

Differential Gene Expression Analysis

Bobby Sims EE282 F24 Final Project

Background

- Dataset from Pohar et al study investigating therapeutic potential of adoptive transfer of engineered anti-inflammatory T regulatory cells for treatment of auto immune inflammatory conditions in murine Encephalitis model
- For non engineered T-cells: Baseline measure of endogenous differential gene expression between conventional and regulatory CD4+ T-cells in health and in response to induced inflammation of the central nervous system
- GEO Omnibus ID: GSE 164460

Groups

- GEO Omnibus ID: GSE 164460
- Conventional T cells (Tconv)
- Regulatory T-cells (Treg)
- Normal (Naïve)
- Disease (EAE)
- Microarray Data
- 20 Samples 6 phenotypes
- 45101 Genes Observed

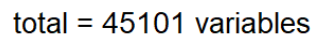
Treg naïve spleen n=3	Tconv naïve spleen n=3
Treg EAE spleen n=3	Tconv EAE spleen n=3
Treg EAE CNS n=3	Tconv CNS n=4

Comparisons Rationale

- Treg vs Tconv Naïve (spleen) baseline homeostatic expression profiles of both (Control)
- Treg vs Tconv EAE (spleen) expression profiles of both at disease peak compared to each other
- Treg Naive vs Treg EAE and Tconv Naïve vs Tconv EAE
- Treg vs Tconv EAE (CNS)
- Compare gene expression among Tregs and Tconv in health and inflammatory state
- Compare expression profiles of Tregs between physiological niches i.e. (EAE in CNS vs Spleen) at disease peak

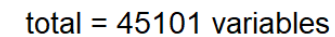
Top 5 up & down genes highlighted

Top 5 up & down genes highlighted



Top 5 up & down genes highlighted

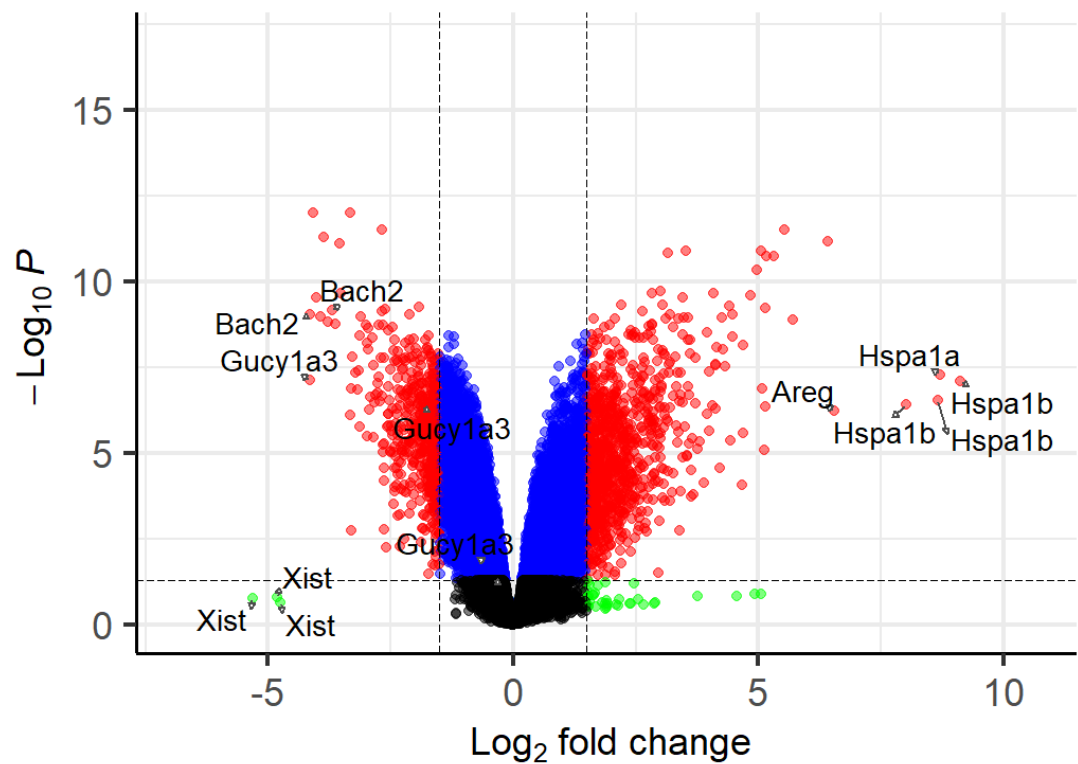
Top 5 up & down genes highlighted



TregEAE_CNS_vs_TregEAE_Sp

Top 5 up & down genes highlighted

● NS ● Log₂ FC ● p-value ● p-value and log₂ FC

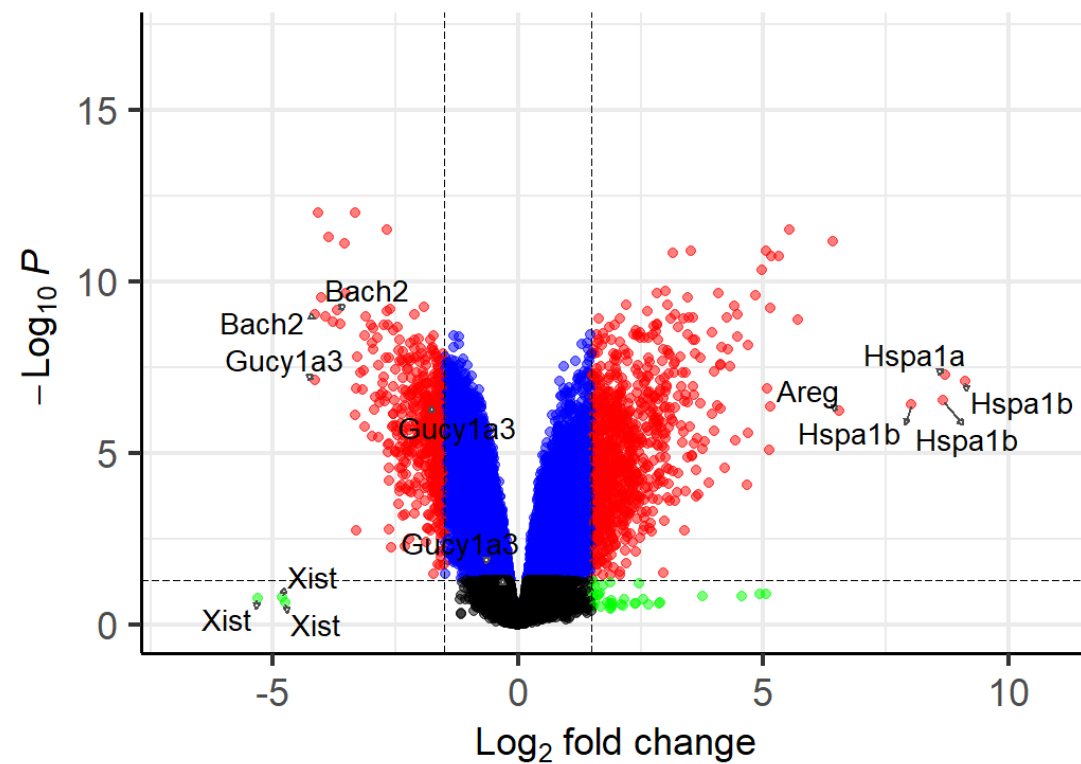


total = 45101 variables

TregEAE_CNS_vs_TregN_Sp

Top 5 up & down genes highlighted

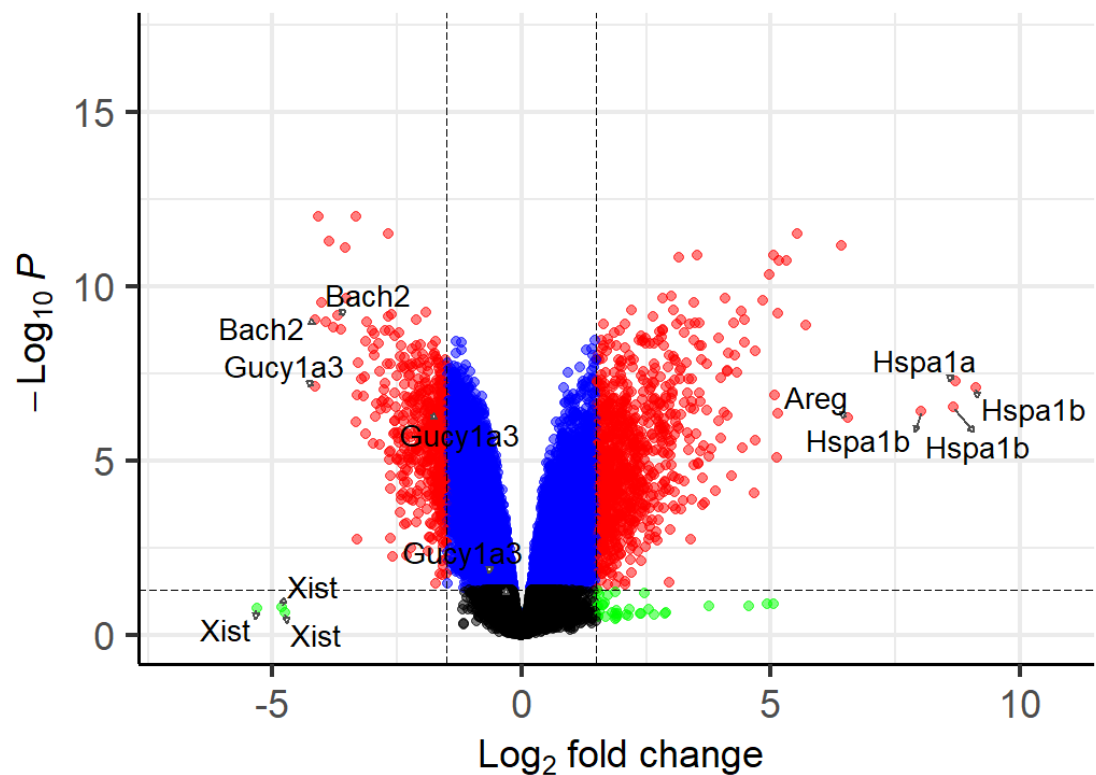
● NS ● Log₂ FC ● p-value ● p-value and log₂ FC



total = 45101 variables

Top 5 up & down genes highlighted

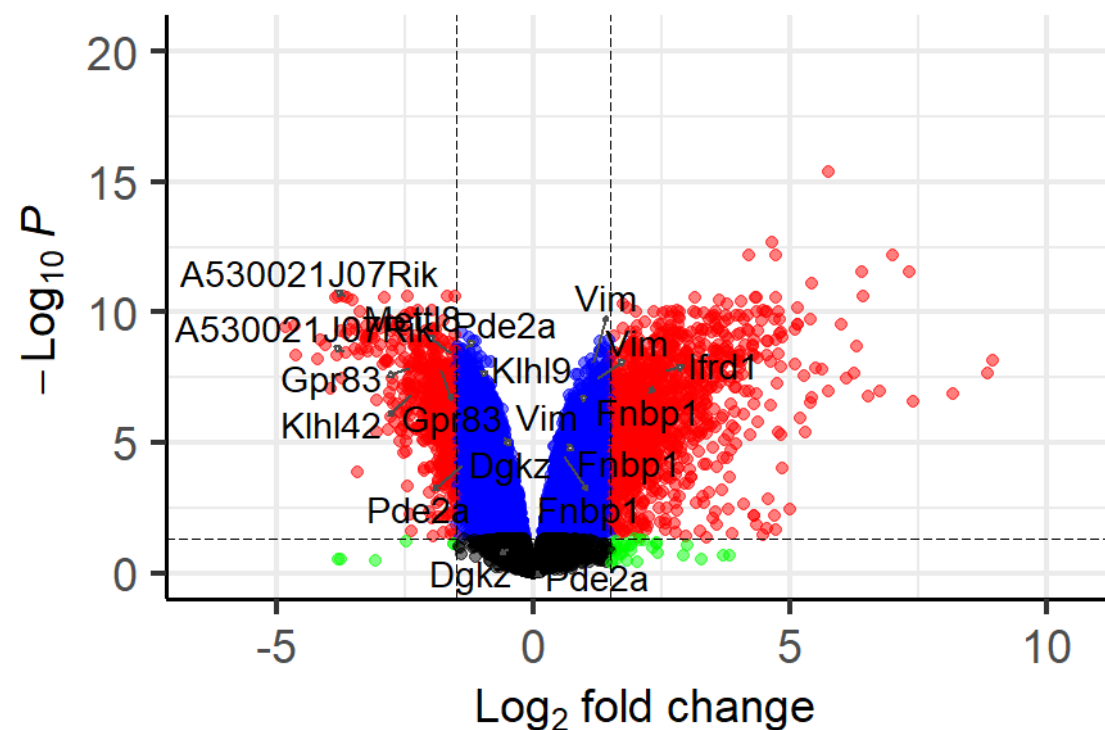
● NS ● \log_2 FC ● p-value ● p-value and \log_2 FC



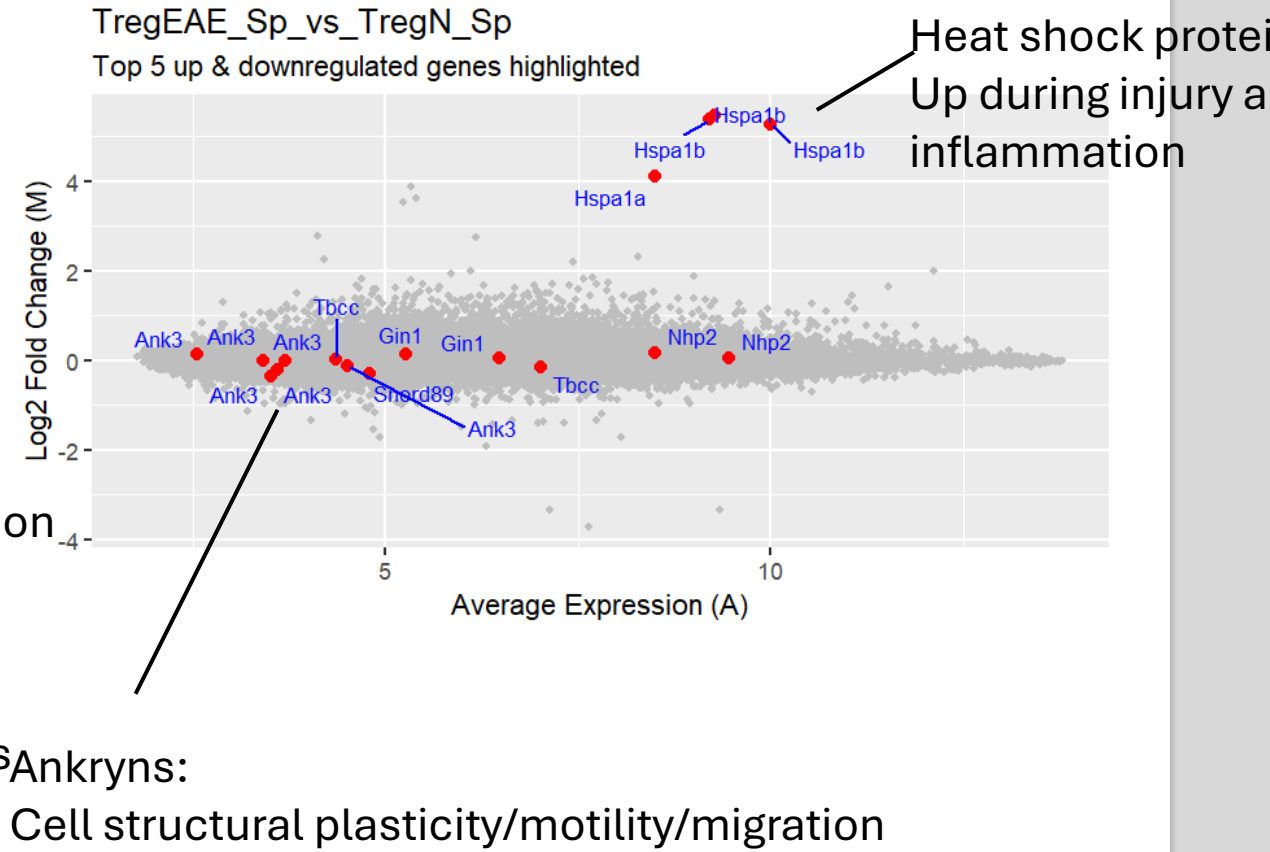
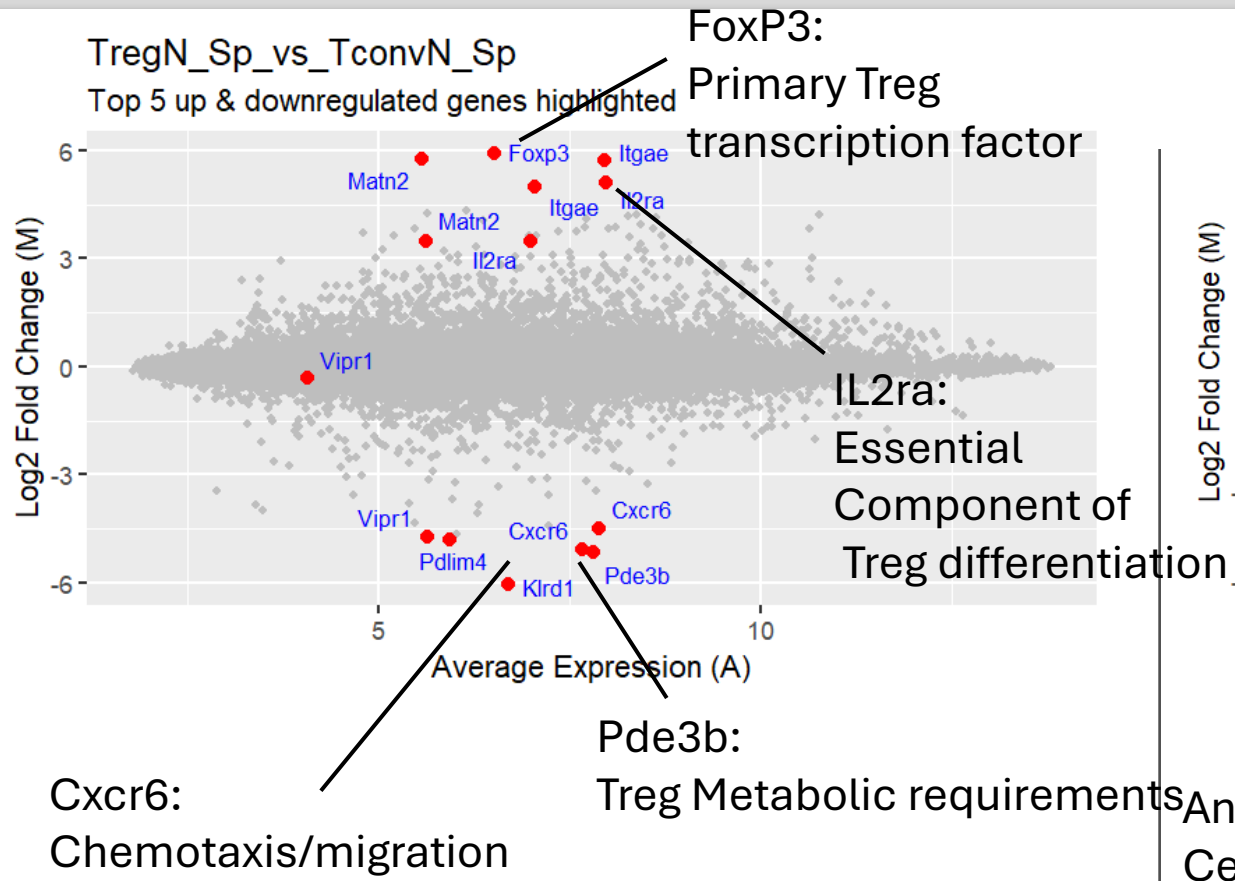
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Top 5 up & down genes highlighted

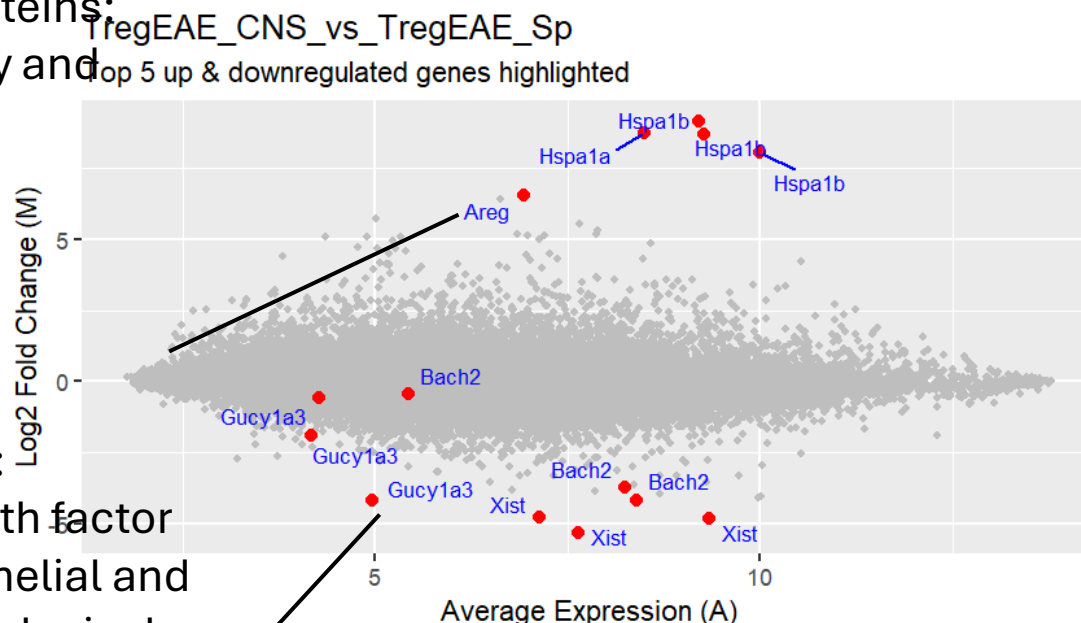
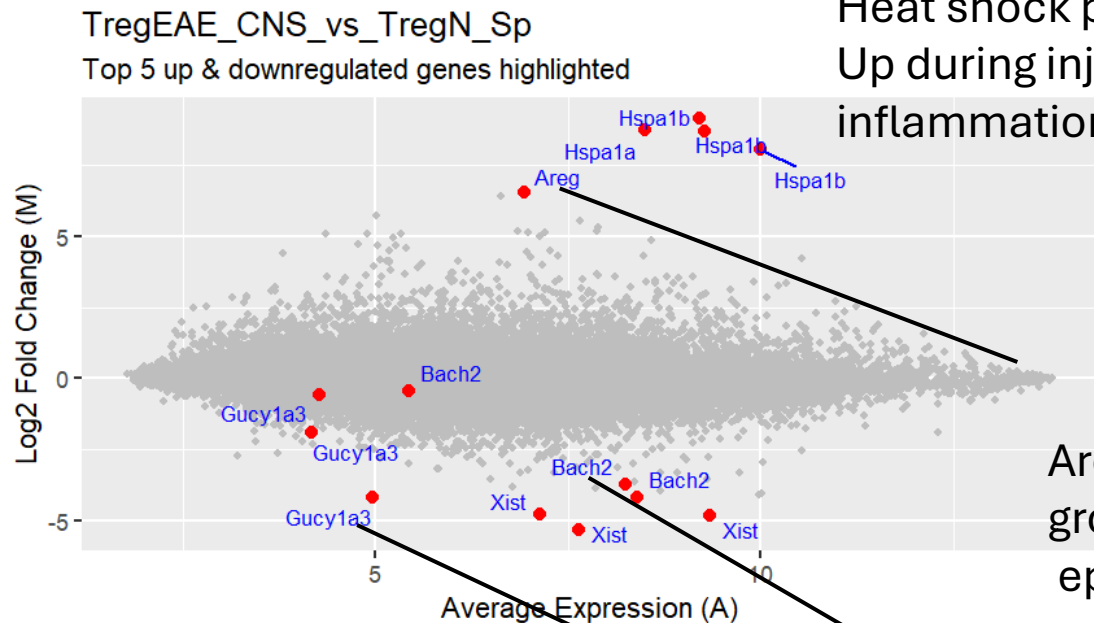
● NS ● \log_2 FC ● p-value ● p-value and \log_2 FC



total = 45101 variables



Heat shock proteins:
Up during injury and
inflammation



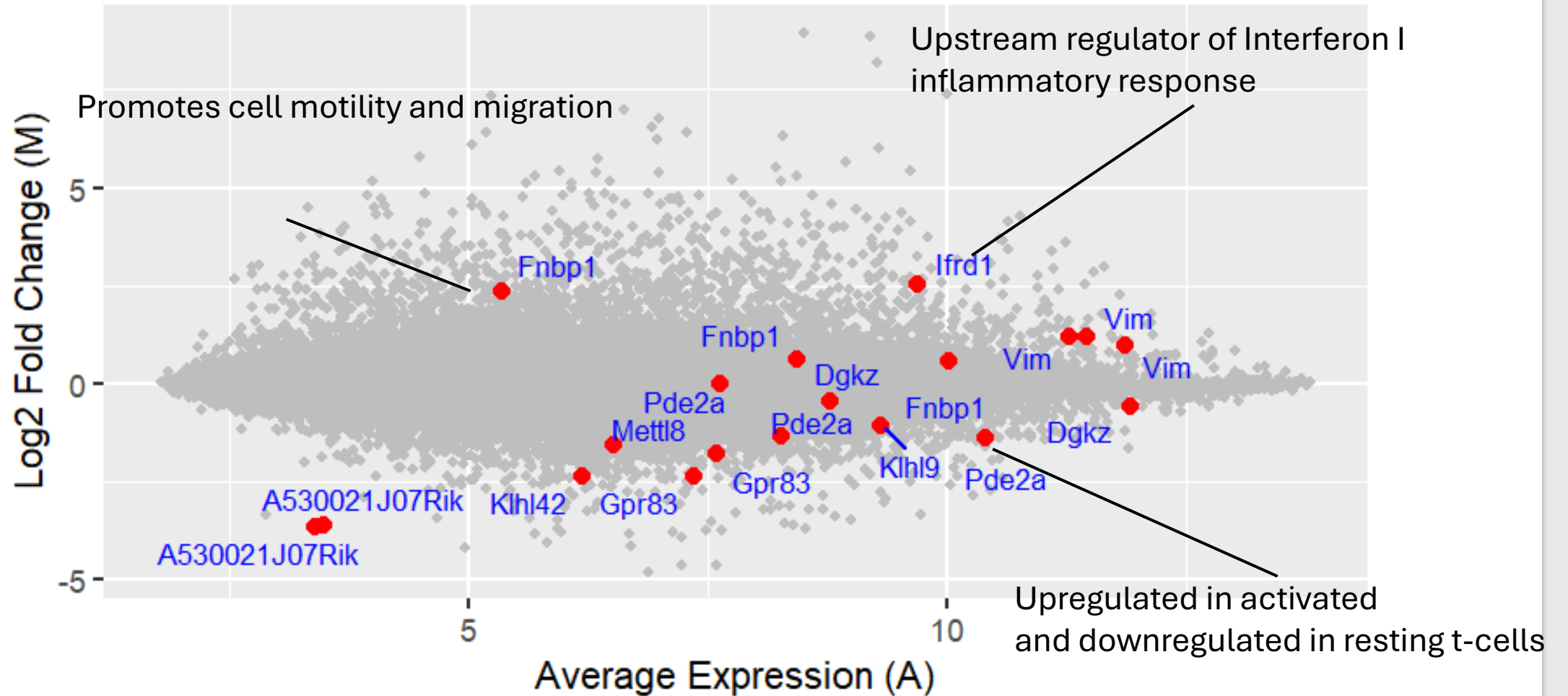
Areg:
growth factor
epithelial and
neurological
tissues

Bach2: Downregulated in peripheral Tregs

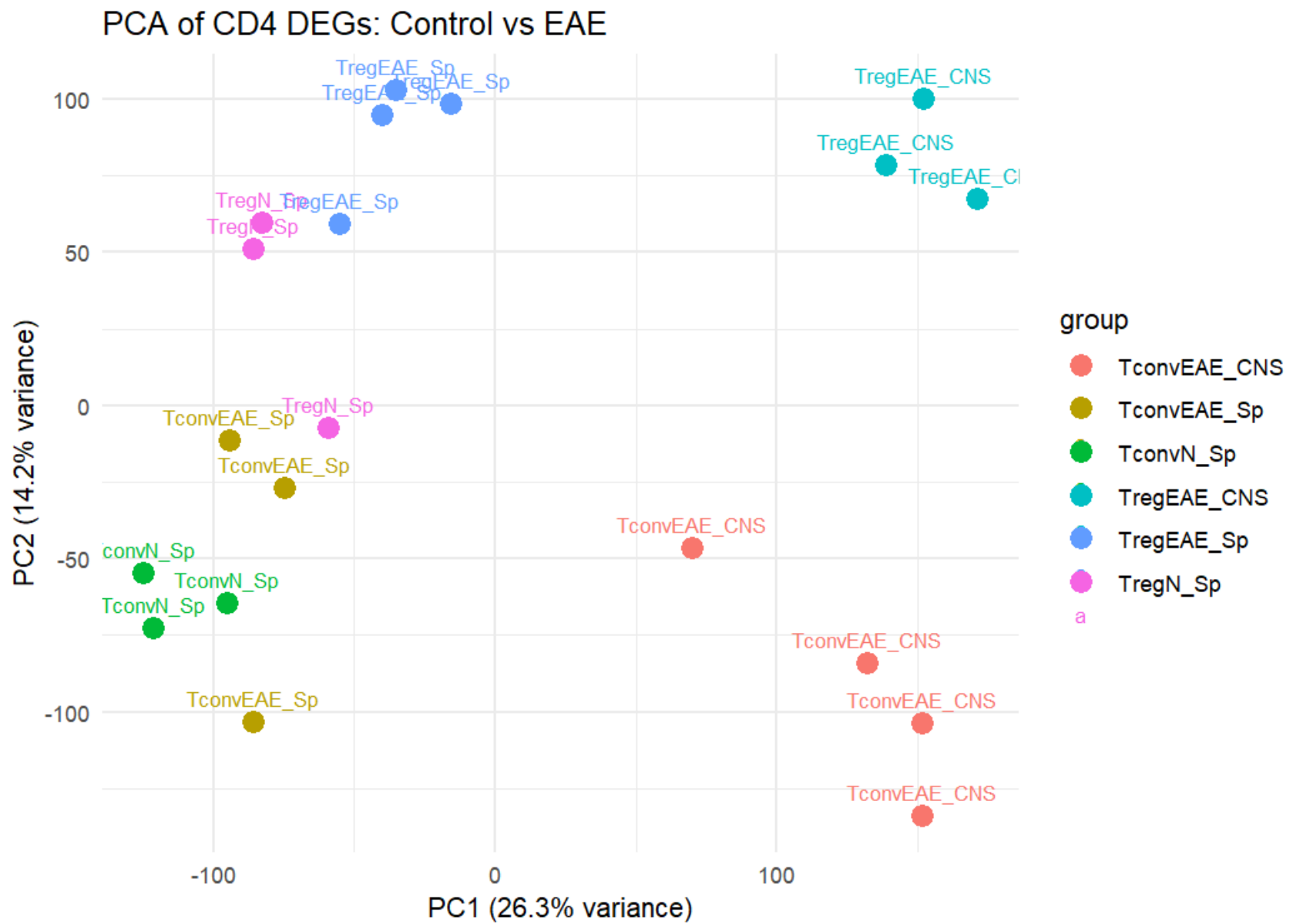
Gucy1a3:
Promotes NO pathway and vasodilation

TconvEAE_CNS_vs_TconvN_Sp

Top 5 up & downregulated genes highlighted

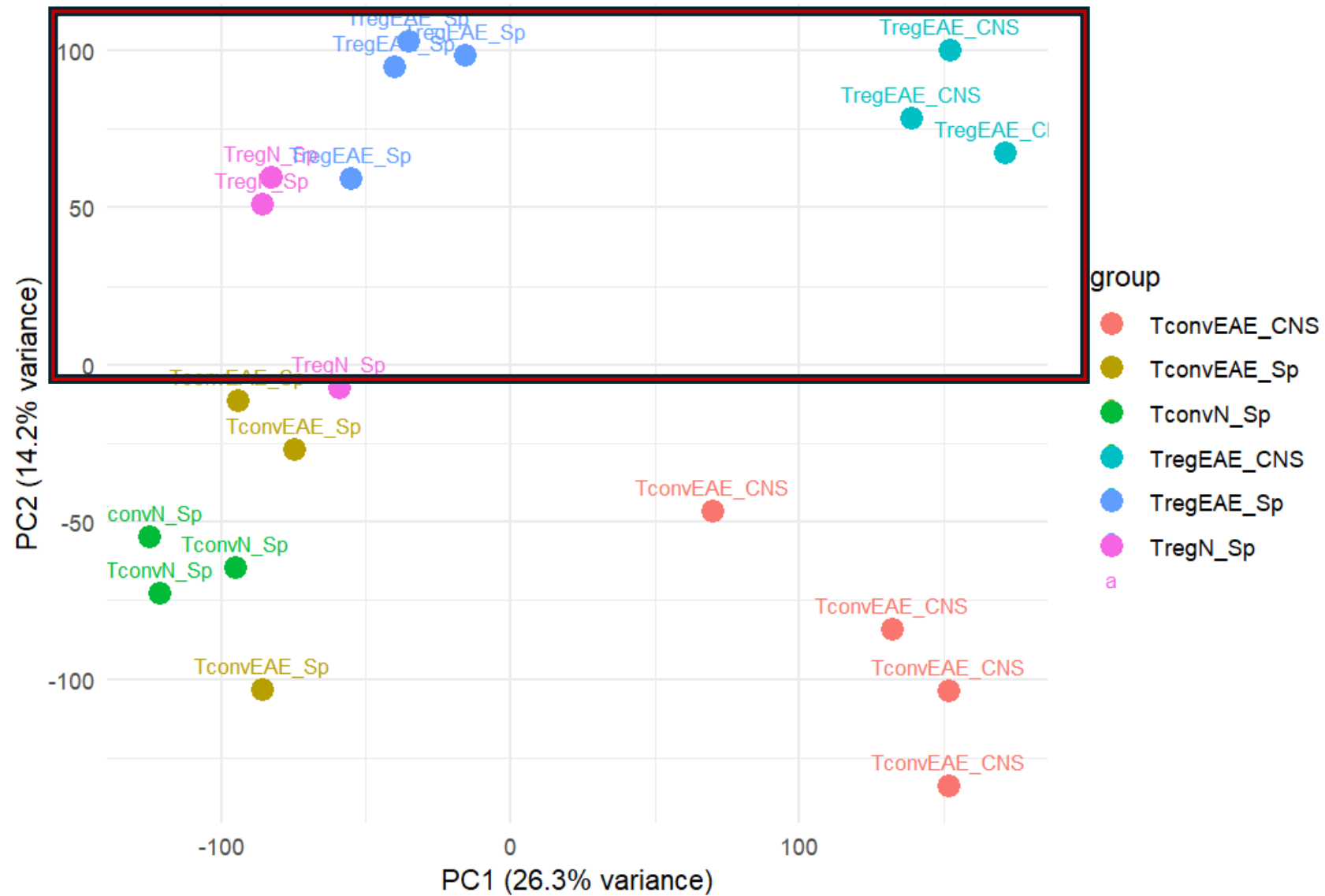


PCA
Of all 45101 genes
across 6 T cell
phenotypes,
Control and EAE



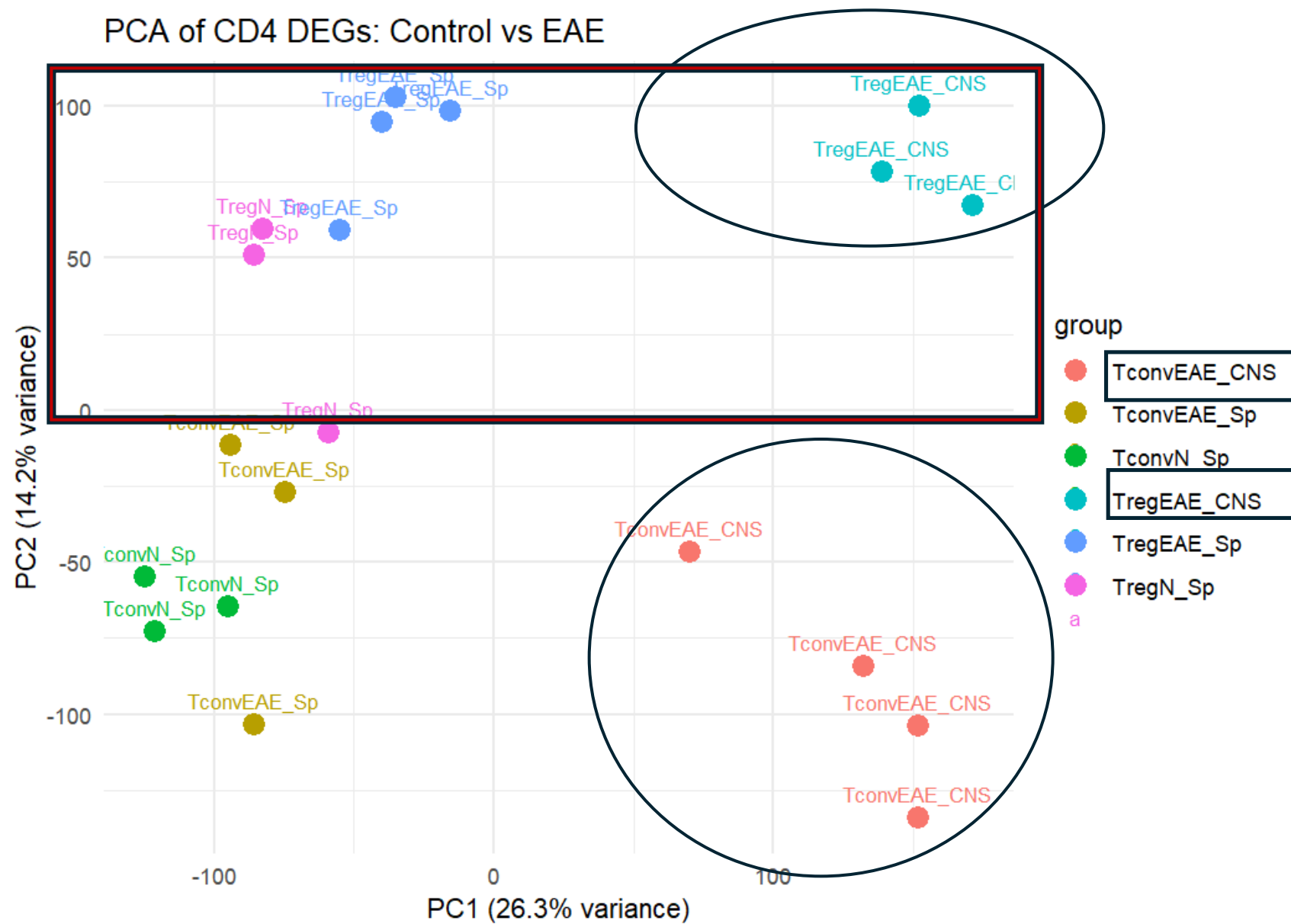
PCA of CD4 DEGs: Control vs EAE

Tregs



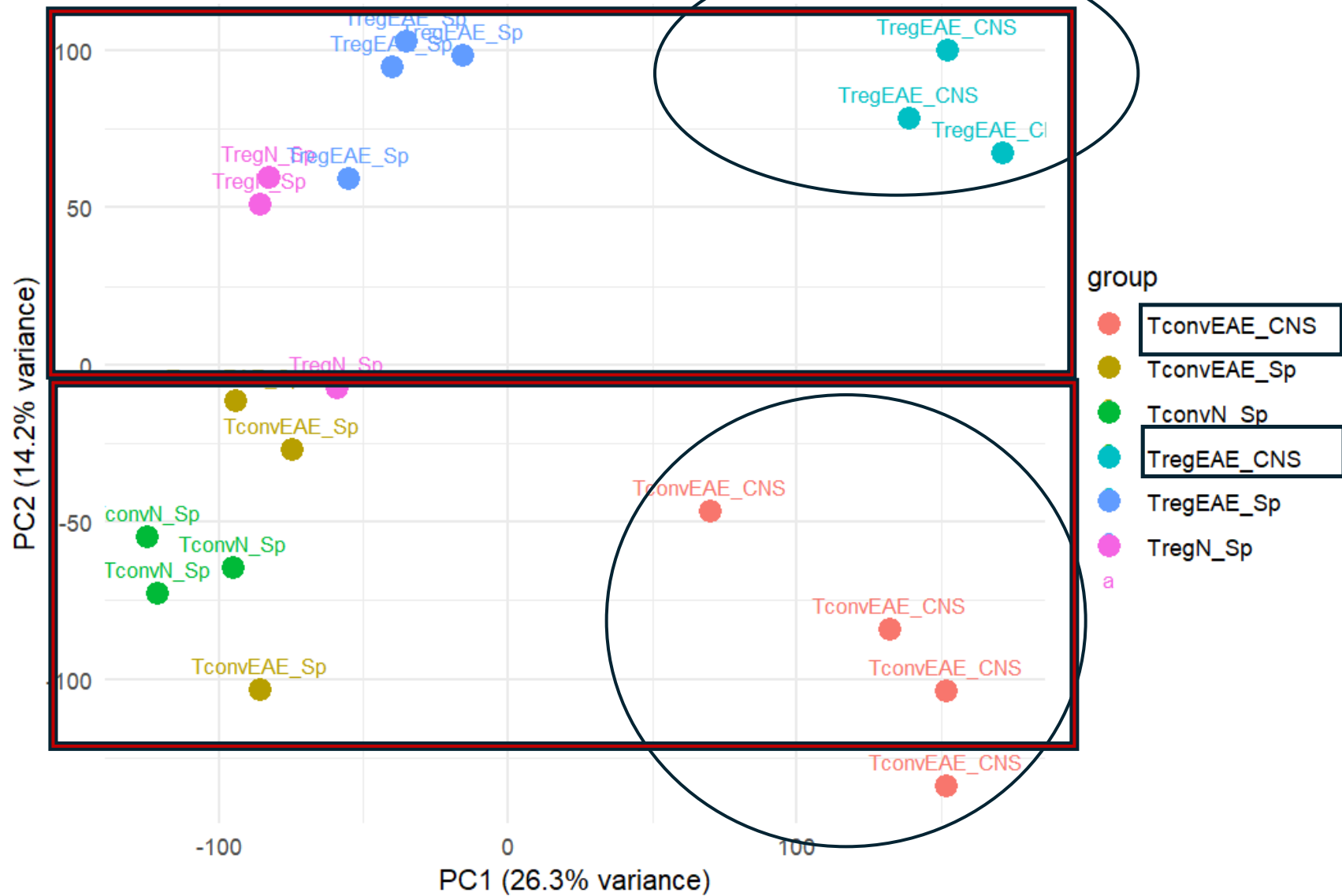
PCA of CD4 DEGs: Control vs EAE

Tregs



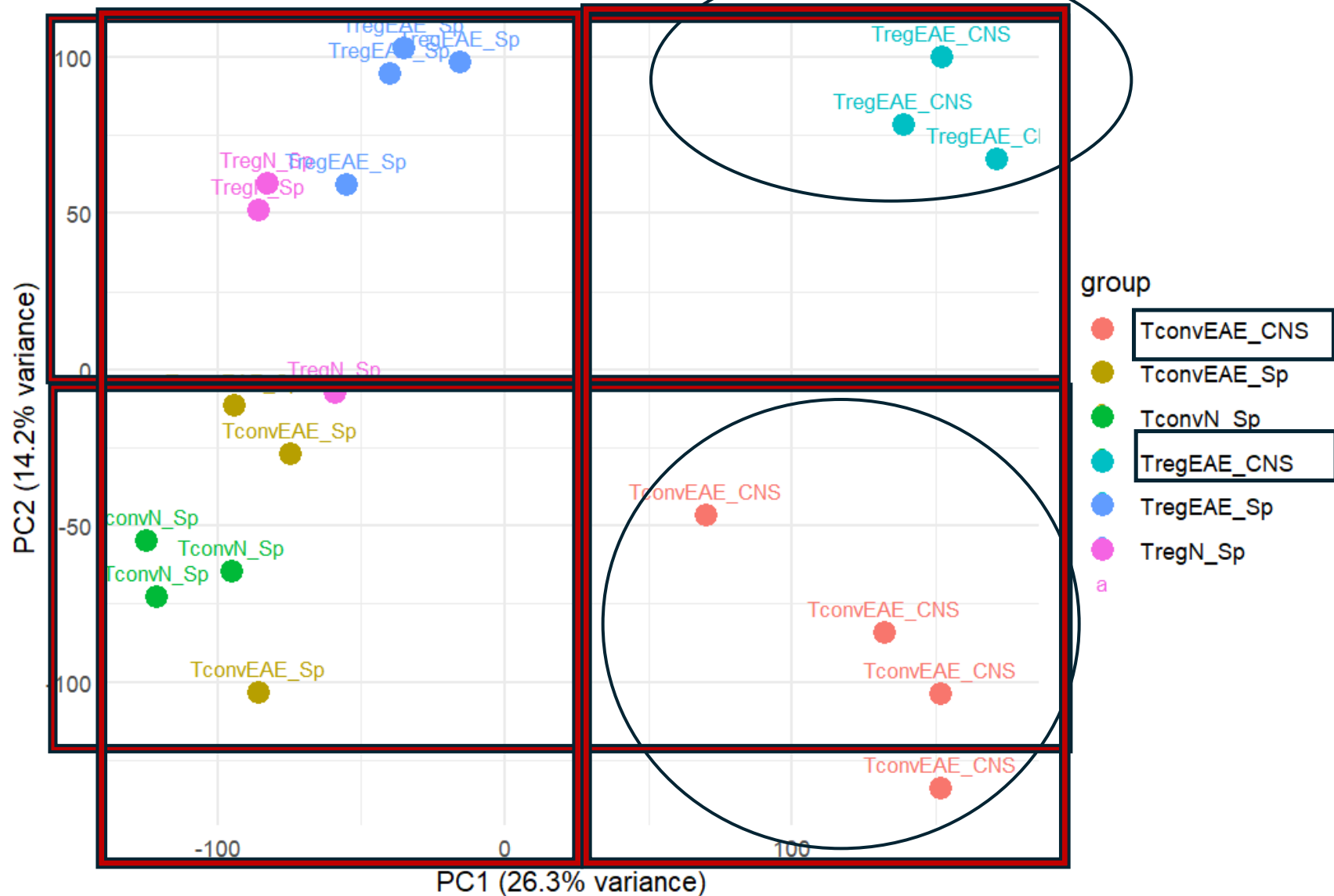
PCA of CD4 DEGs: Control vs EAE

Tregs



PCA of CD4 DEGs: Control vs EAE

Tregs

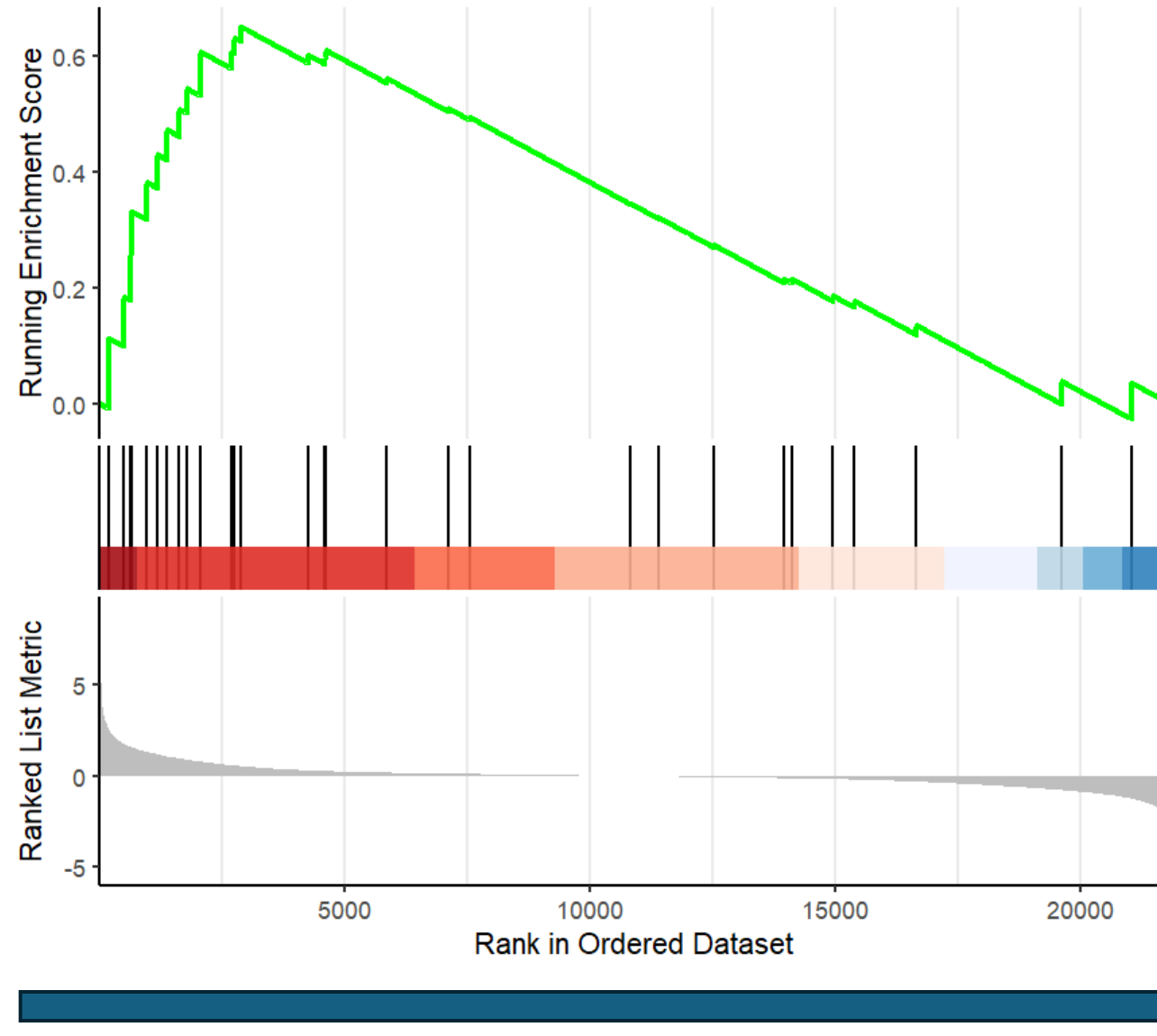


Spleen

Central Nervous System

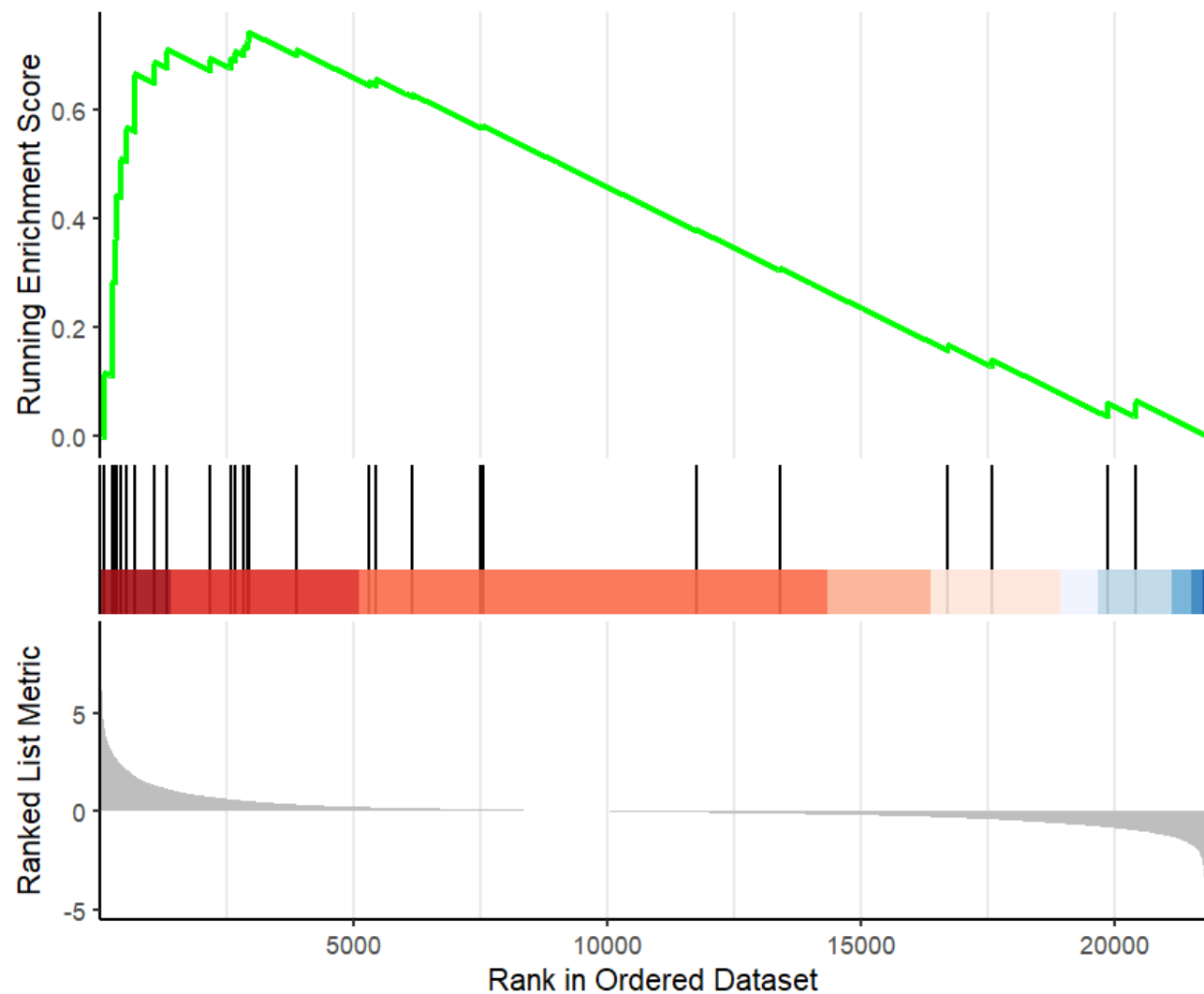
GSEA Murine Inflammatory Pathway

DGE of Tregs from EAE CNS vs Naïve Splenic Tregs



GSEA Murine Inflammatory Pathway

DGE of Tconv from EAE CNS vs Naïve Splenic Tconv



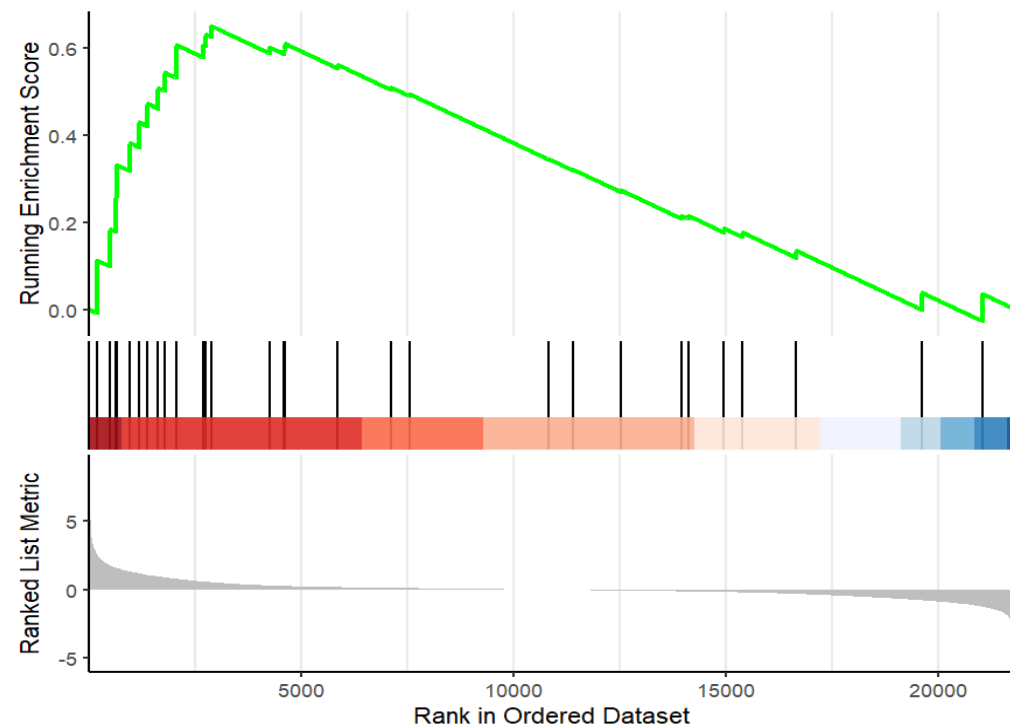
Gene set enrichment analysis (GSEA)

- How differential gene expression among observed groups align with canonical functional expression pathway?
- Groups used (only 2 contrasts from study)
 - Tregs: CNS EAE (Disease focal point) vs Spleen (Naïve, Healthy)
vs Murine Inflammatory Response Pathway
 - Tconv: CNS EAE (Disease focal point) vs Spleen (Naïve, Healthy)
vs Murine Inflammatory Response Pathway

[WP_INFLAMMATORY_RESPONSE_PATHWAY](#)

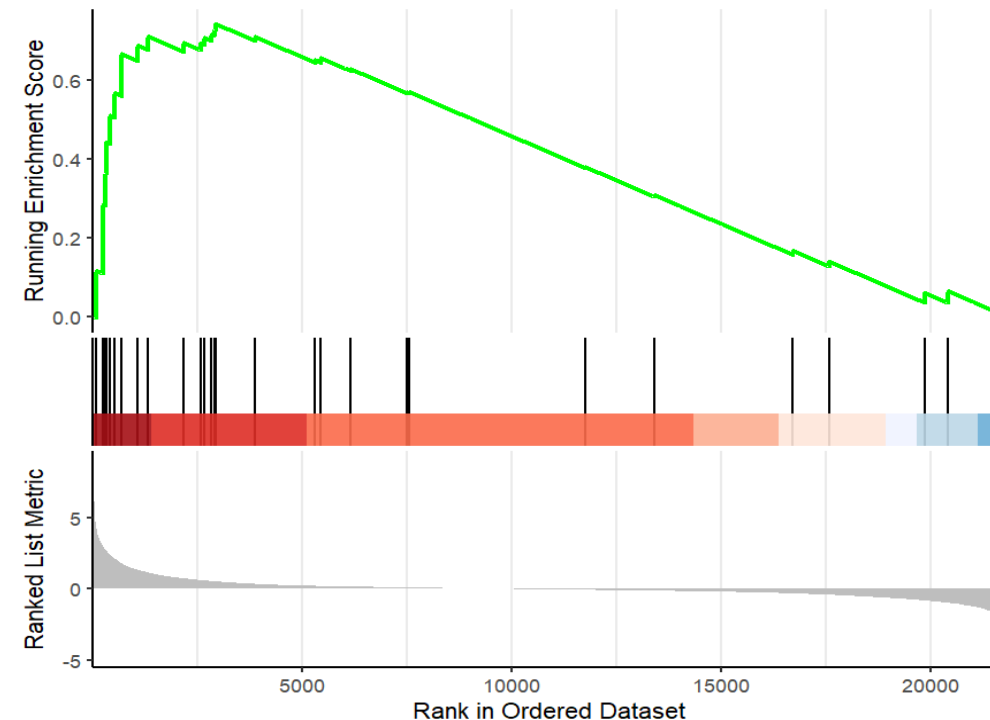
GSEA Molecular signature database

GSEA Murine Inflammatory Pathway
DGE of Tregs from EAE CNS vs Naïve Splenic Tregs



Enrichment Score	Normalized Enrichment Score	P-value adjusted
0.6509818	1.750969	0.001945999

GSEA Murine Inflammatory Pathway
DGE of Tconv from EAE CNS vs Naïve Splenic Tconv



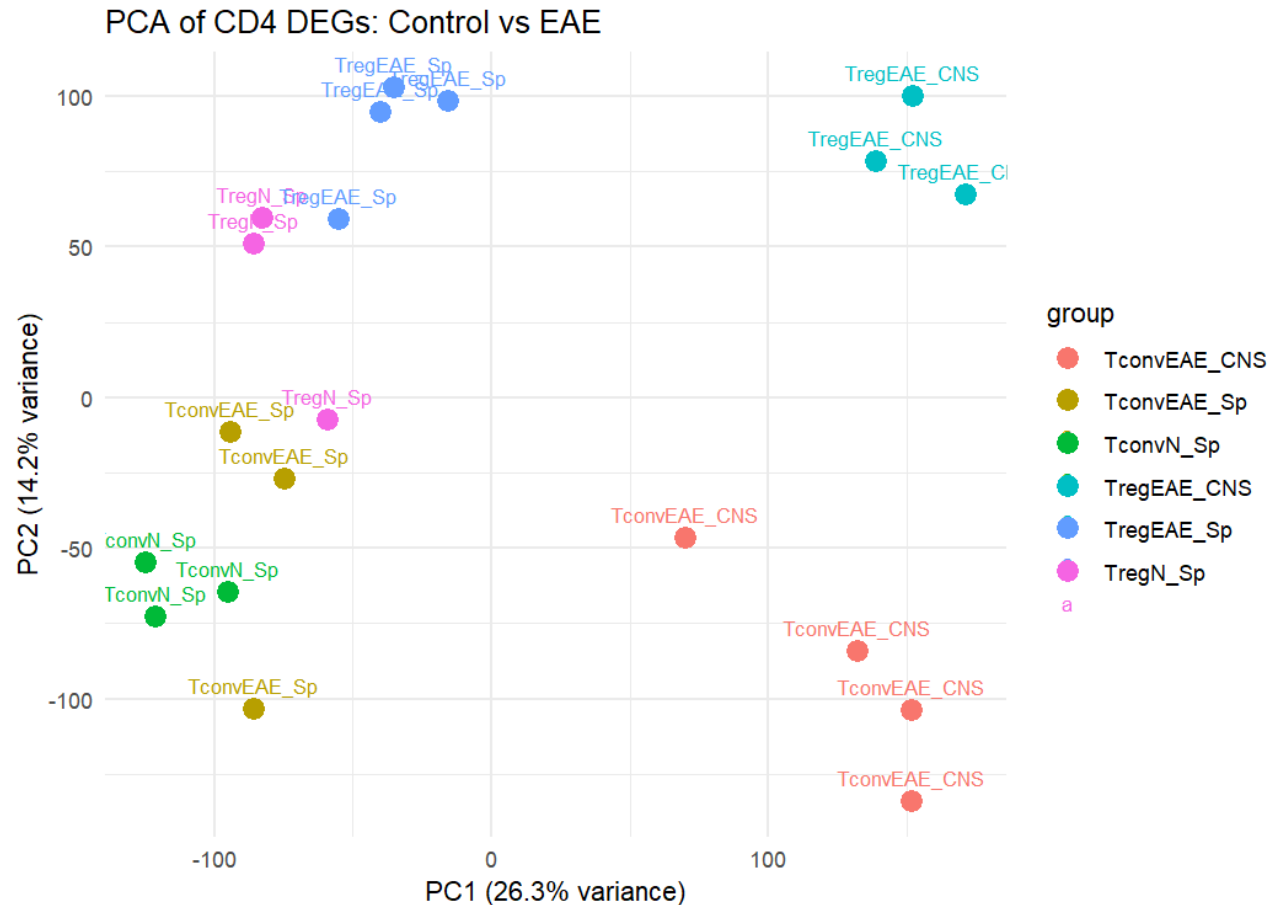
Enrichment Score	Normalized Enrichment Score	P-value adjusted
0.7439637	1.954822	0.0001704259

Pro inflammatory pathway more enriched in Tconv

Analysis and summary DEGs : Volcano and MA Plot

- Treg upregulation of canonical regulatory genes vs Tconv
- Downregulation of motility and migration genes in splenic Tregs irrespective of inflammation status
- Inflammation at disease focal point: CNS Tregs exclusively upregulate epithelial and neurological tissue regeneration factor AREG

Analysis and summary PCA



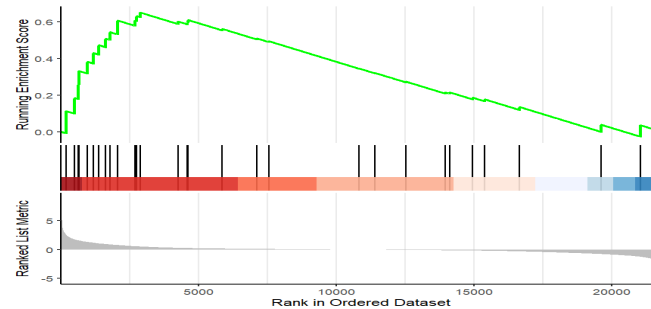
- PC1 appears to indicate CNS
- PC2 appears to indicate Treg identity
- Clustering trends
- Q1: Splenic Tregs Naïve and EAE
- Q2: CNS EAE Tregs
- Q3: Splenic Tconv Naïve and EAE
- Q4: CNS EAE Tconv
- T-cells that take up residency in spleen and CNS have distinct expression profiles
- Greater similarity between T-cell classes than tissue type and disease state.
- Clustering hierarchy
- 1) Class: Treg vs Tconv
- 2) Tissue residency
- 3) Disease state

Analysis and Summary: GSEA

- Vs canonical murine inflammatory pathway: greater enrichment in Tconv than Tregs at focal point of infection (CNS)

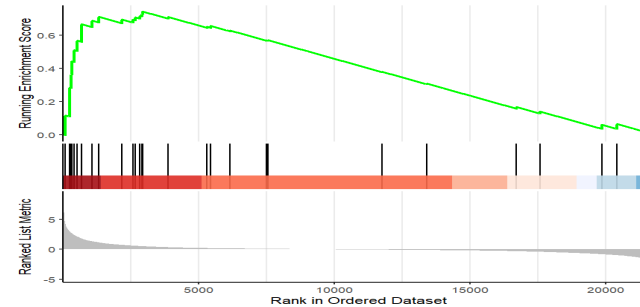
GSEA Murine
Inflammatory Pathway

DGE of Tregs from EAE CNS vs
Naïve Splenic Tregs



Enrichment Score	Normalized Enrichment Score	P-value adjusted
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DGE of Tconv from EAE CNS vs Naïve Splenic
Tconv



Enrichment Score	Normalized Enrichment Score	P-value adjusted
0.7439637	1.954822	0.0001704259

Reference

- Pohar, J., O'Connor, R., Manfroi, B., El-Behi, M., Jouneau, L., Boudinot, P., Bunse, M., Uckert, W., Luka, M., Ménager, M., Liblau, R., Anderton, S. M., & Fillatreau, S. (2022). Antigen receptor-engineered Tregs inhibit CNS autoimmunity in cell therapy using nonredundant immune mechanisms in mice. *European journal of immunology*, 52(8), 1335–1349.
<https://doi.org/10.1002/eji.202249845>