

# **Immunological Atlas of Pancreatic Lymph Nodes in Type 1 Diabetes**

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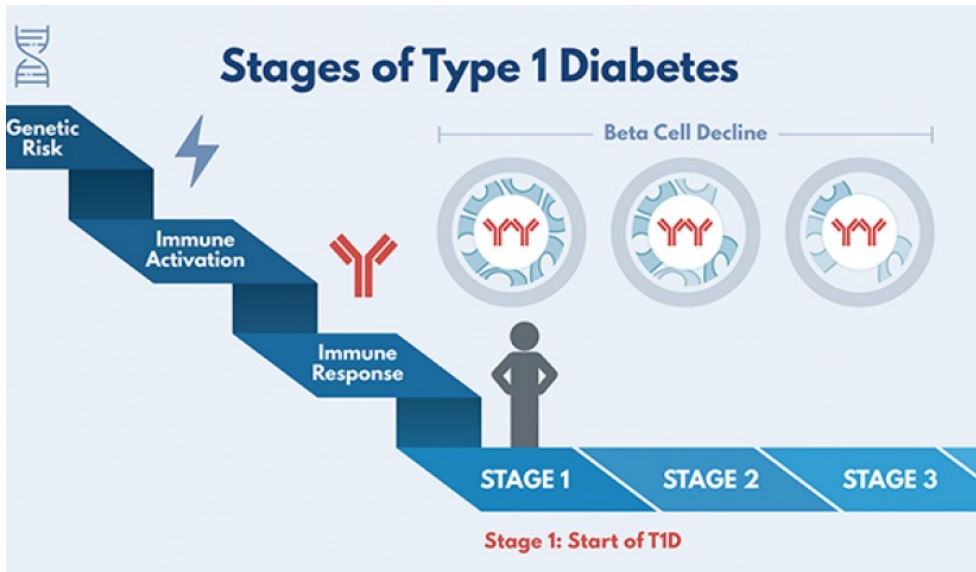
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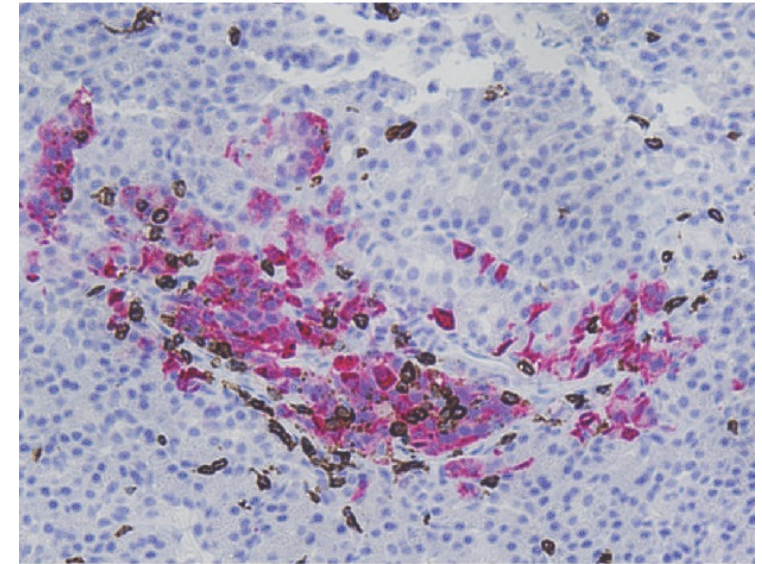
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# T1D onset has multiple stages over time



- Immune activation
- Immune attack of  $\beta$  cells
- Development of single autoantibody (AAb)

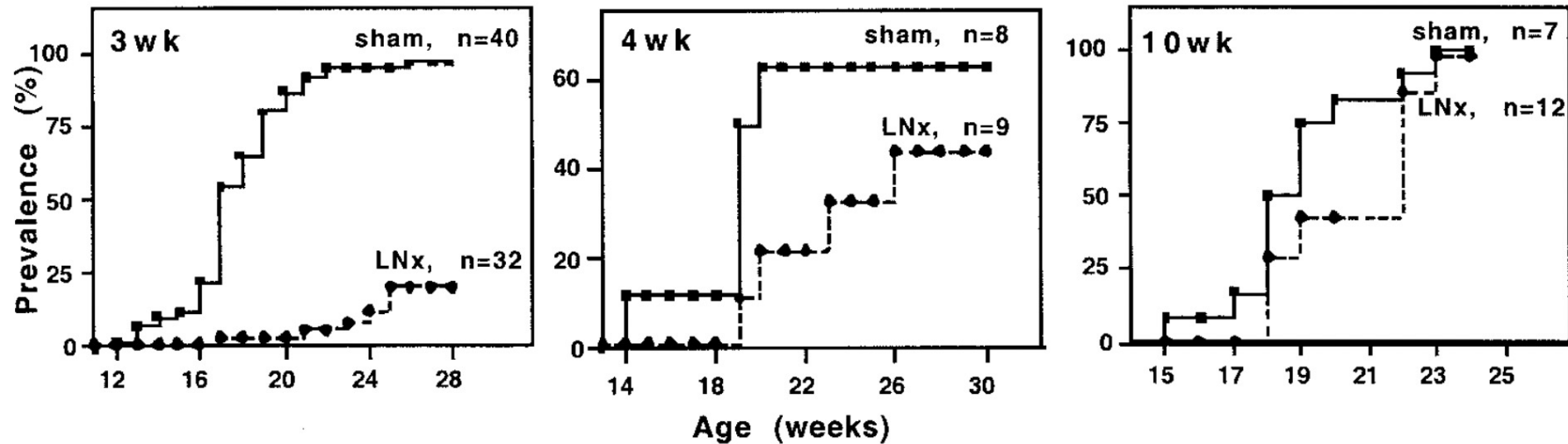
CD8 Glucagon



HPAP-020: 4 AAb+, <1 year onset

- Where is autoimmunity coordinated?
- What immune perturbations occur?
- How early can these perturbations be detected?

# Pancreatic lymph nodes (pLN) may be essential for T1D development



- In non-obese diabetogenic (NOD) mouse model, removal of pLNs early in development abrogates disease
- LN removal has no effect later in disease development

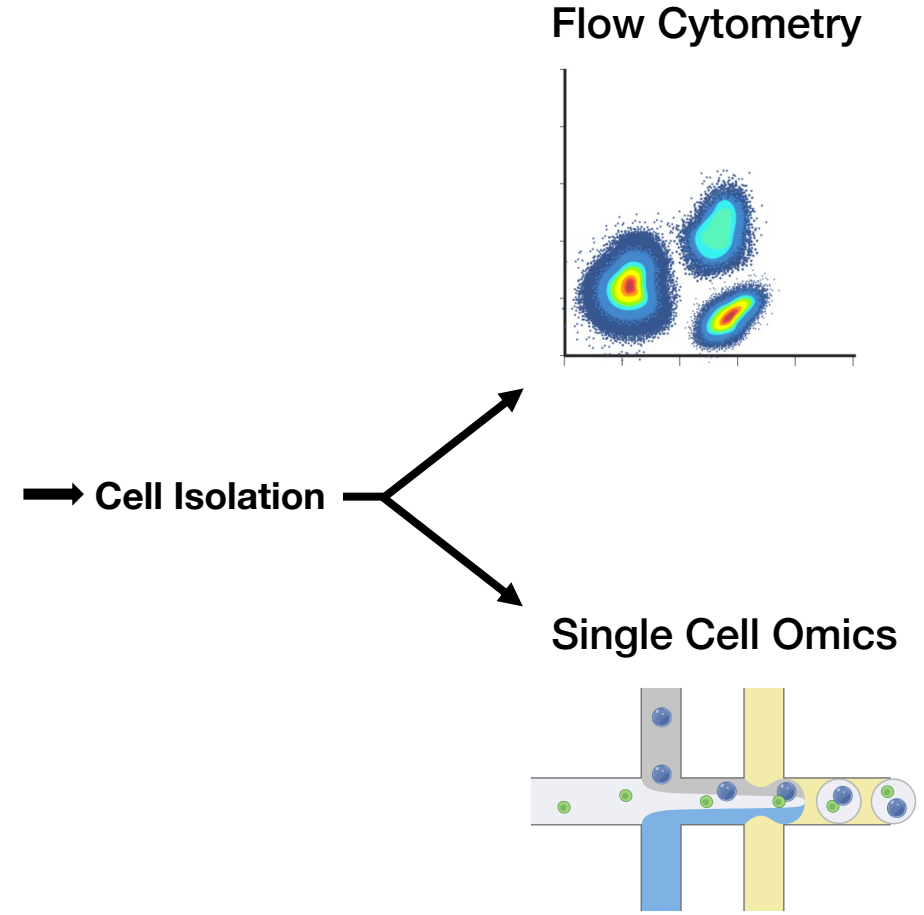
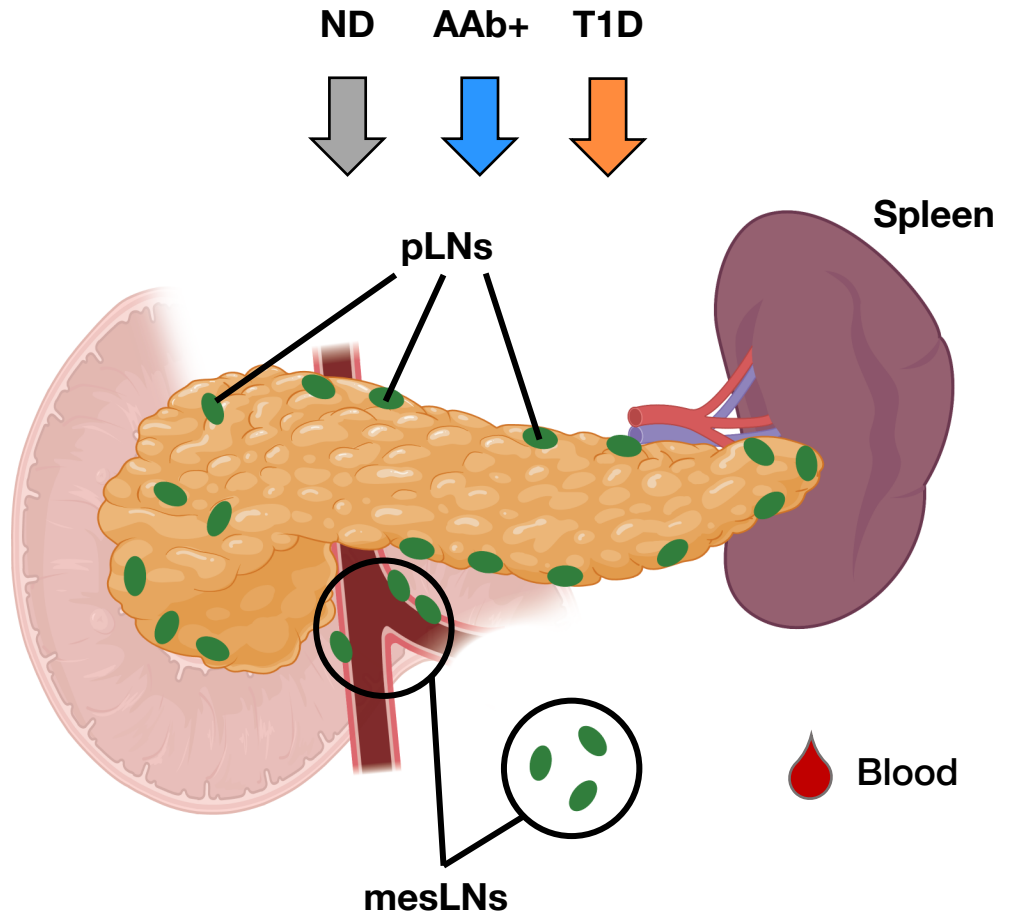
Human pLN immunology data in T1D is understudied

# A survey of immunological tissues in T1D

- Immune population profile of pLN throughout human T1D development?
- Shared immunophenotypes found in other lymphoid organs and tissues?

# Tissues isolated for immunological assays

## Human Pancreas Analysis Program (HPAP)



# Immunological lineage panel

Filter	Marker	Fluorochrome
B 515/20	CD15	FITC
B 780/60	CD4	BB790

R 660/20	CXCR5	AF647
R 730/45	CD45	AF700
R 780/60	CCR7	APC Cy7

UV 379/28	CD11c	BUV395
UV 515/30	CD8	BUV496
UV 586/15	CD45RA	BUV563
UV 660/20	CD38	BUV661
UV 740/35	CD25	BUV737
UV 820/60	CD3	BUV805

V 450/40	PD1	BV421
V 470/15	CD14	BV480
V 515/20	Viability	Aqua blue
V 586/15	CD56	BV570
V 610/20	HLA-DR	BV605
V 660/20	CD27	BV650
V 710/50	CD16	BV711
V 800/30	CD19	BV785

YG 586/15	CD123	PE
YG 610/30	CD127	PE-CF594
YG 660/40	CD69	PE-Cy5
YG 710/50	CD34	PE-Cy5.5
YG 780/60	CD21	PE-Cy7

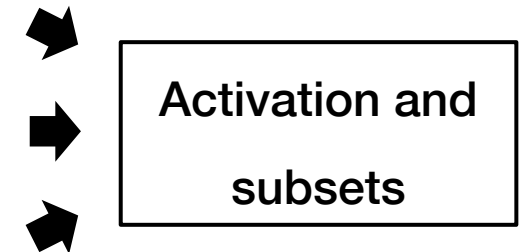
- **Innate**

- Neutrophils
- Eosinophils
- DCs
- Monocytes
- NK cells
- Hema. stem cells
- ILCs

- **T cells**

- CD4+ and CD8+

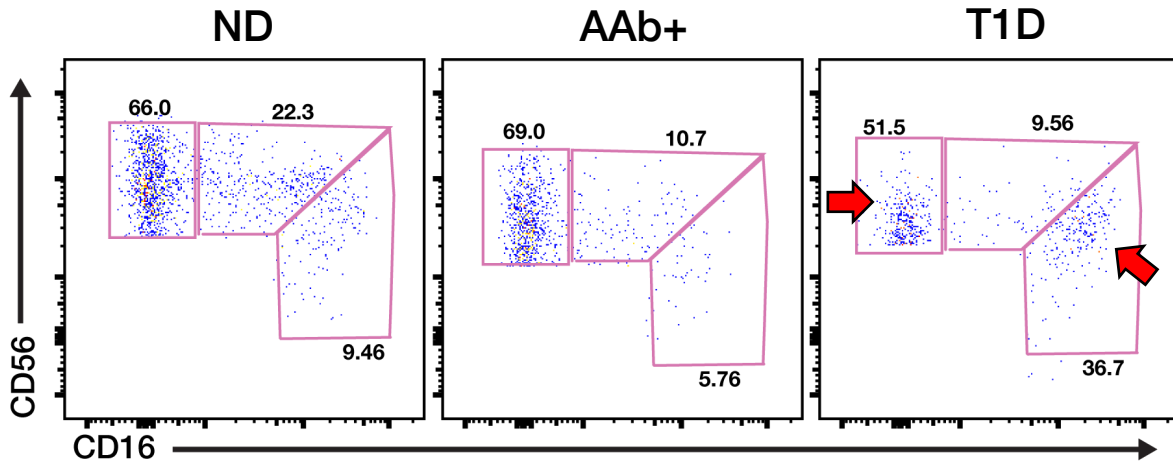
- **B cells**



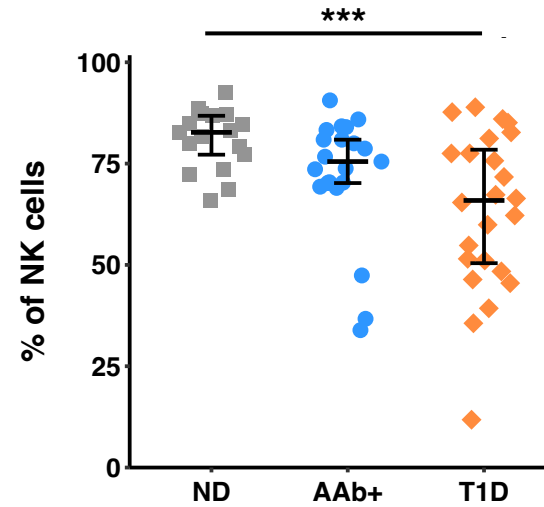
Major immune populations did not change in AAb+, T1D pLN

# Shift in pLN NK cell phenotype in T1D

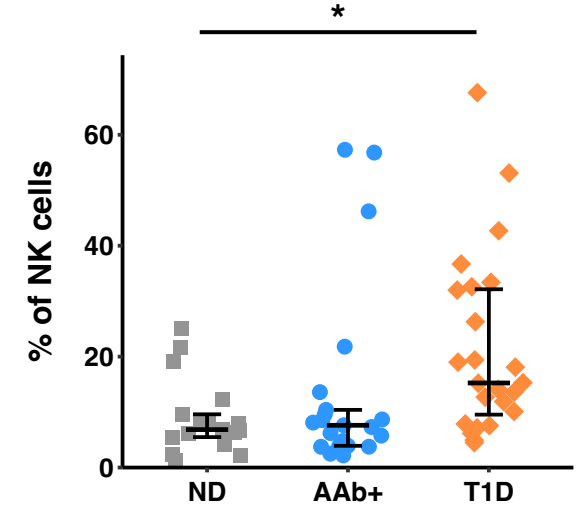
pLN NK cells



CD56<sup>bright</sup>CD16<sup>-</sup>  
“Regulatory”

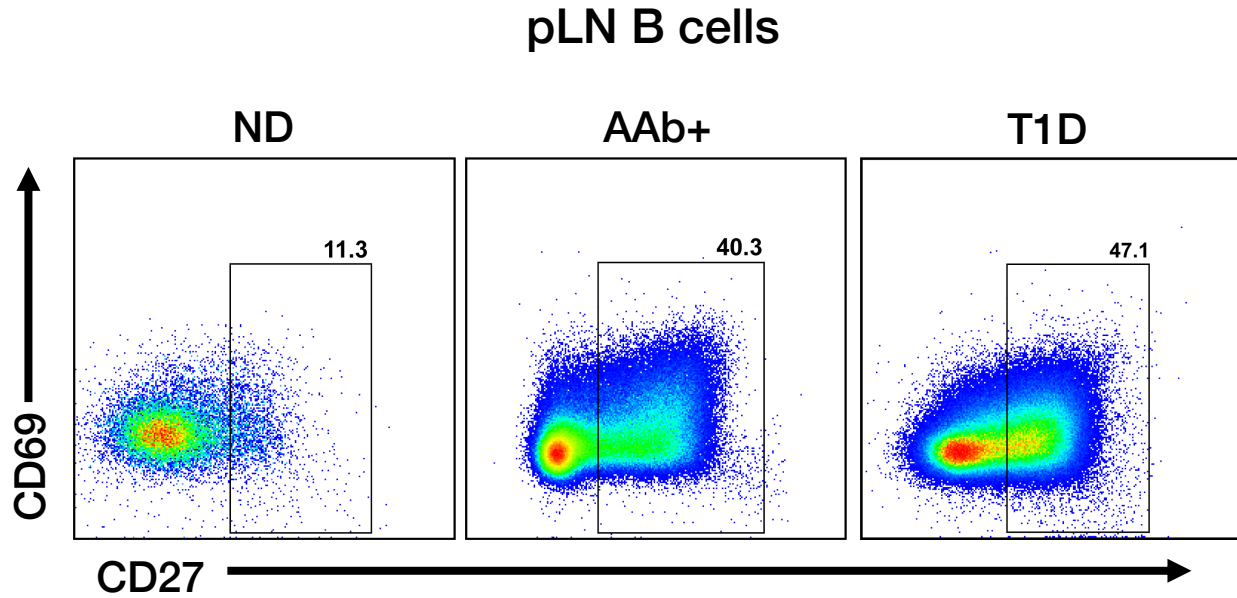


CD56<sup>dim</sup>CD16<sup>+</sup>  
“Cytotoxic”

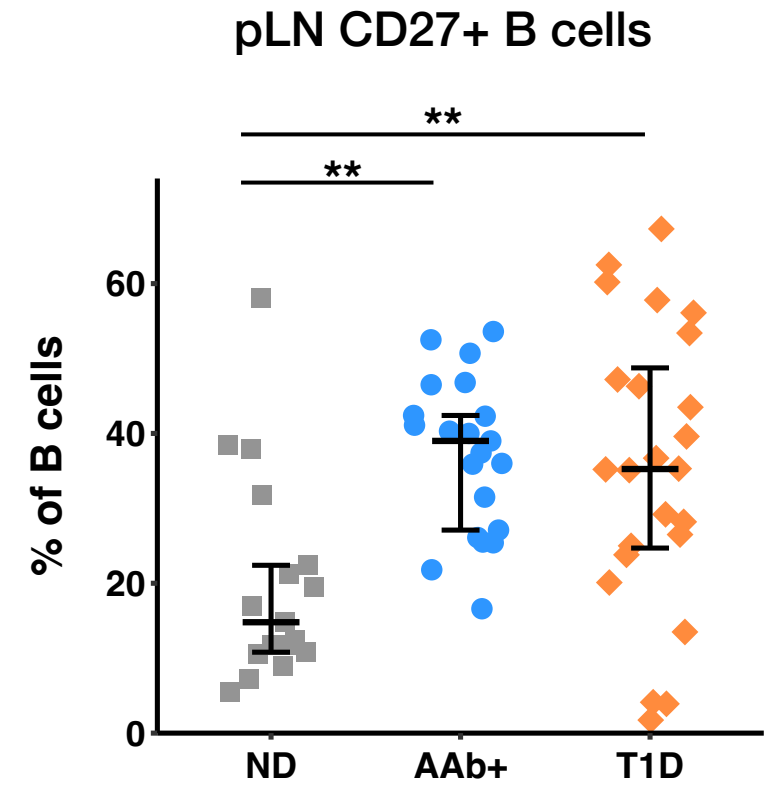


No other innate immune cell phenotypes observed

# pLN B cells have increased CD27 expression

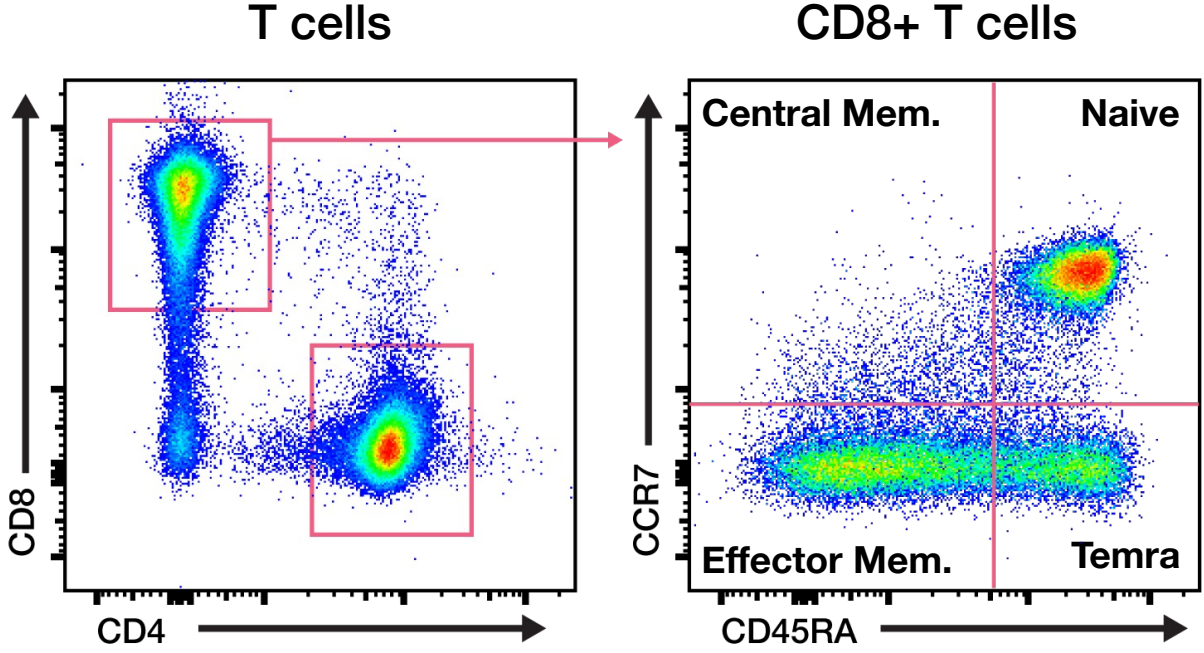


- CD27 is a memory B cell marker



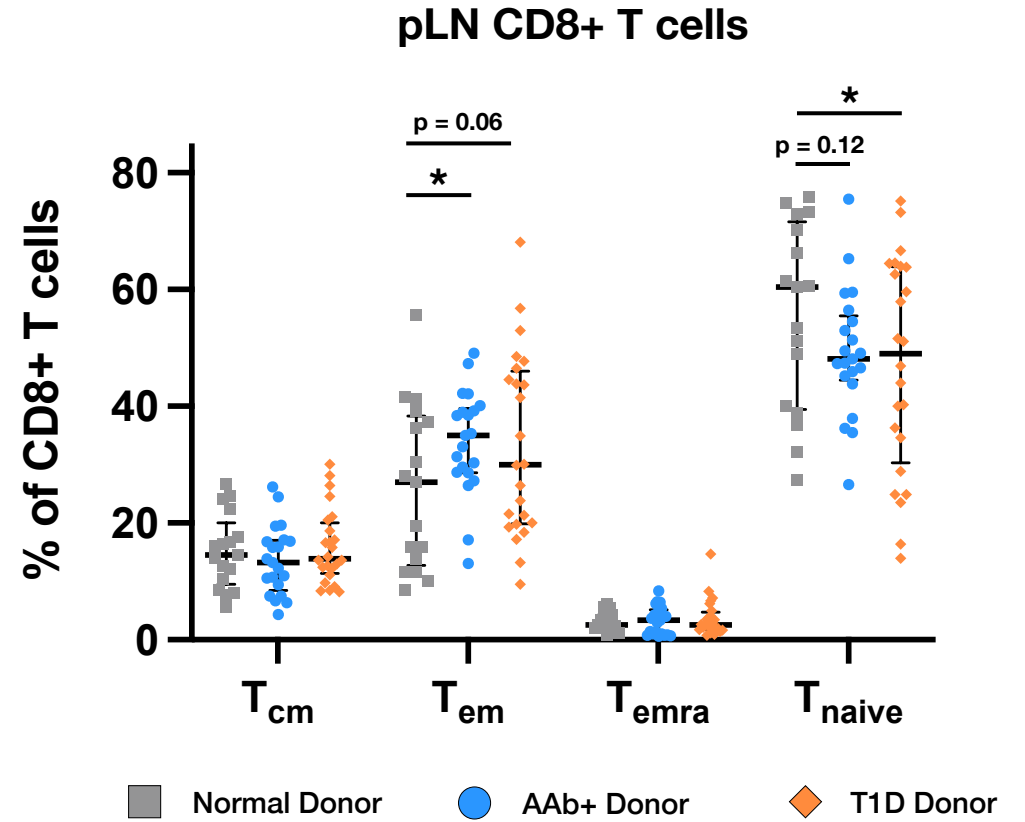
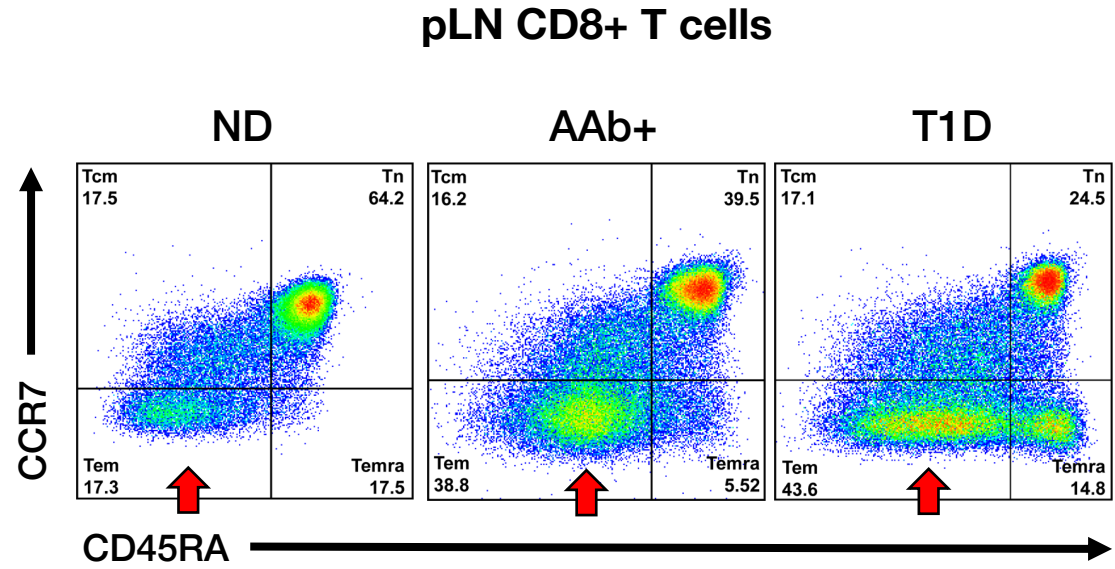


# Gating for T cells

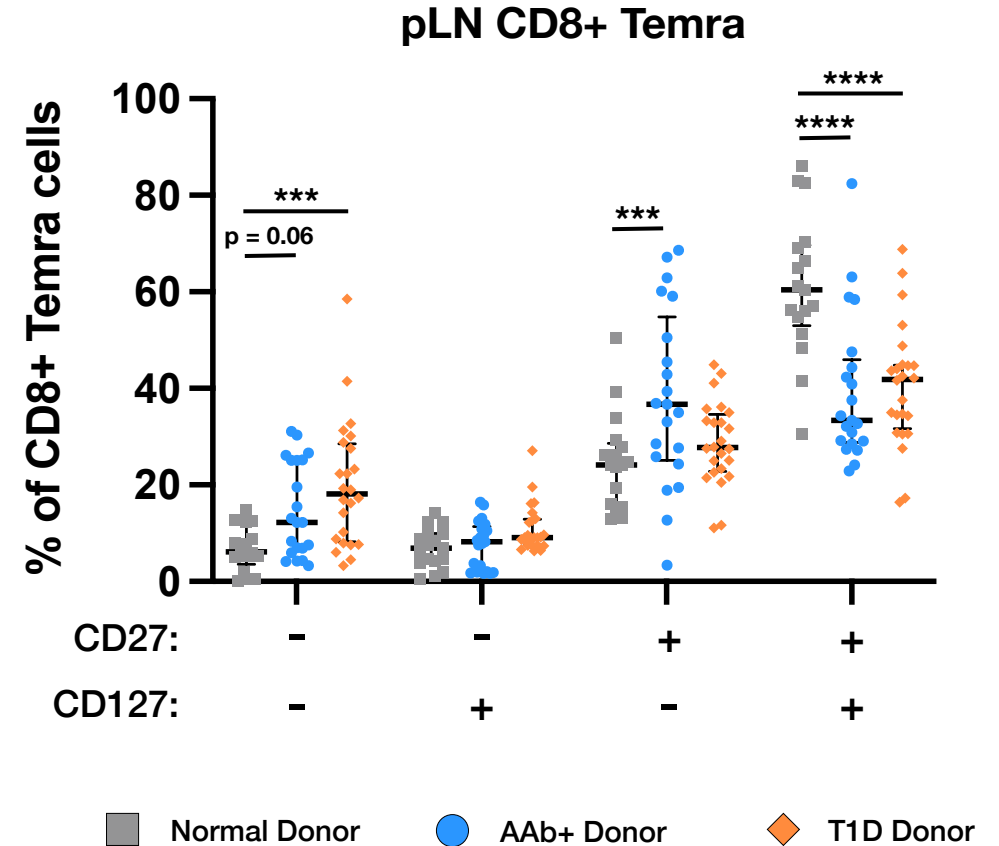
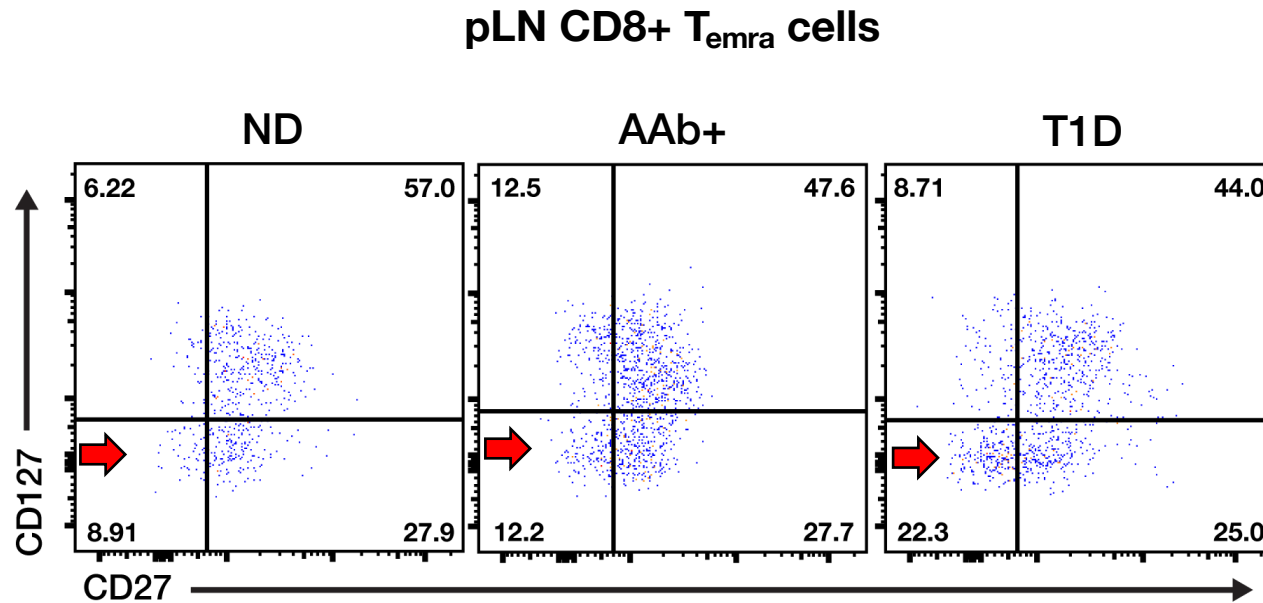


<u>CCR7</u>	<u>CD45RA</u>	
-	-	“Effector” (Tem)
-	+	“Temra”
+	-	“Central” (Tcm)
+	+	“Naive” (Tnaive)

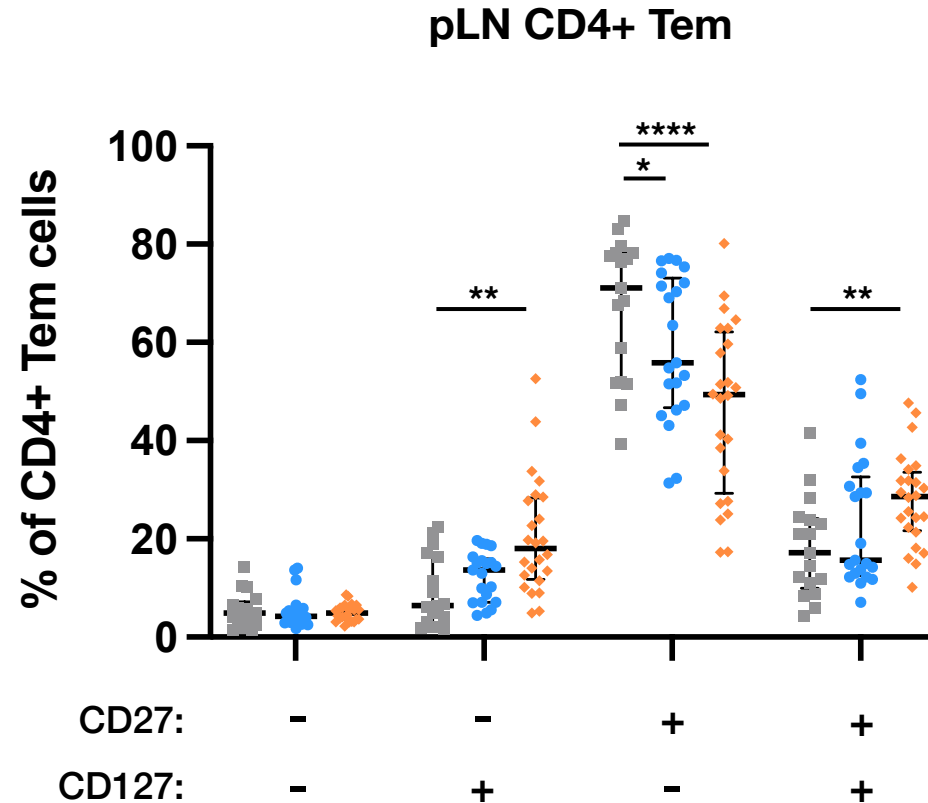
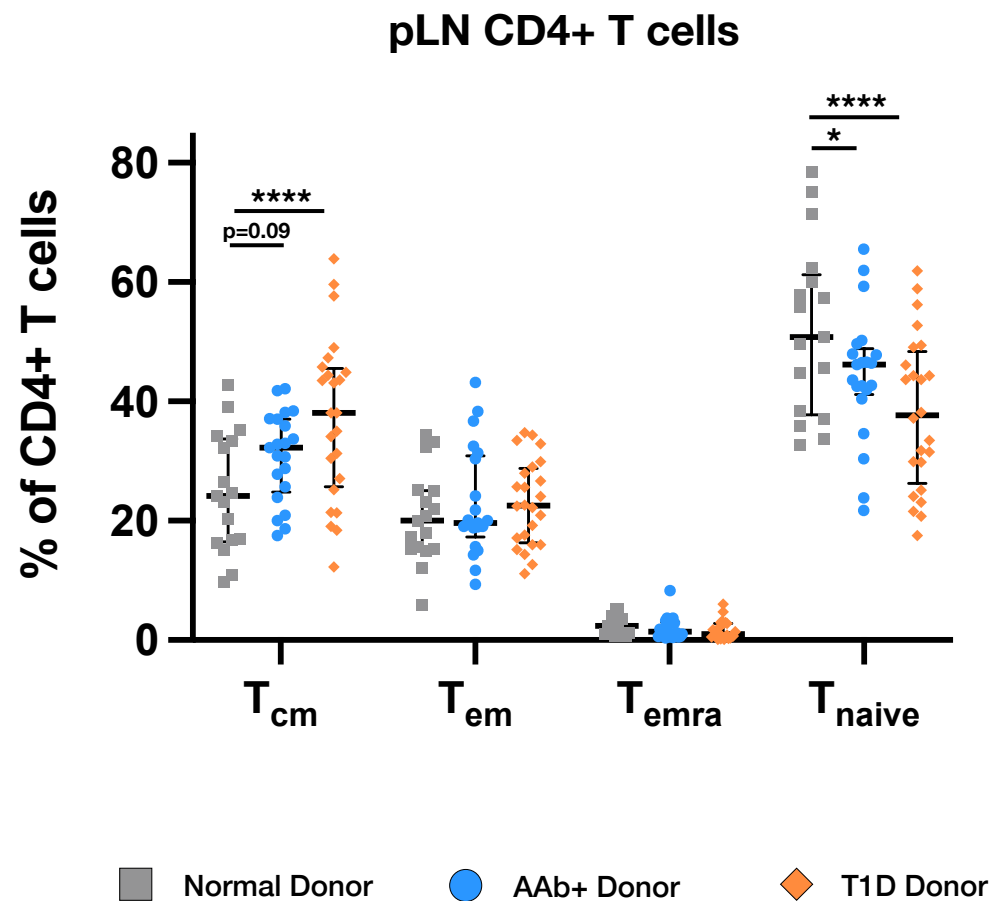
# CD8+ Tem are more frequent in AAb+ and T1D pLN



# CD8+ Temra are increasingly CD27- & CD127-

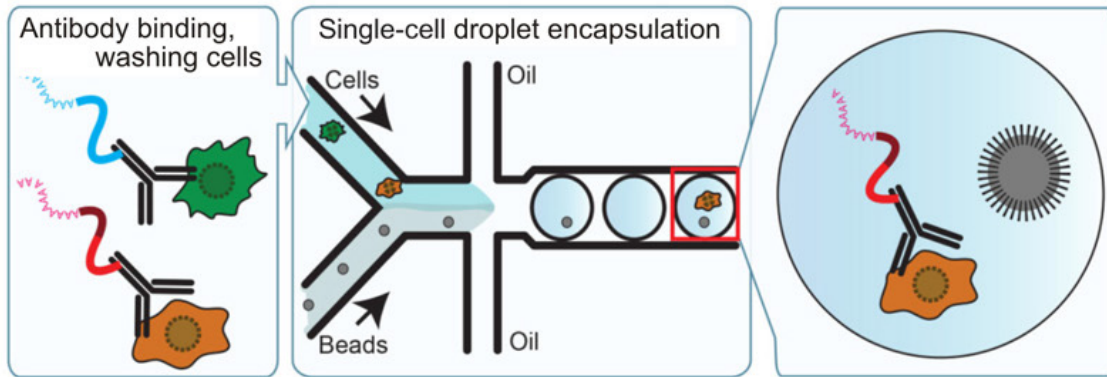


# CD4+ T<sub>cm</sub> & T<sub>em</sub> cells altered in AAb+ & T1D



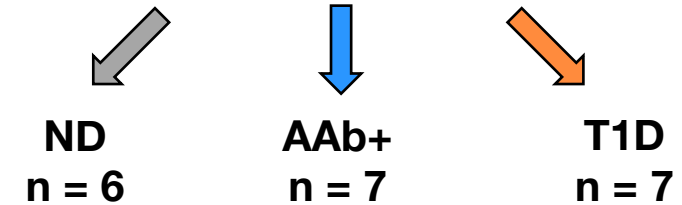
# CITEseq on HPAP tissue for deep immunophenotyping

## CITEseq –RNAseq and surface epitopes in single cells



- 3' scRNAseq
- Totalseq-A Cocktail (Biolegend)

20 Donors



- pLNs, mesLNs, splenocytes
- 49 total samples
- 650,083 cells passing QC

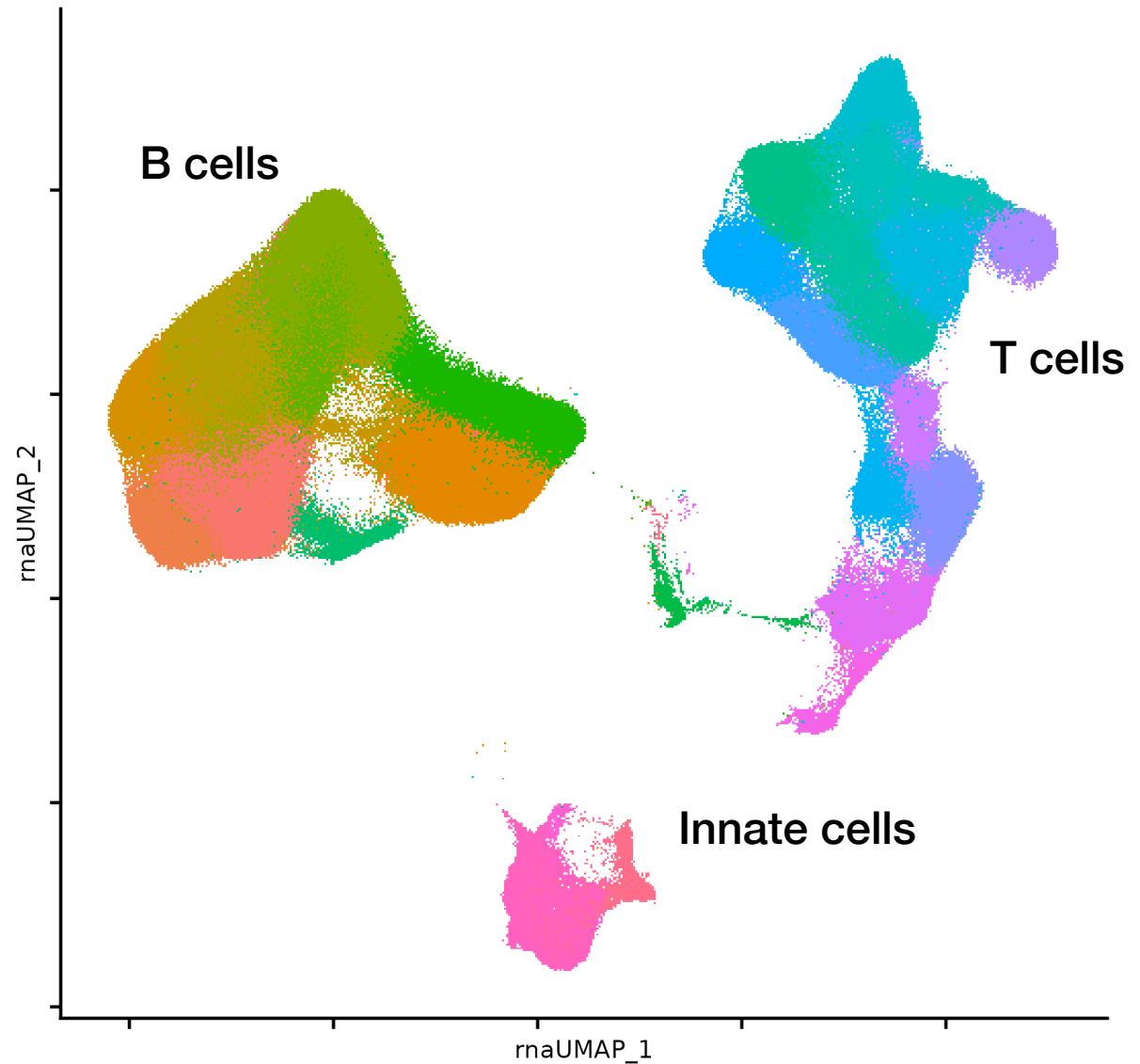
All data publicly available

PANC-DB: [hpap.pmacs.upenn.edu](http://hpap.pmacs.upenn.edu)

GEO: accession GSE221787

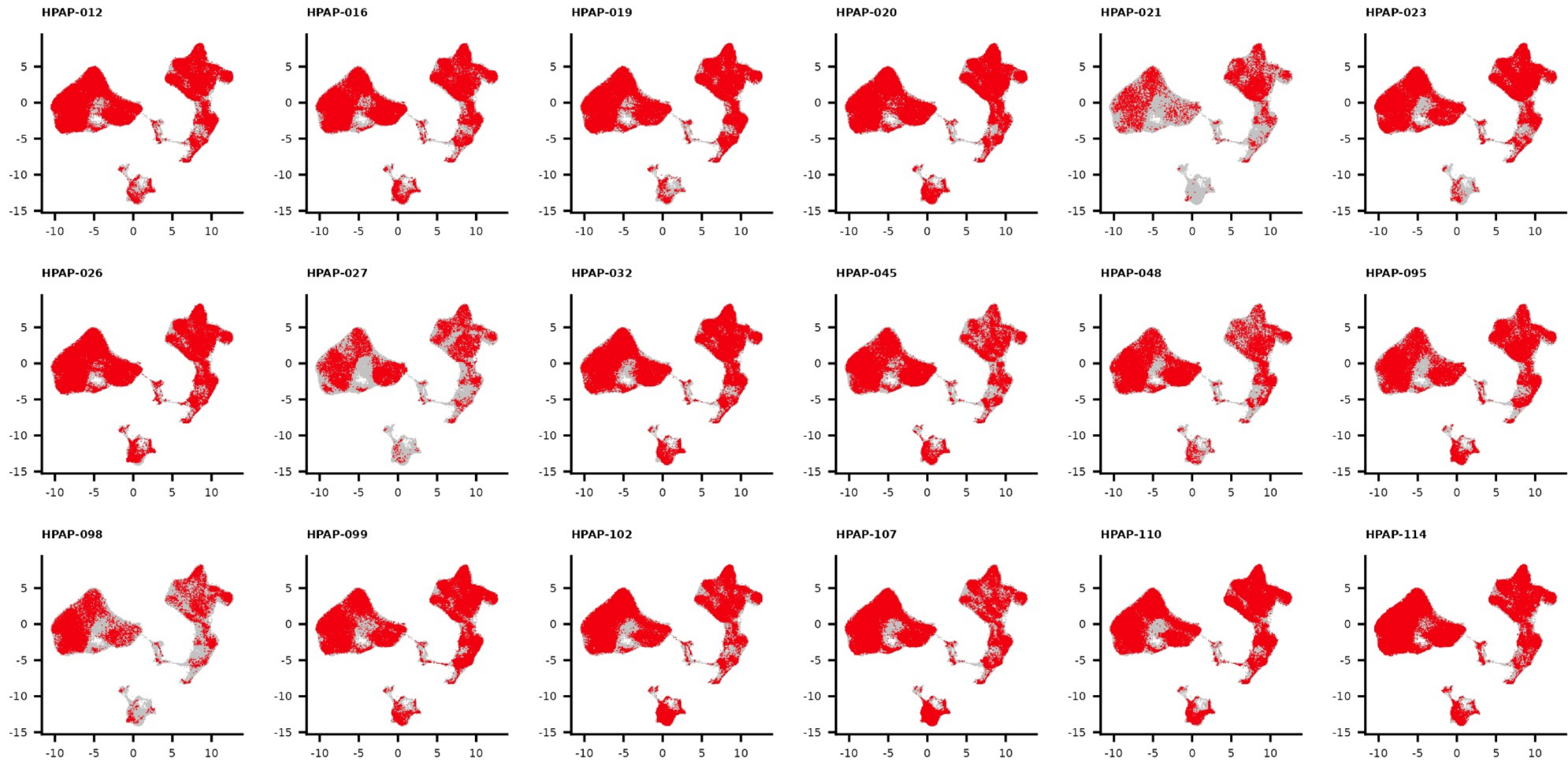
# Clusters identified from CITEseq

- B class switched memory #1
- B class switched memory #2
- B IgM+ memory #2
- B IgM+ memory #3
- B IgM+ memory #4
- B IgM+ memory/marginal zone like #1
- B IgM+ memory/marginal zone like #2
- B naïve #1
- B naïve #2
- B naïve #3
- B plasma cell/plasmablast
- B Tbet+/ABCs
- T CD4 naïve #1
- T CD4 naïve #2
- T CD4 Tcm/Tem
- T CD4 Tcm/Treg
- T CD4 Tem/activated
- T CD8 gdT/Helios+
- T CD8 naïve #1
- T CD8 naïve #2
- T CD8 Tem/Temra
- T CD8 Tem/Trm #1
- T CD8 Tem/Trm #2
- NK
- NK/ILC
- DC
- Monocyte #1
- Monocyte #2
- Monocyte #3/Innate Mix



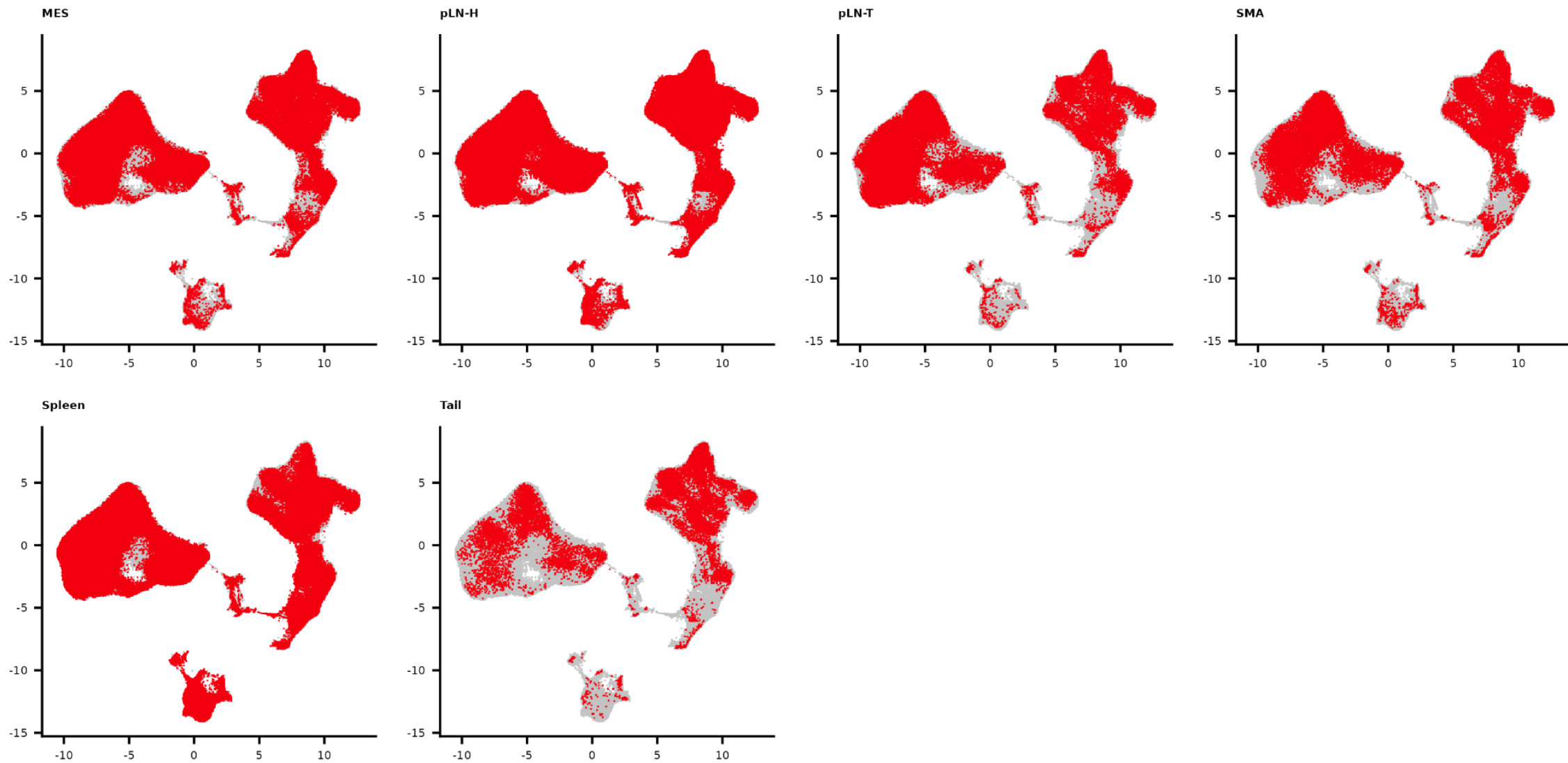
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# Cells by Donor



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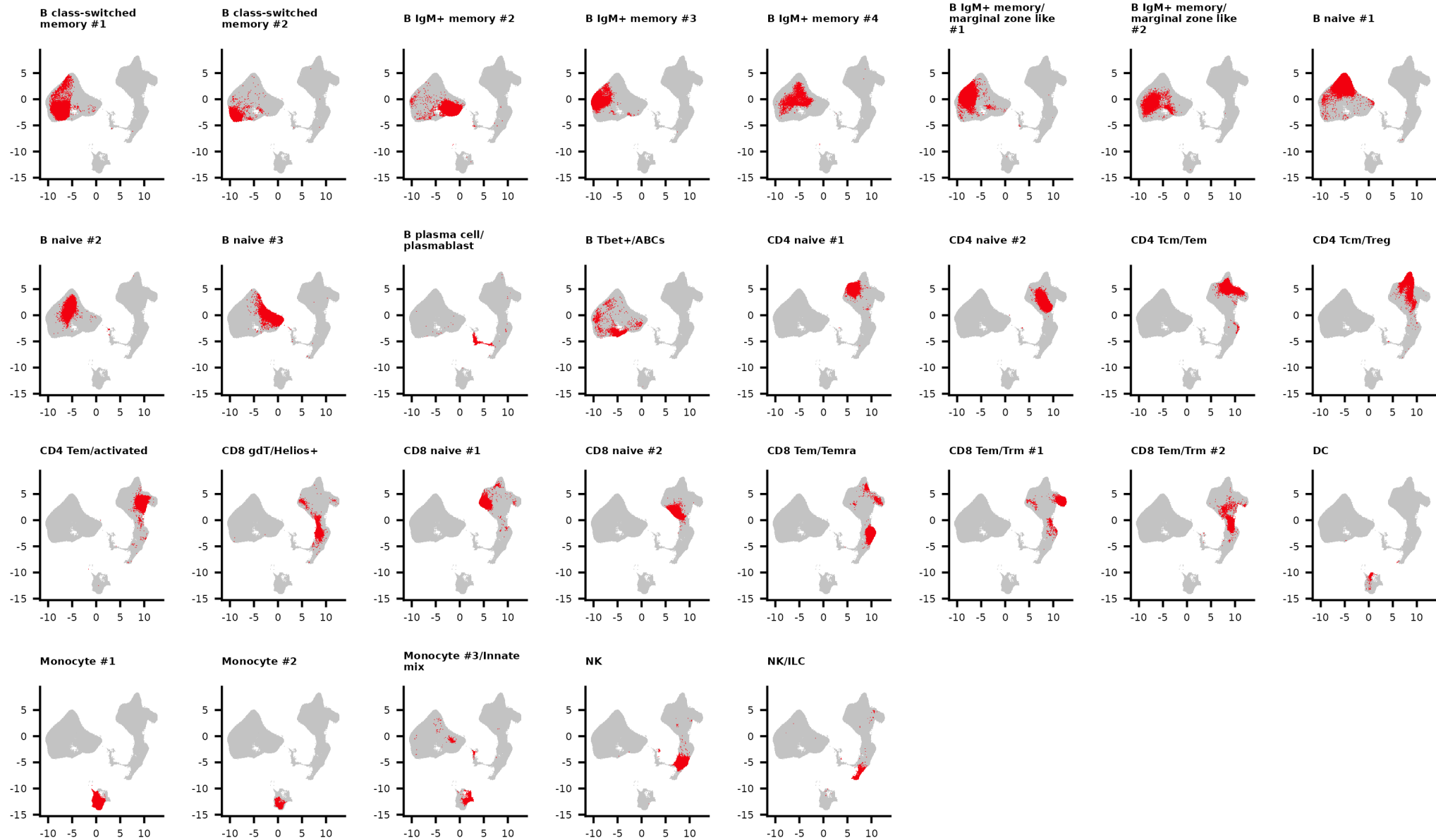
# Cells by Tissue



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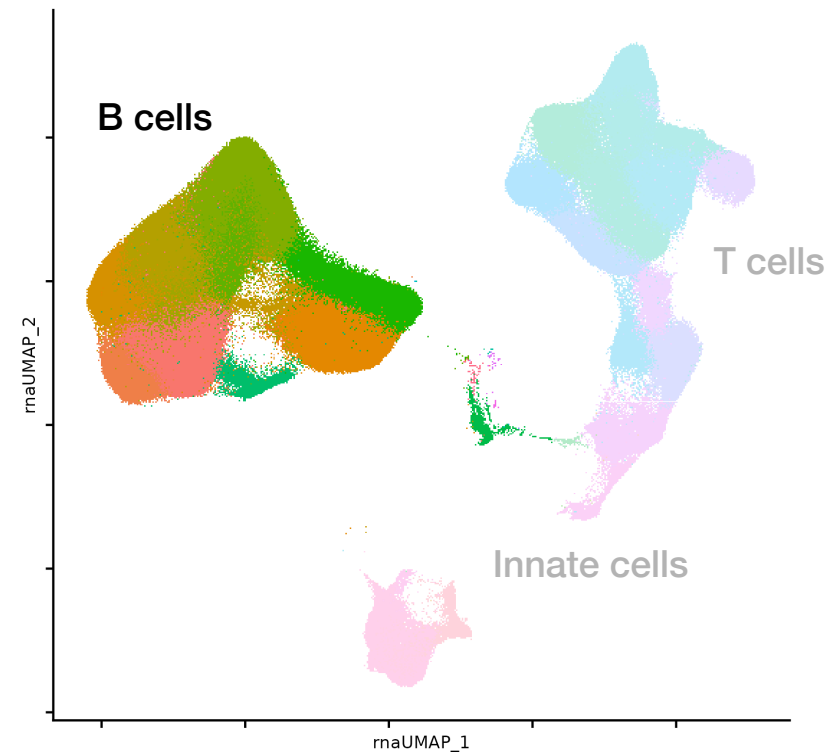
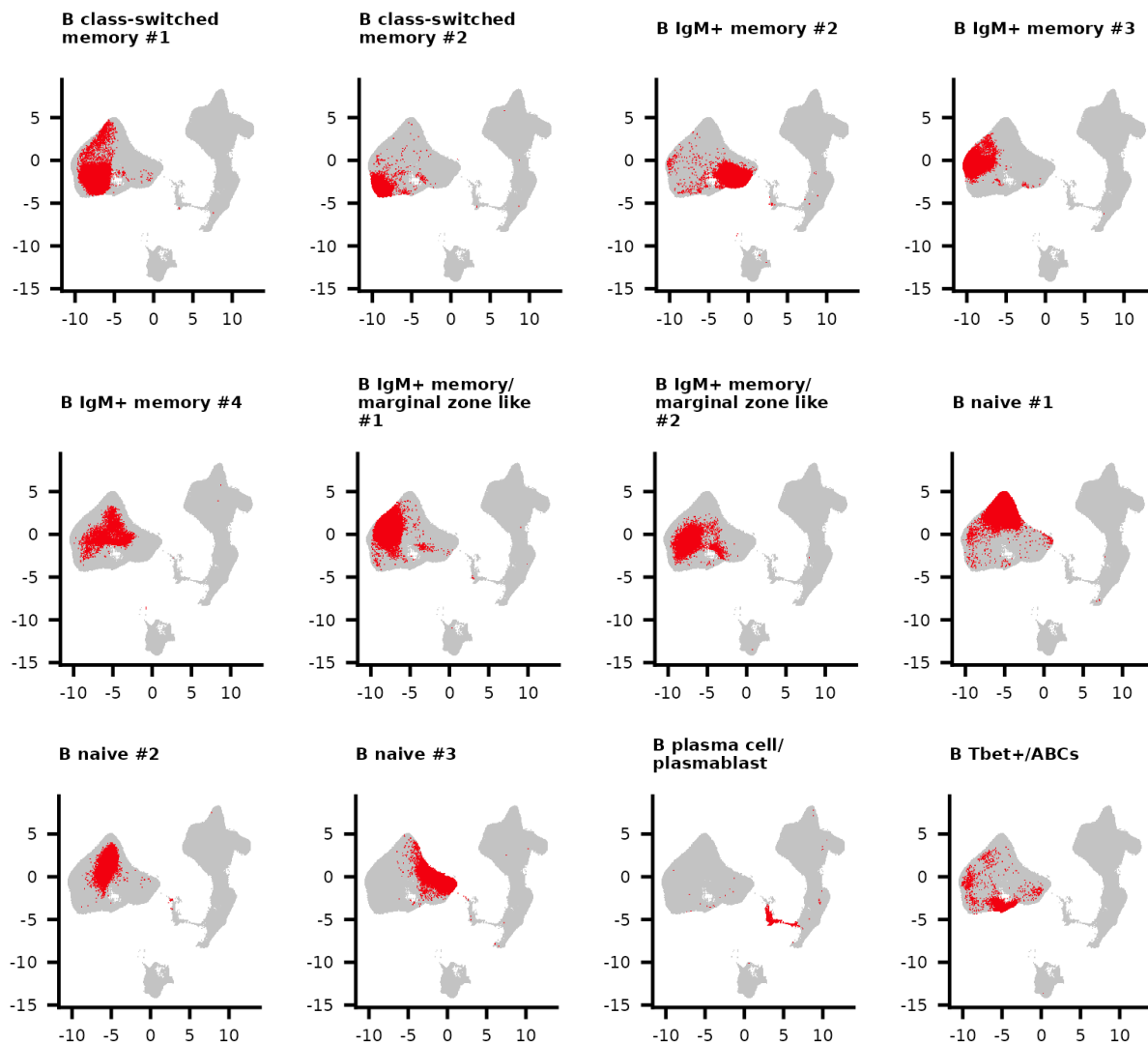


# Cells by Cluster



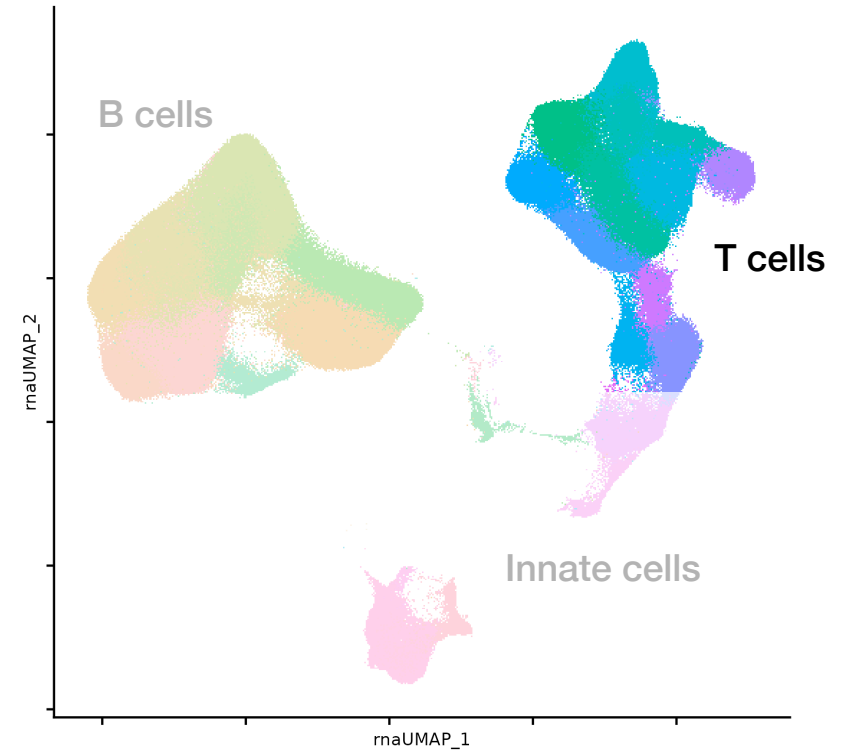
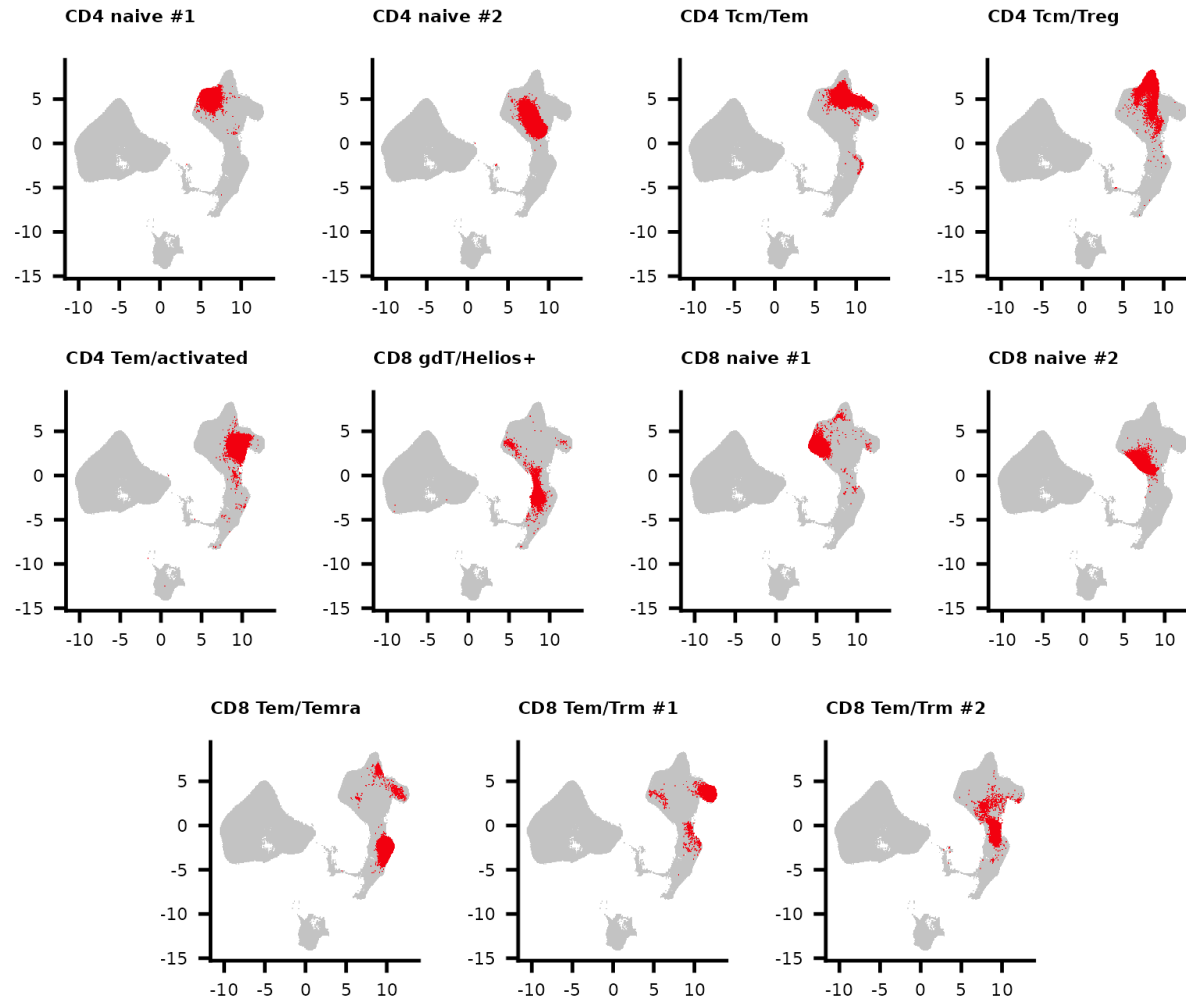
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# B cell clusters



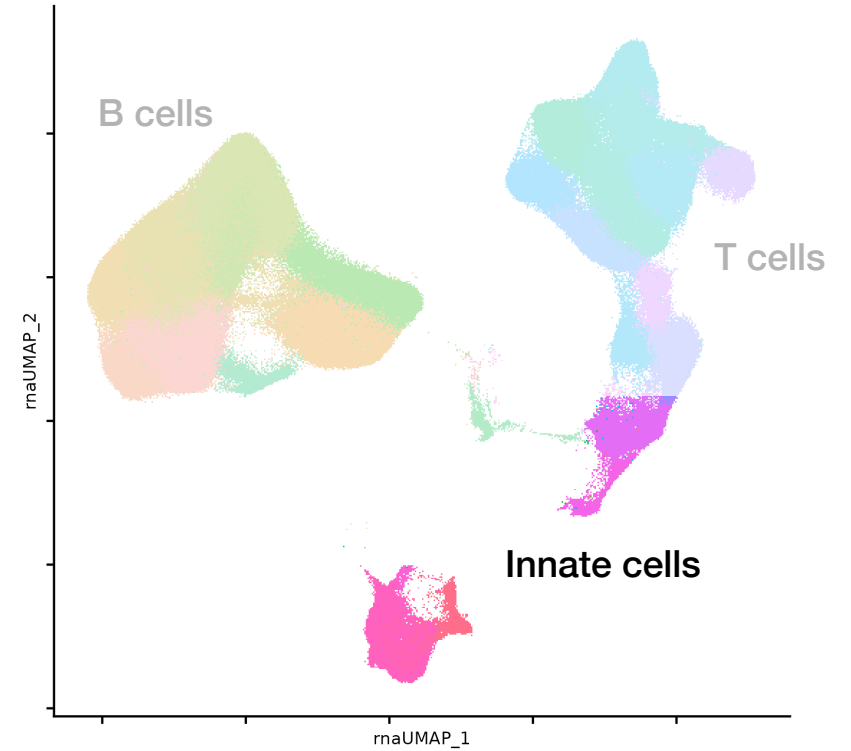
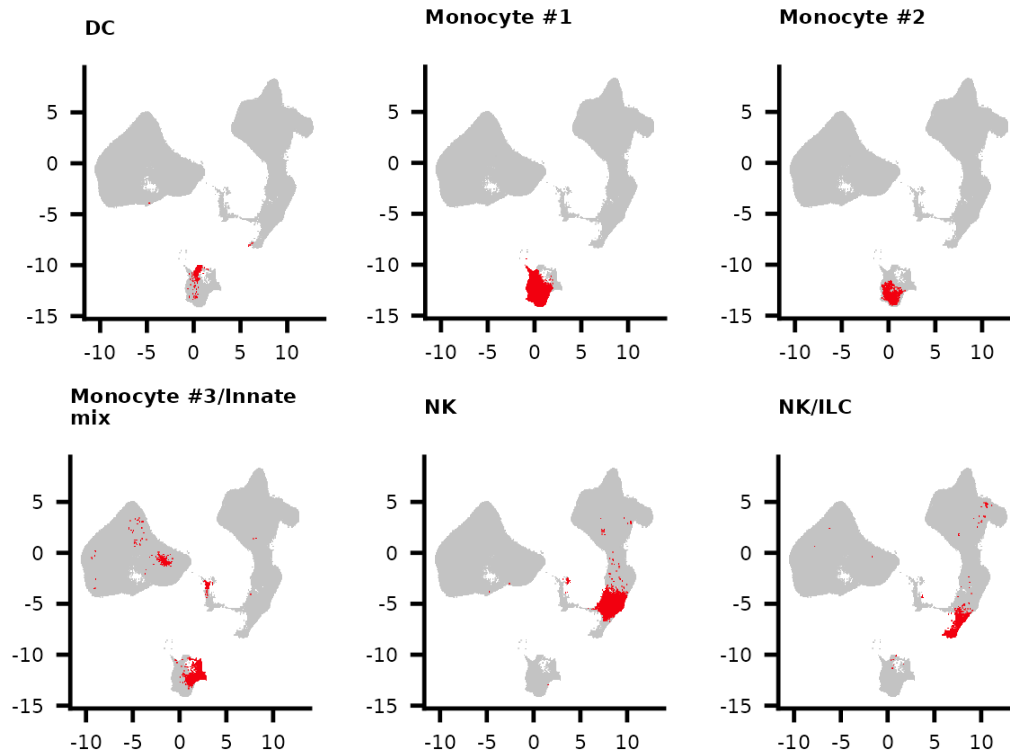
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# T cell clusters



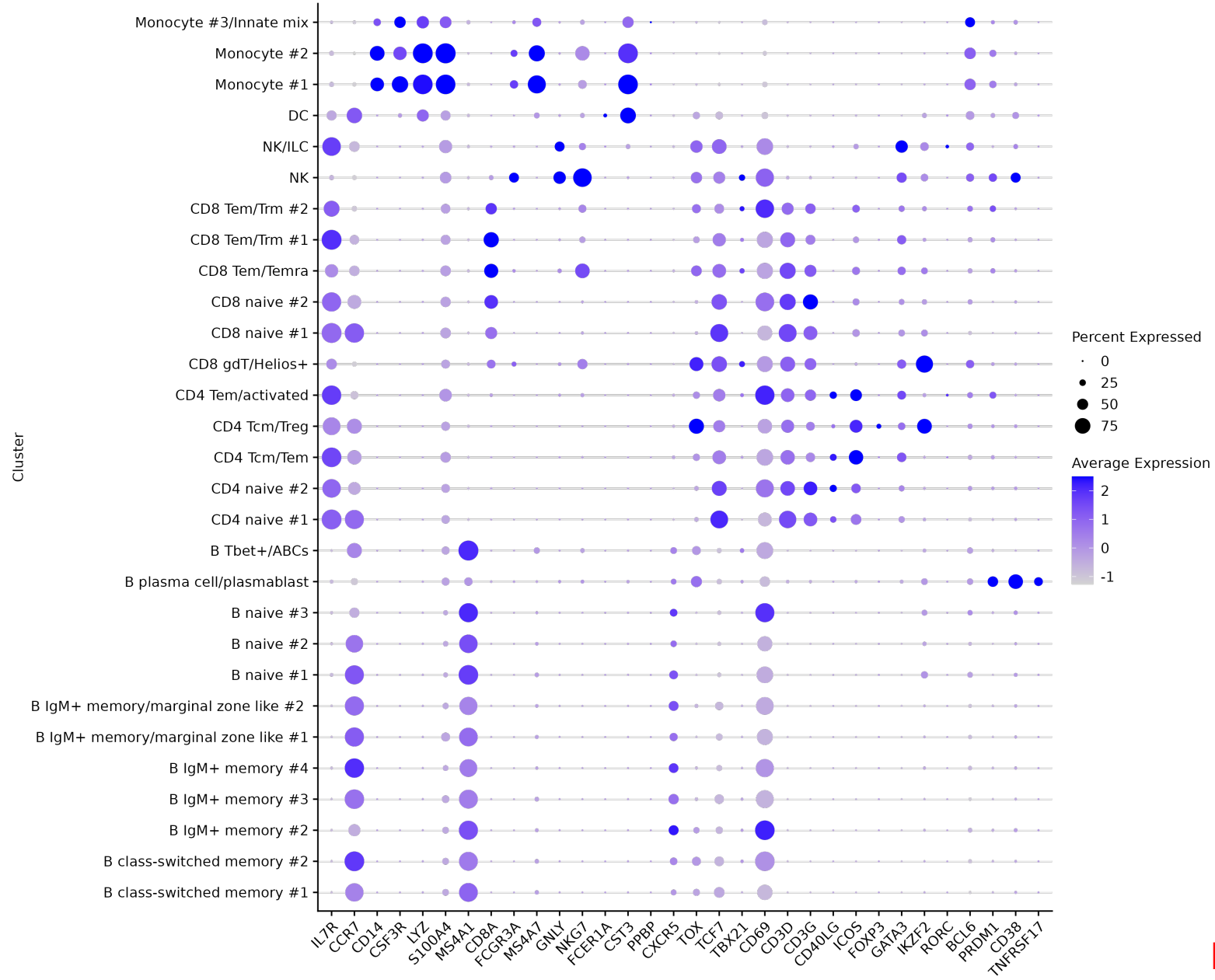
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# Innate cell clusters



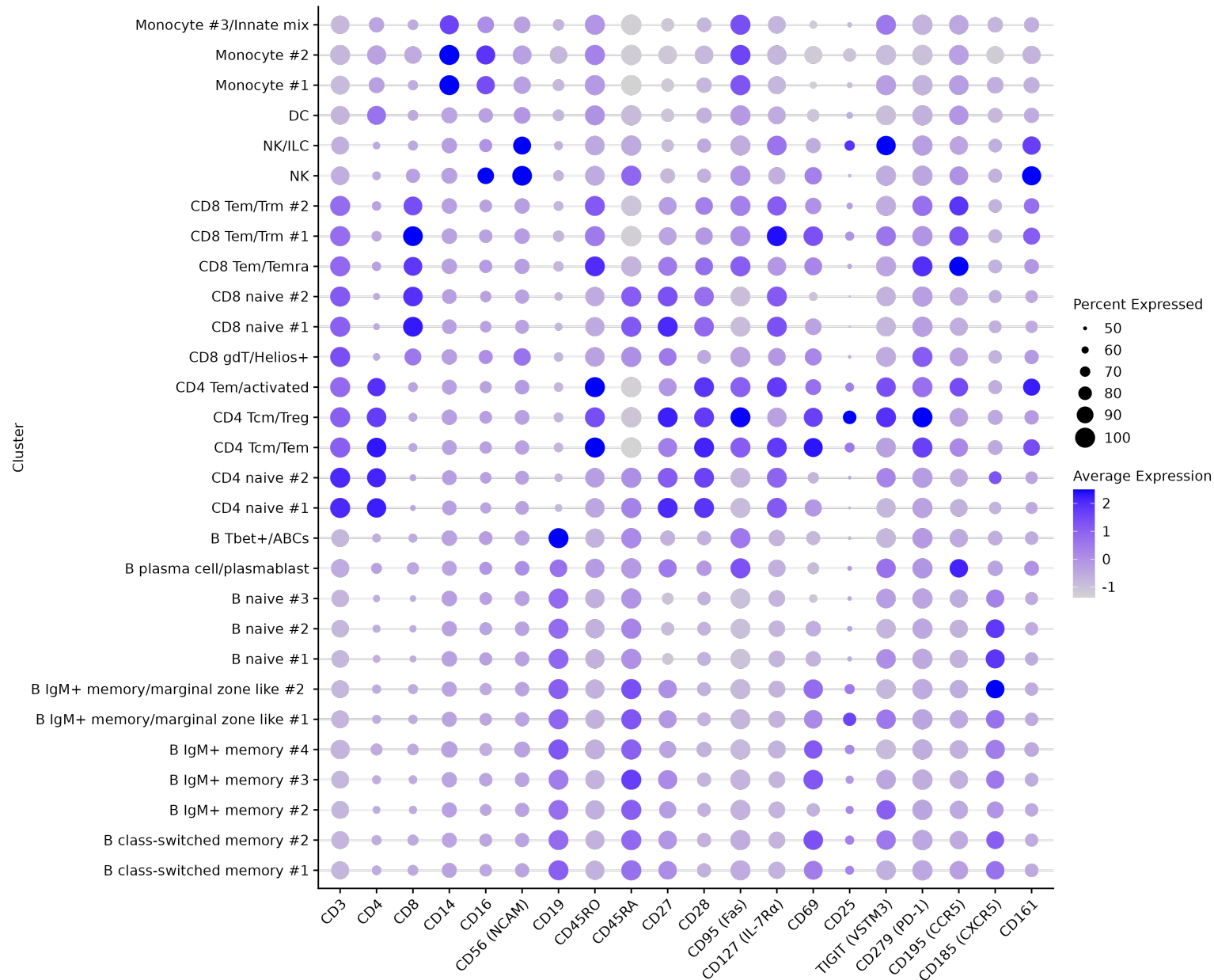
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# Gene (RNA) Expression by Cluster



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# Surface Epitope Expression by Cluster



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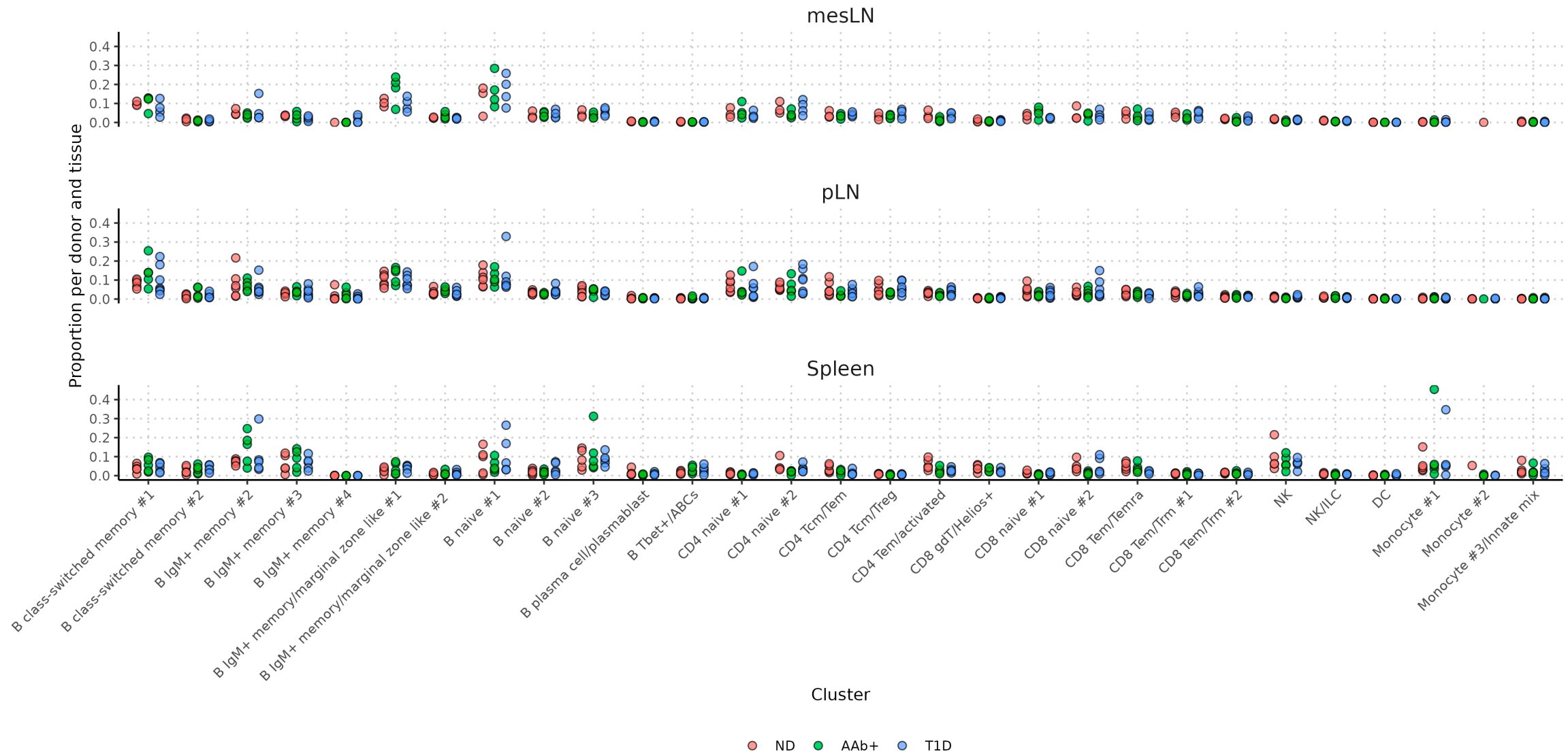


# Gene Expression Modules by Cluster



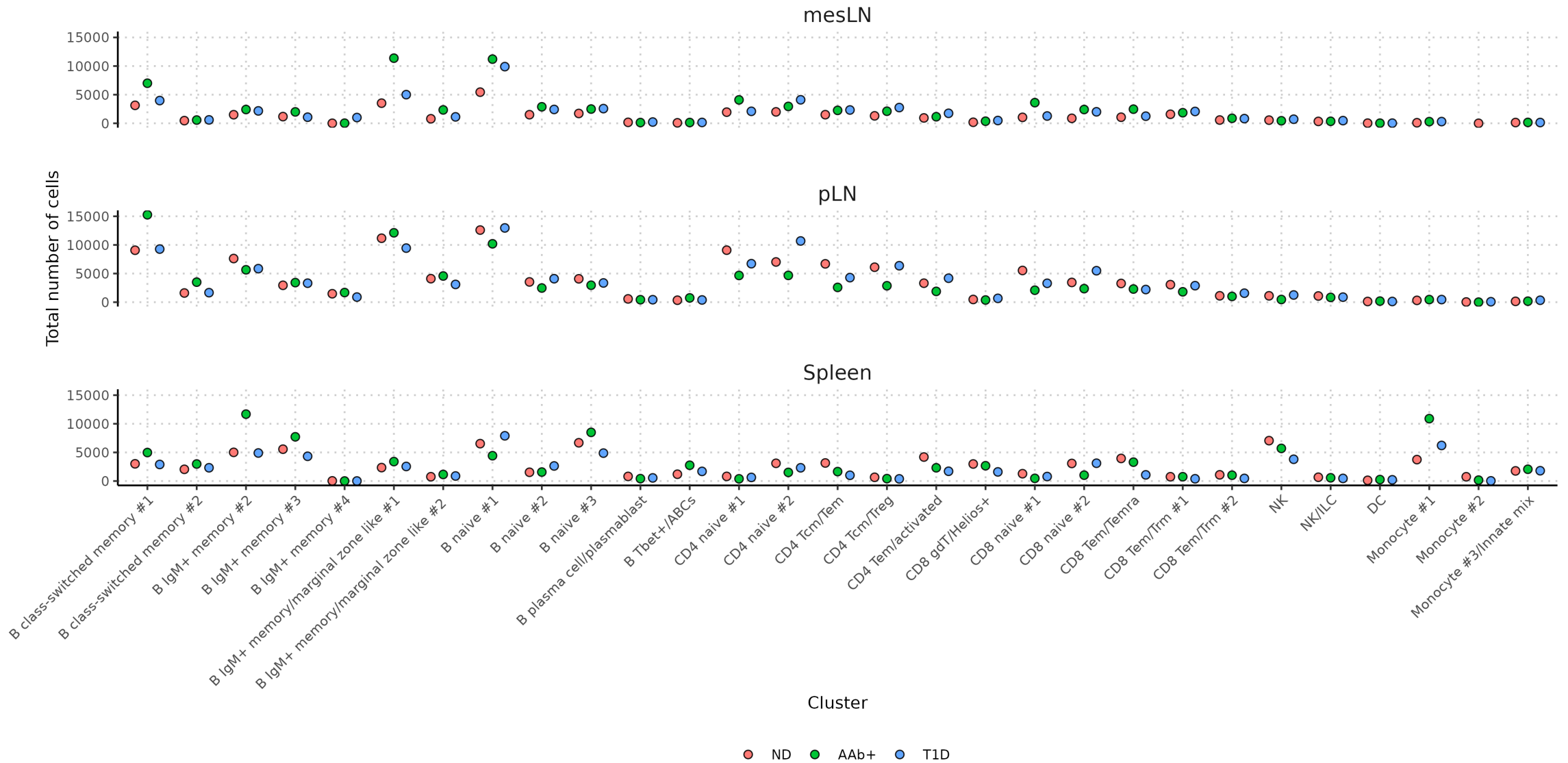


# Clusters across disease states



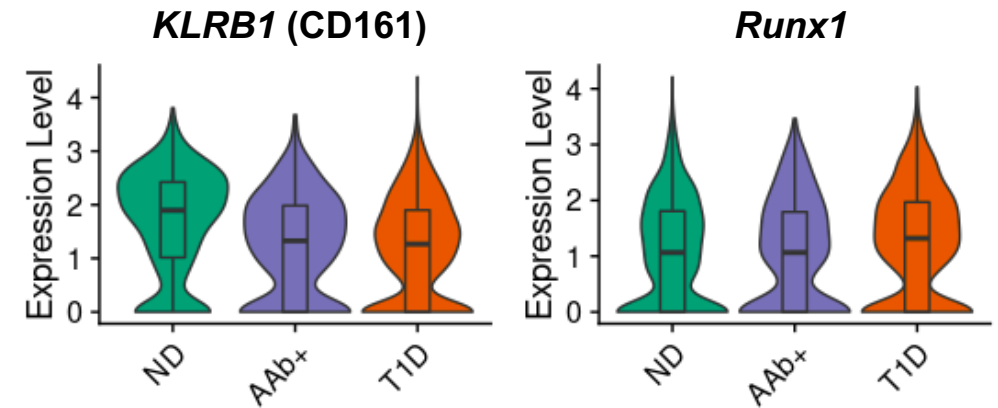
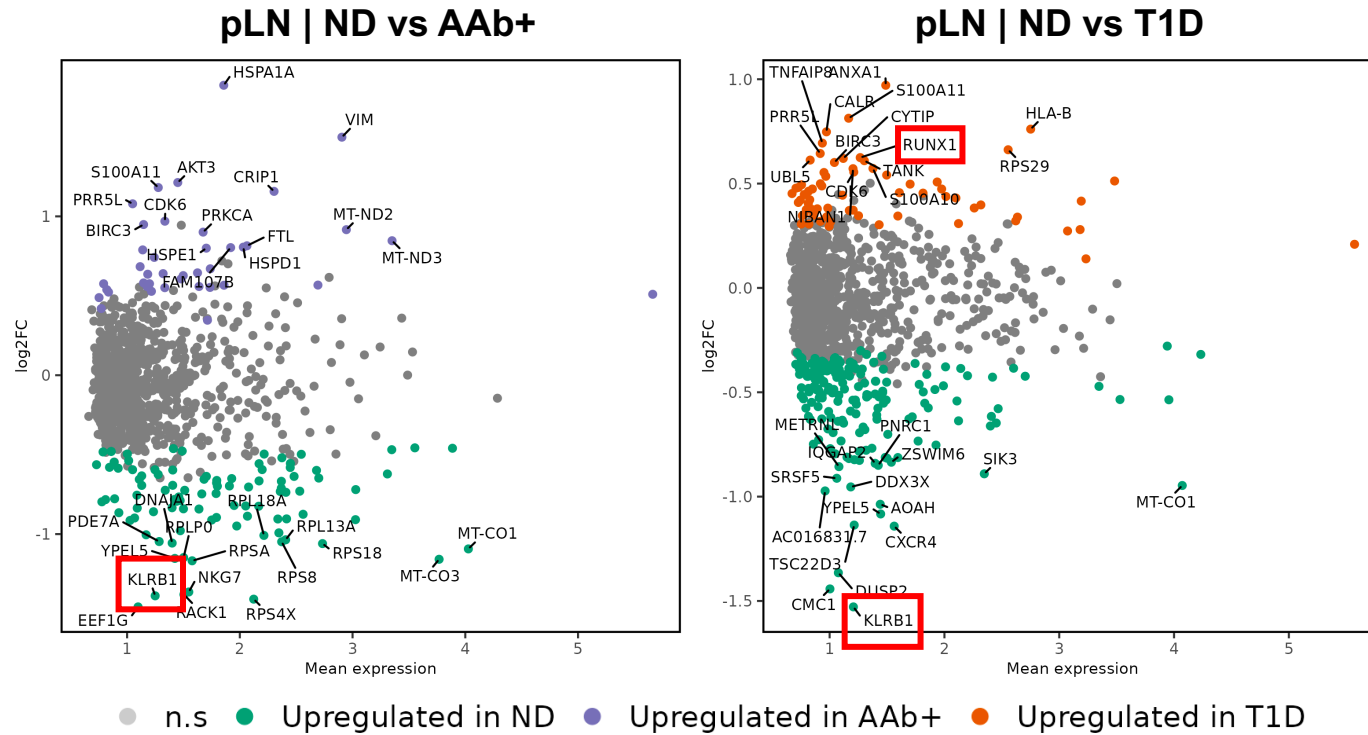
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# Number of cells across clusters



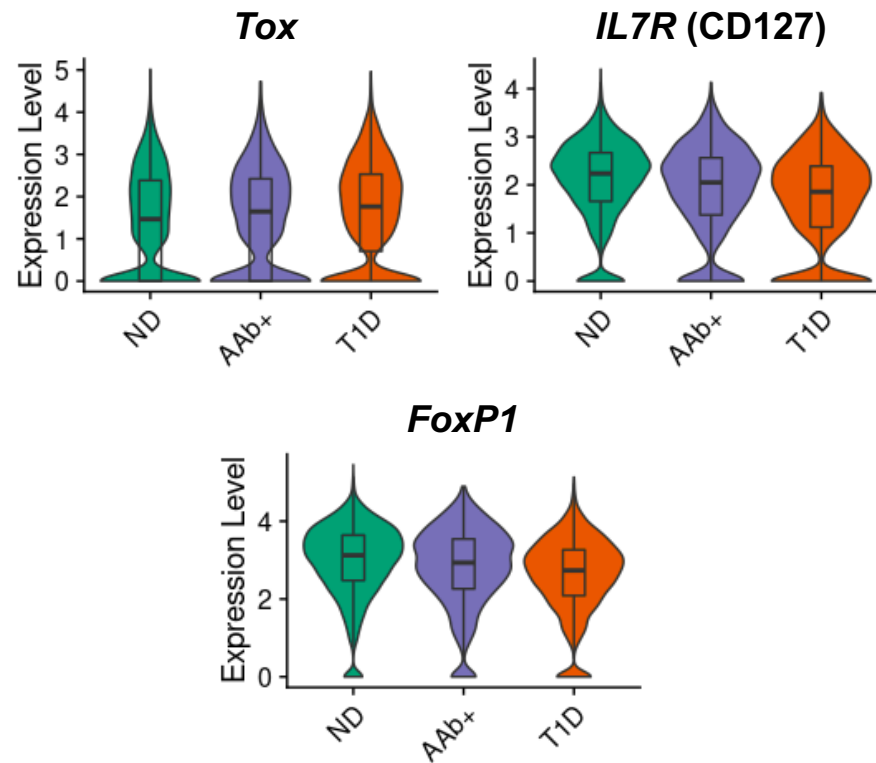
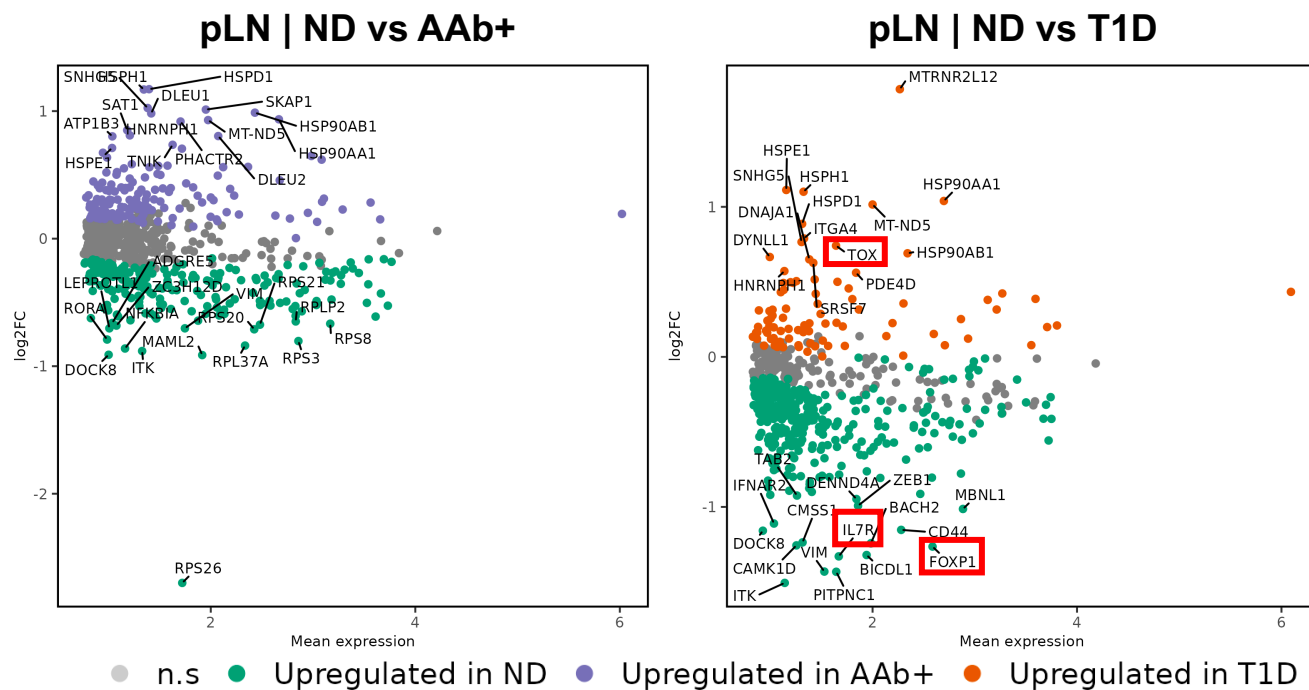
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# Differential gene expression: NK cell cluster



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# Differential gene expression: CD4 Tcm/Treg cluster



# Immune Atlasing: Take-Aways and Future Directions

- **Main Finding – pLN in AAb+ and T1D individuals do have altered immune profiles**
  - NK cells more cytotoxic in T1D
  - B cells have increased CD27 expression in AAb+, T1D
  - T cells tend to have increased memory populations, more differentiated/activated
  - initial indications of overlapping phenotypes in CITEseq/flow datasets
  - pLN have evidence of “immunological aging” or inflammatory state
- **Ongoing Work**
  - Data analysis!
  - Examination of mesLNs, spleen
  - Correlating phenotypes to clinical data

**All data publicly available**

**PANC-DB: [hpap.pmacs.upenn.edu](http://hpap.pmacs.upenn.edu)**

**GEO: accession GSE221787**

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# Acknowledgements

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### Luning Prak Lab

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Nina Luning Prak

### Locci Lab

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Nicolai M. Doliba	

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