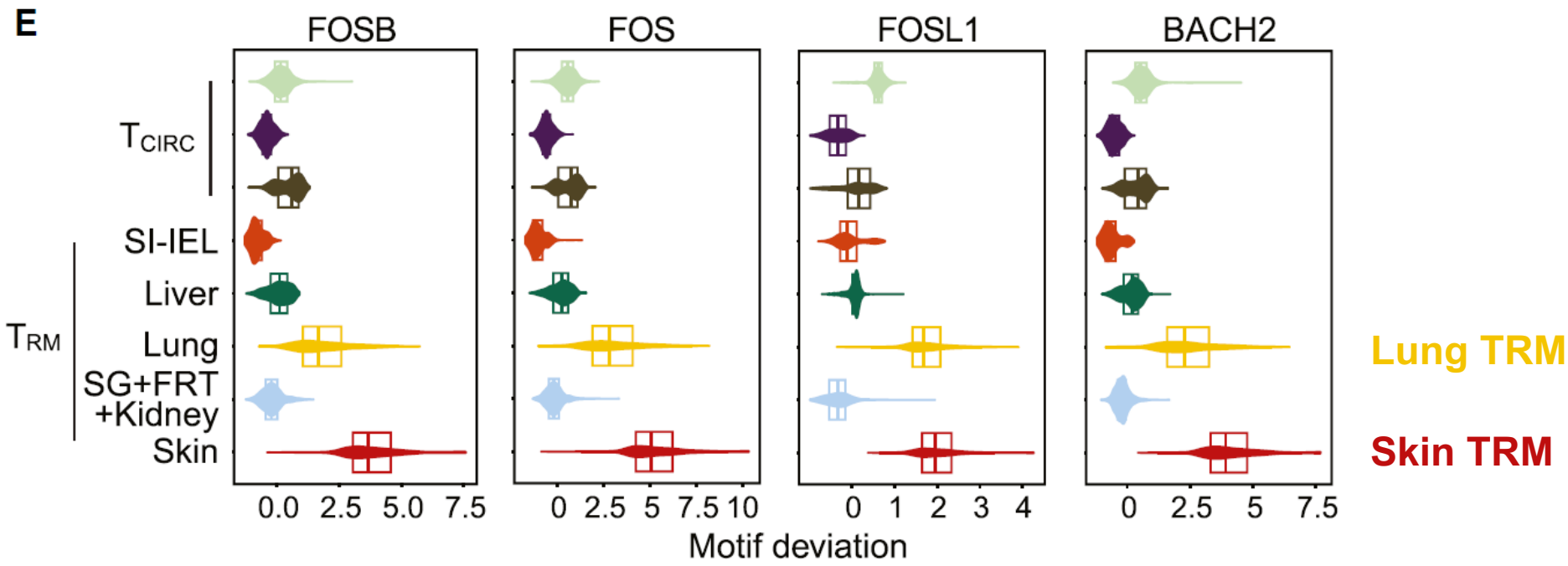
Immunity

CellPress

Resource

Distinct epigenomic landscapes underlie tissue-specific memory T cell differentiation

Frank A. Buquicchio,[1,2,6,7] Raissa Fonseca,[3,7] Patrick K. Yan,[1,2] Fangyi Wang,[1,2] Maximilien Evrard,[3] Andreas Obers,[3] Jacob C. Gutierrez,[1,2] Colin J. Raposo,[1,2] Julia A. Belk,[1,4] Bence Daniel,[1] Pirooz Zareie,[3] Kathryn E. Yost,[1] Yanyan Qi,[1] Yajie Yin,[1,2] Katherine F. Nico,[1,2] Flora M. Tierney,[1,2] Michael R. Howitt,[1,2] Caleb A. Lareau,[1,2,5,6] Ansuman T. Satpathy,[1,2,5,6,8,*] and Laura K. Mackay[3,8,9,*]



Buquicchio *et al.*, Immunity 2024