

Introduction

Baboons infected with simian immunodeficiency virus (SIV) share qualities with elite controllers of HIV (see graphic below). The mechanisms underlying elite control in baboons remain largely undefined. Our group has shown CD8+ cells, which includes CD8 T and NK cells, dampen SIV infection in baboon CD4 cells in vitro through aspects of innate immunity. Furthermore, CD8+ cell depletion in baboons in vivo elevates the frequency of activated CD4 T cells and increases SIV loads to pathogenic levels seen in rhesus macaques. Identifying the phenotype of baboon CD8+ cells involved in this observed SIV control could inform the development of novel HIV therapies. In this work, we sought to identify unconventional CD8+ cell types that expand following peak viremia in SIV-infected baboons by applying a cytometric pipeline, including algorithmic analysis.

Baboons infected with SIV display features seen in elite controllers of HIV-1, including the ability to:



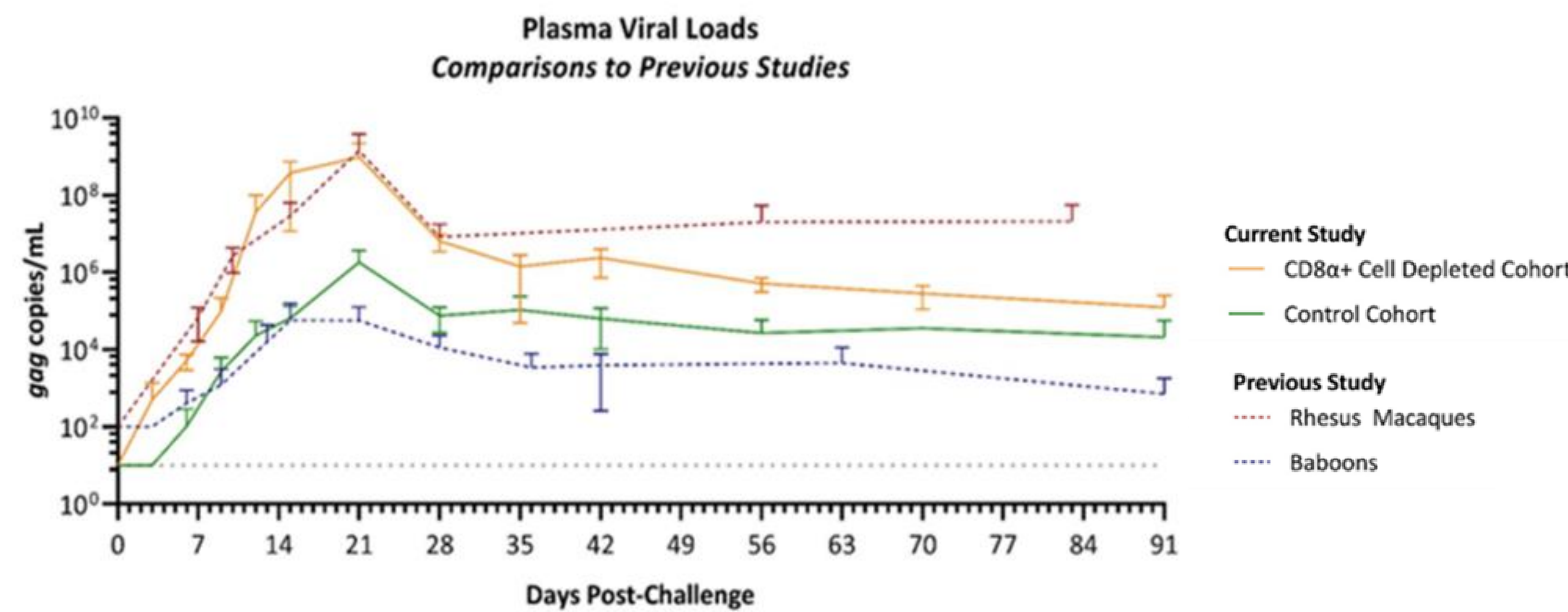
	Elite Controller	Olive Baboon
Maintain CD4 T cell counts	✓	✓
Avoid clinical disease progression	✓	✓
Suppress viral loads	✓	✓
Regulate immune cell activation	✓	✓
Mount an immune response	✓	✓

Methods

- Baboons ($n=4$) were challenged intravenously with SIVmac251.
- PBMC were stained with a 17-color panel, acquired on the BD FACSymphony™ A5 Cell Analyzer and analyzed in FlowJo™ Software.
- Technical anomalies were removed with PeacoQC¹. CD8+ cells were used for algorithmic analysis.
- We applied the machine learning workflow Tracking Responders Expanding (T-REX), which performs dimensionality reduction (tSNE), identifies areas of difference between two conditions, and clusters cells (DBSCAN) in the areas of difference².
- Marker Enrichment Modeling (MEM) was applied to clusters and used to merge clusters with similar scores (threshold of 1)³.

Results: The role of CD8+ cells in immune control of SIV

1 Plasma viral loads in CD8 α + cell-depleted baboons



2 Summary of CD8-depletion studies in non-human primates infected with SIV

Non-natural host



Ex: *rhesus macaque*

Marked increase in viremia following CD8+ cell depletion⁴
Viral inhibition by CD8+ cells from SIV-exposed macaques

Strong role for CD8 cells in adaptive immune control

Natural host



Ex: *sooty mangabey*

No major changes in viremia during the period of CD8+ cell deficiency⁵

Limited role for CD8 cells in immune control

Baboons

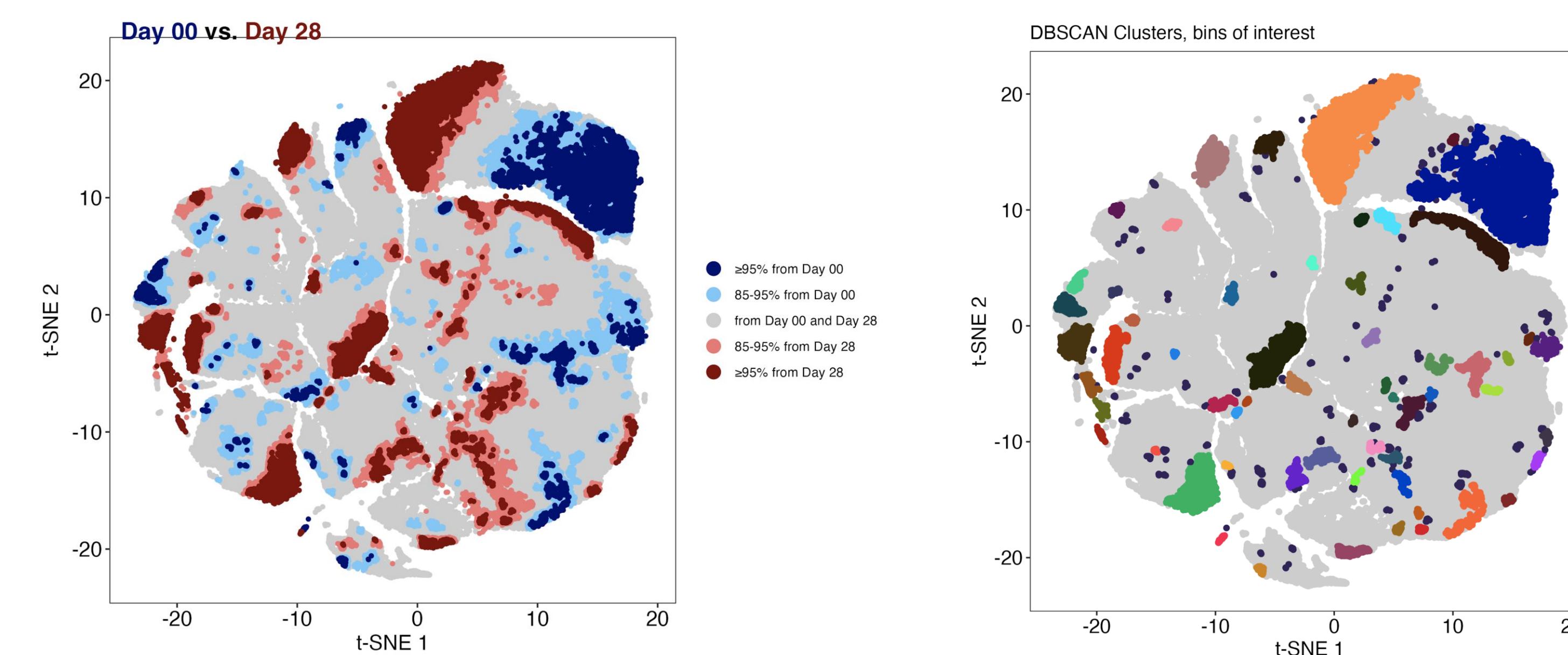


Marked increase in viremia following CD8+ cell depletion
Viral inhibition by CD8+ cells from SIV-unexposed baboons⁵

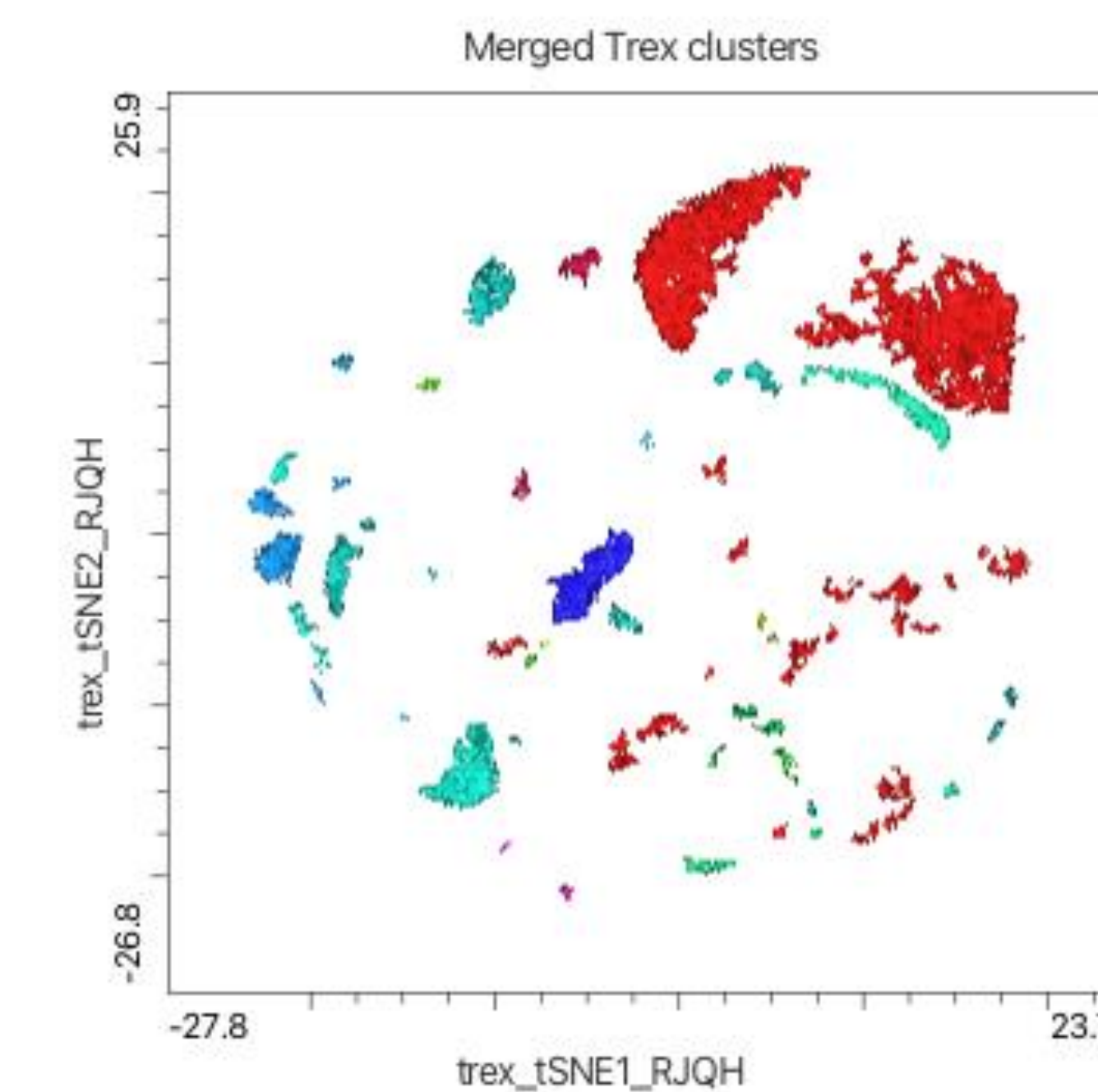
Strong role for CD8 cells in innate immune control

Results: Tracking changes in the CD8+ compartment following SIV infection

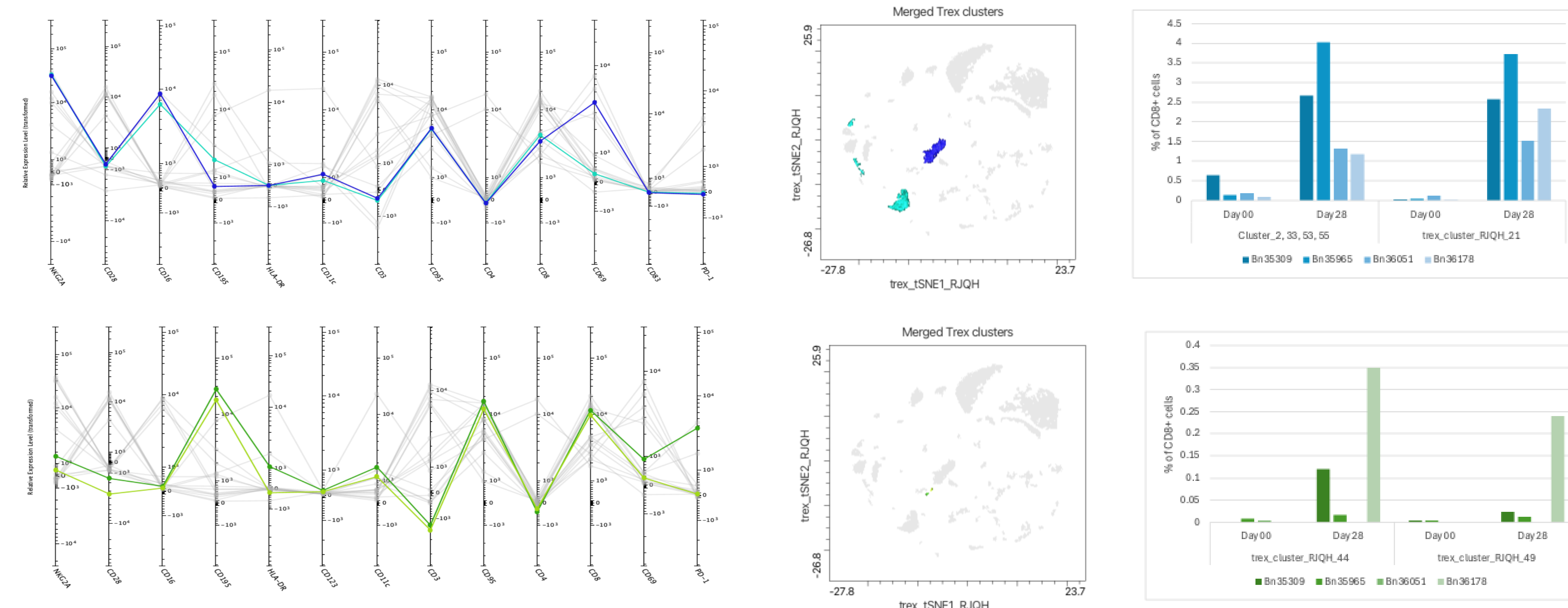
3 Areas of difference and clusters identified by T-REX workflow



4 Final clusters after merging on MEM scores



5 Phenotype and frequency of NK8-like clusters



Summary

- Following peak viremia, we observed an increase in the frequency of four clusters of NK8-like cells, identified as CD3⁺CD14⁻CD20⁻CD8^{lo}CD95⁺.
- The clusters could be subdivided as CD16⁺NKG2a⁺CD69^{+/+} and CD195⁺PD-1^{lo}.
- Though elevated from 0 dpi, the frequency of these clusters at 28 dpi was <4% of CD8+ cells; CD195⁺PD-1^{lo} NK8-like clusters were as low as 0.01% at 28 dpi.
- The rarer NK8-like clusters had not previously been identified by manual gating and Louvain-based clustering; their identification in the T-REX workflow has shed light on a possible role for NK8 cells in early control of viral loads, working in synergy, but perhaps sequentially, with CD8 T cells to dampen viremia.

References

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