

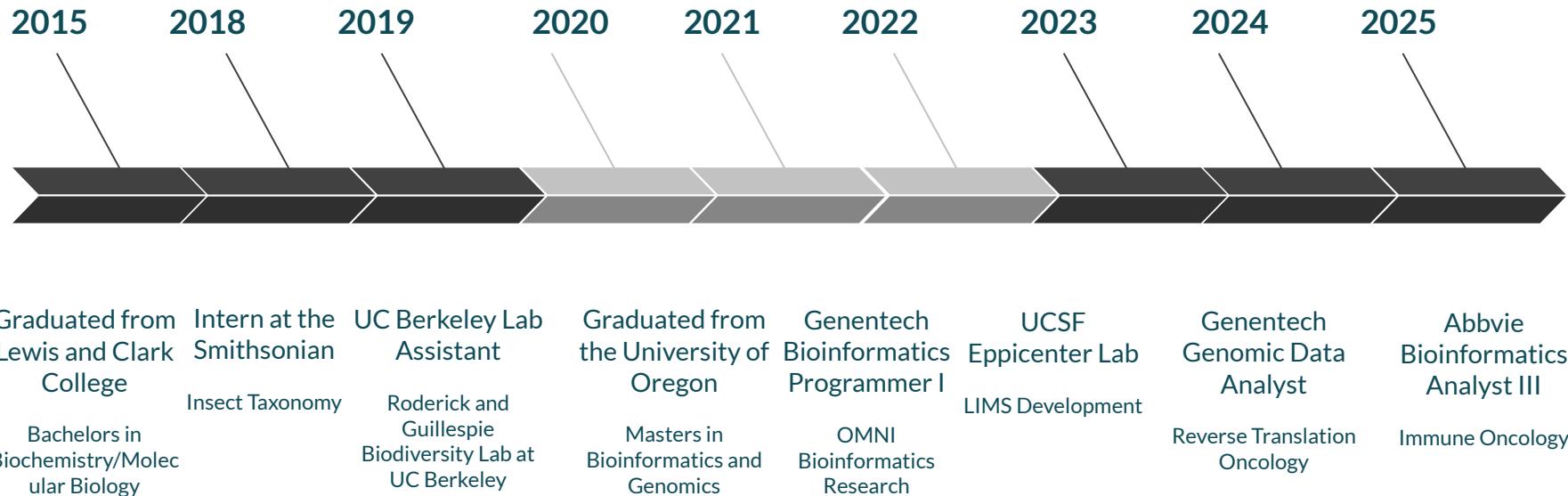


ONCOLOGY
REVERSE TRANSLATION

Methods Overview of BRIM Trial Analysis

Severiano Villarruel June 29, 2023

Timeline



Introduction

Graduated
Biochemistry/Molecular
Biology - Lewis and
Clark College

Smithsonian
Intern -
Insect
Taxonomy

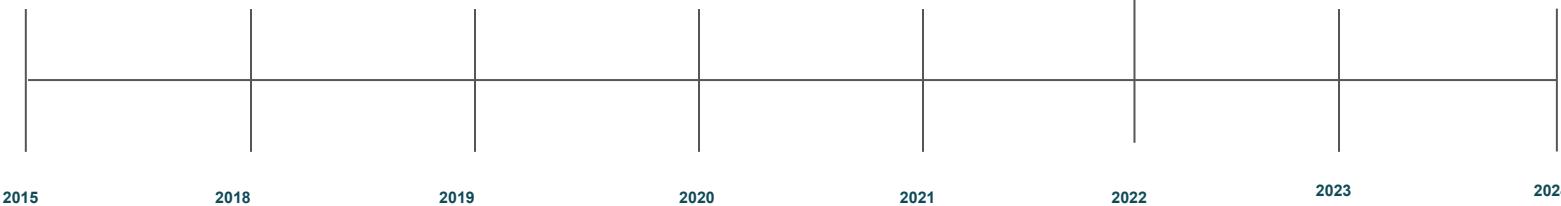
Worked in
Roderick/
Guillespie Lab - UC
Berkeley,
Biodiversity

U Oregon
Bioinformatics
and Genomics
MS

Genentech:
Pipeline Dev for
cfDNAmt data

UCSF:
LIMS Dev

Genentech:
RT Oncology



Background

Goals

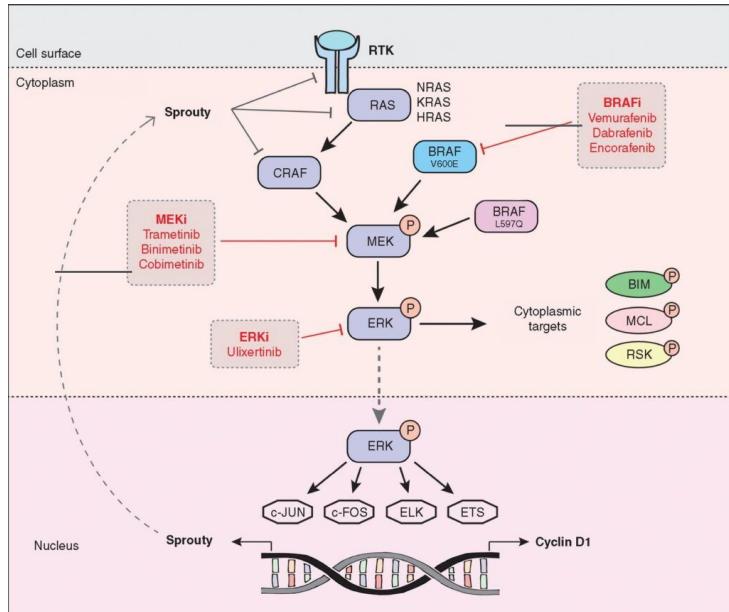
- Demonstrate example of using an advanced method to analyze data

Analysis Overview

- Clinical trial data/metadata
- scRNAseq Processing Method
- Differential abundance testing
- Differential gene expression

Background

Inhibitor Model

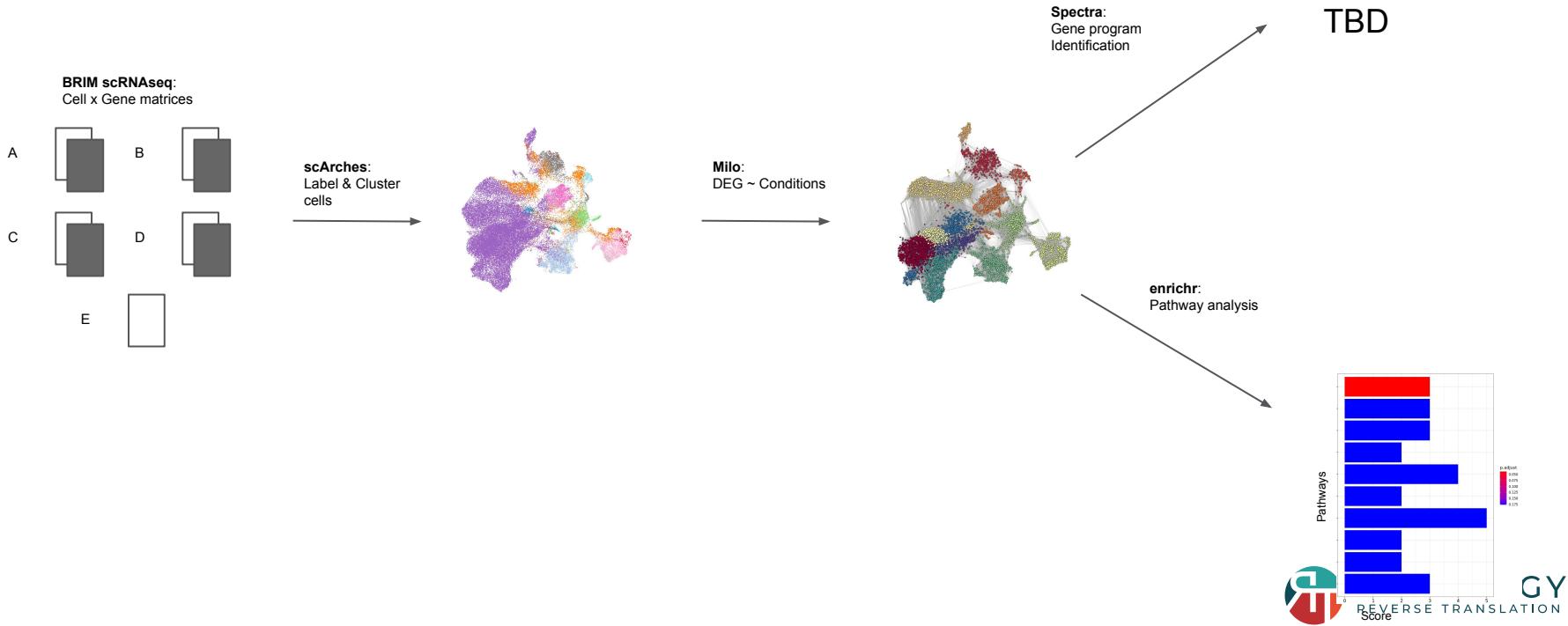


Clinical Metadata

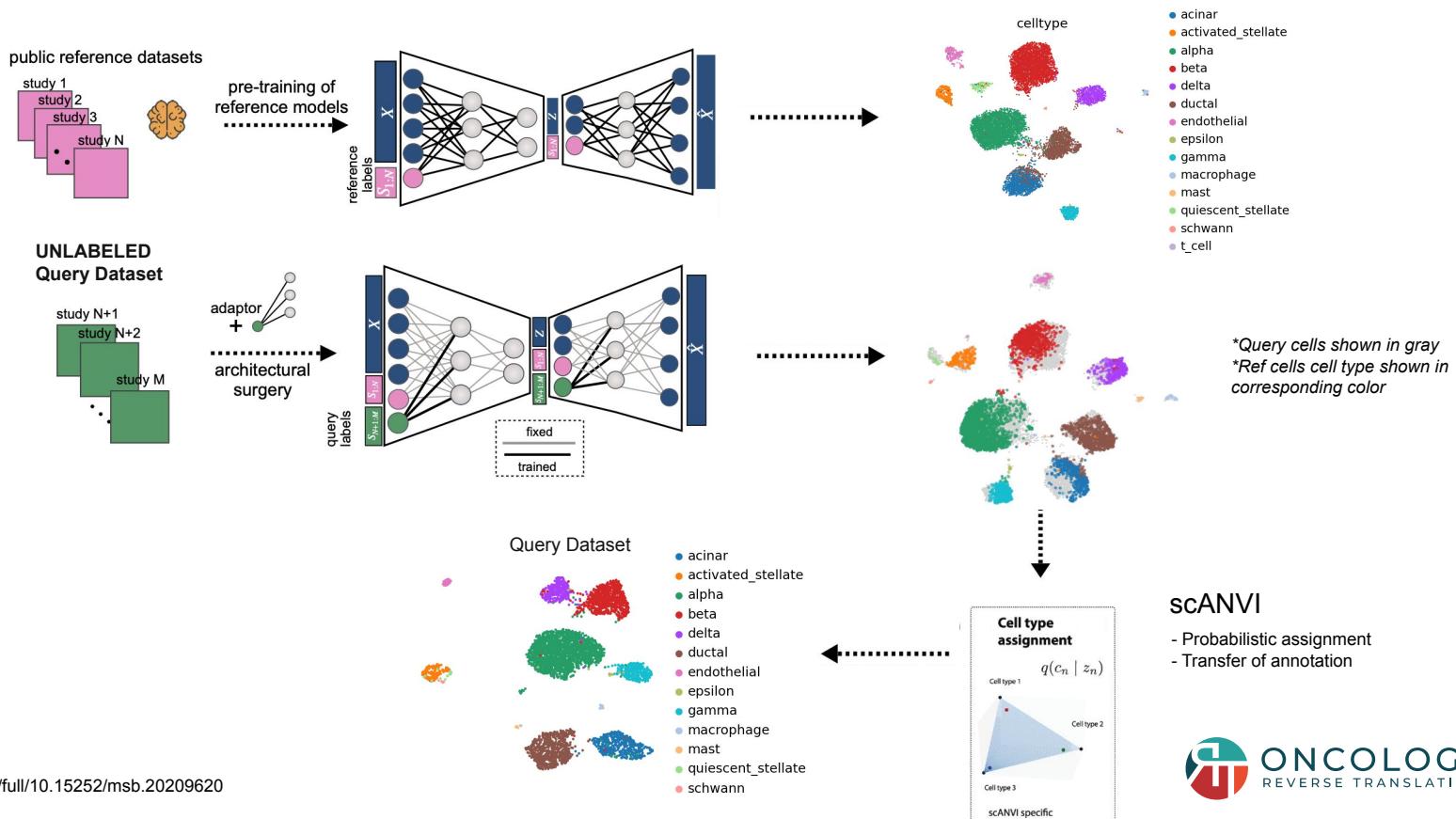
Response	Patient Id	Sample stage	BRAF mutation	Treatment	BORR
PD/SD	E Baseline	V600E	V(720)+C(60)	SD	
	D Baseline	V600E	V(960)+C(60)	SD	
	D C2D15				
	C Baseline	V600E	Prior BRAFi, C(60)	SD	
	C C2D15				
CR/PR	B Baseline	V600E	V(960)+C(60)	CR	
	B C2D15				
	A Baseline	V600E	V(720)+C(60)	PR	
	A C1D14				

Smalley and Smalley, Cancer Discovery, 2018

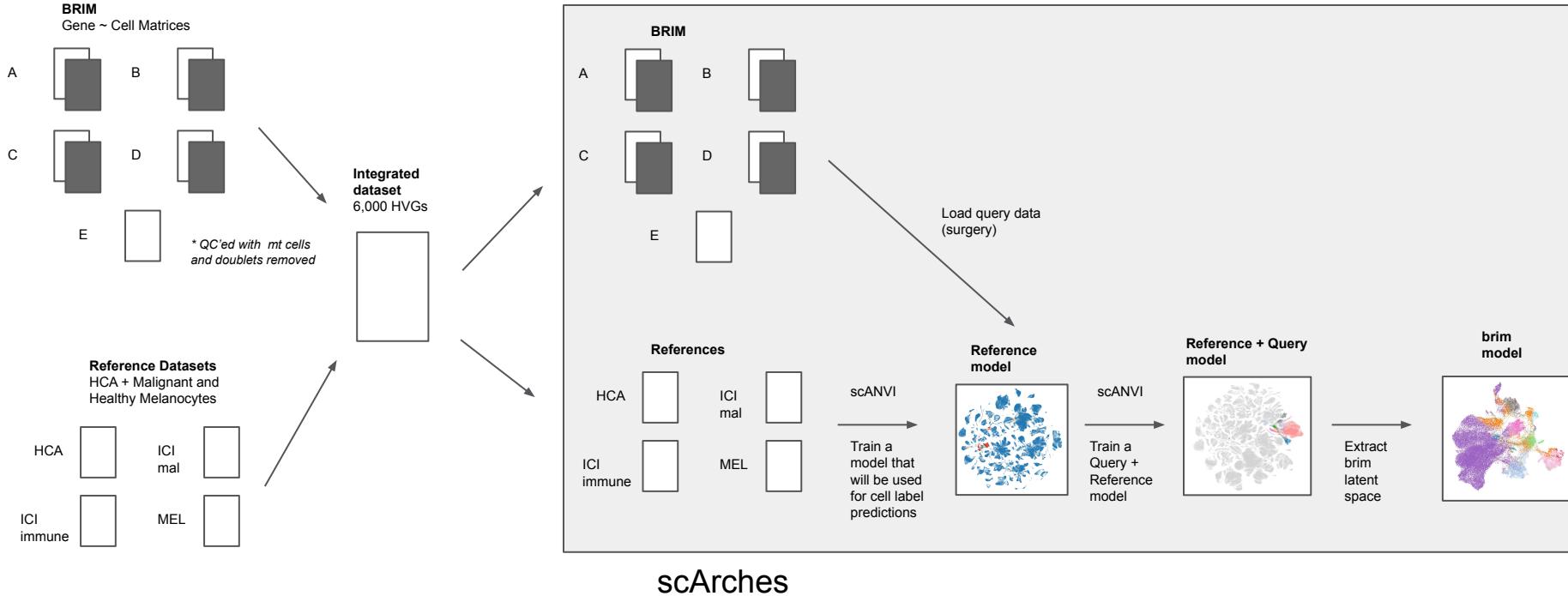
Processing + Analysis Overview



scArches: Map query cells to reference latent space

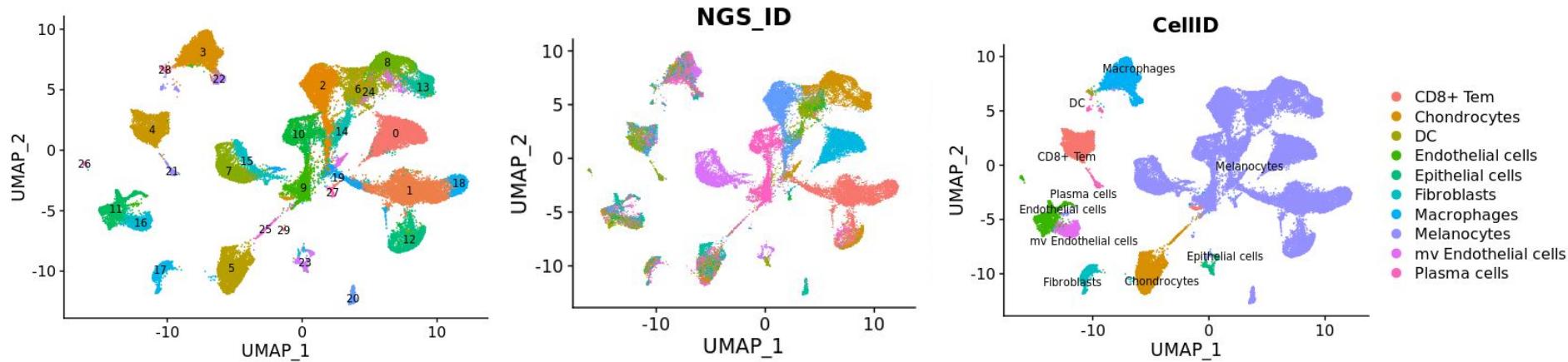


Label and Cluster Cells with scArches



Validation: Integration using Seurat v3 reciprocal PCA integration

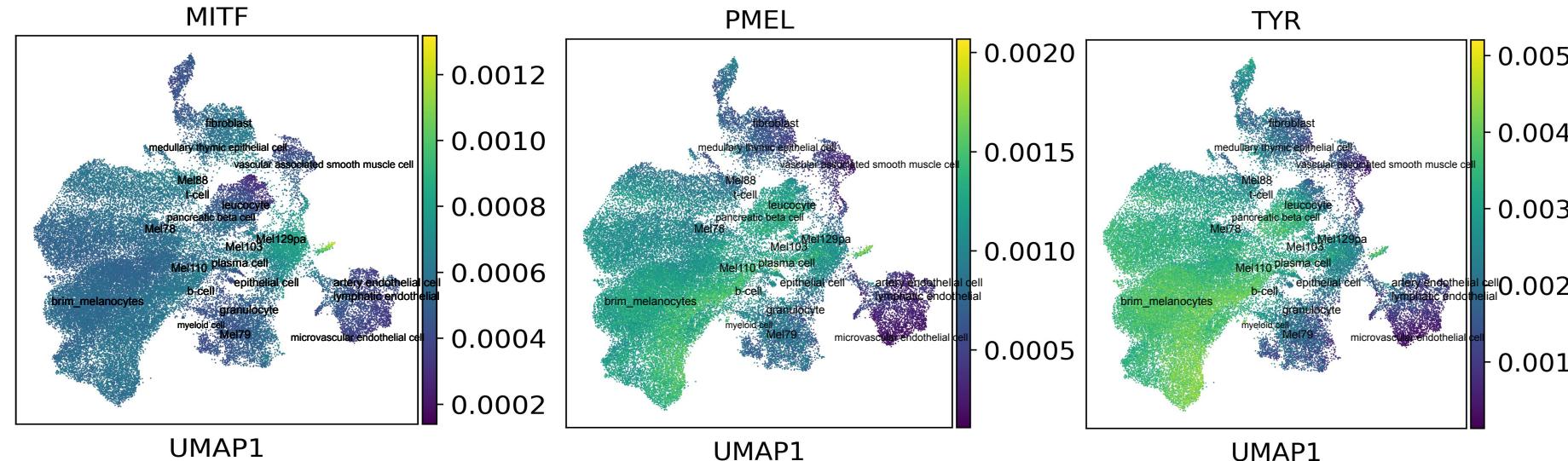
2 Samples with the best resolution used as reference datasets (27-652044 & 27-652047)



Small patient numbers (2 responders vs. 2 non-responders) represent a challenge uncovering robust biology associated to response.

Cell annotations were added with BlueprintEncode reference using SingleR

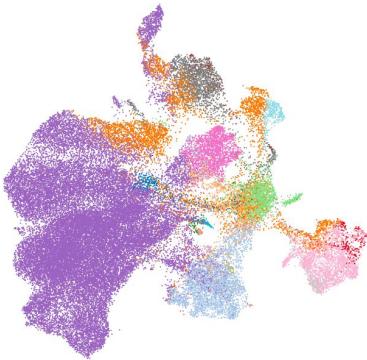
Validation: Melanoma marker gene expression (MITF, PMEL, TYR)



*using normalized (decoded)
gene expression from scANVI
model

BRIM Metadata Analysis: UMAP embedding of latent space from model post surgery

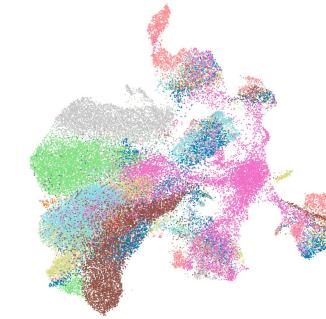
predictions



- Mel78
- Mel79
- Mel88
- Mel103
- Mel110
- Mel129pa
- endothelial cell
- b-cell
- brim_melanocytes
- epithelial cell
- fibroblast

- granulocyte
- leucocyte
- lymphatic endothelial
- medullary thymic epithelial cell
- microvascular endothelial cell
- myeloid cell
- pancreatic beta cell
- plasma cell
- t-cell

pat_labs



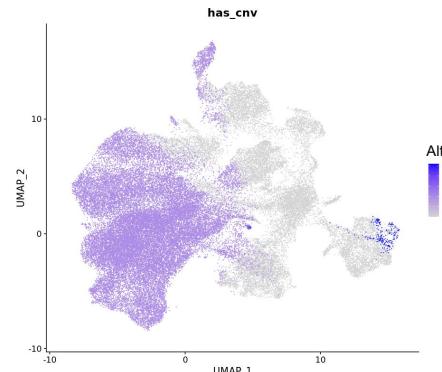
- A_Baseline_PR
- A_C1D14_PR
- E_Baseline_SD
- C_Baseline_SD
- C_C2D15_SD
- B_Baseline_CR
- B_C2D15_CR
- D_Baseline_SD
- D_C2D15_SD

brim_Treatment



- Prior BRAFi C(60)
- V(720)+C(60)
- V(960)+C(60)

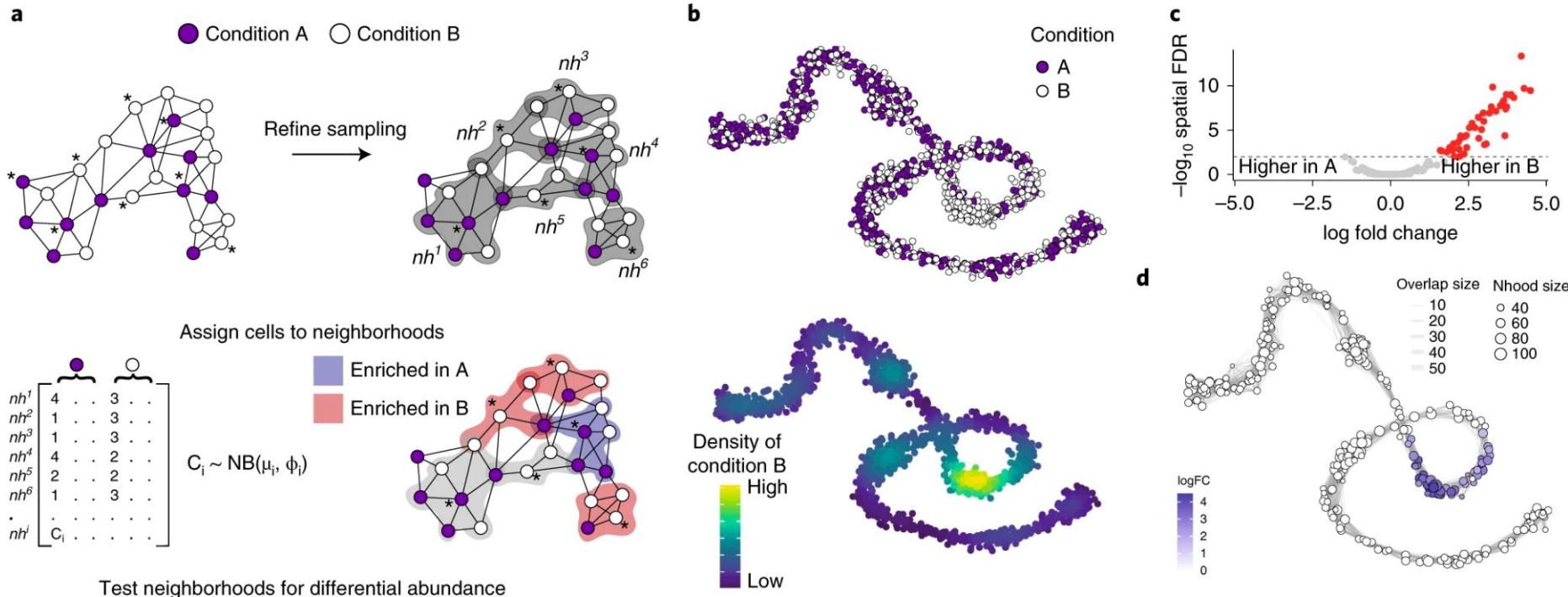
Cells with CN alteration



Transition to DA



Milo Overview: Differential Abundance Testing using a Condition Matrix



Differentially abundance between CR_PR vs SD_PD

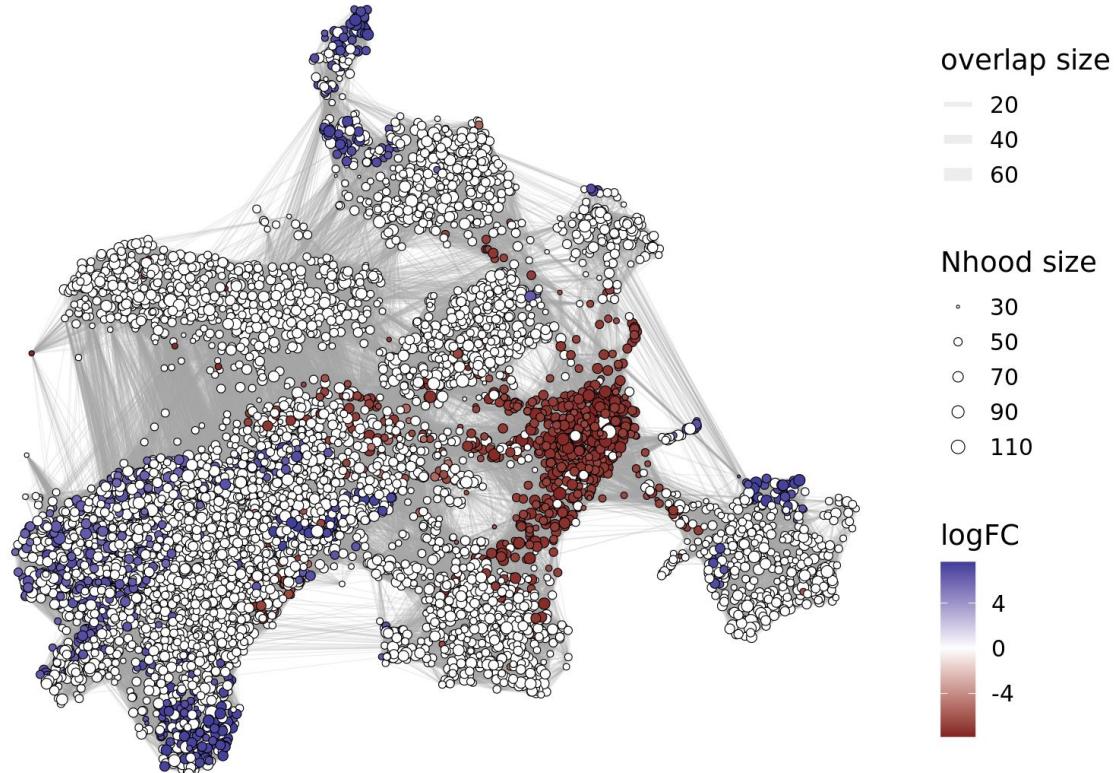
Design = ~ Response

Condition Matrix

Patient	sample	stage	Response
D	Baseline		SD PD
A	Baseline		CR PR
A	FollowUp		CR PR
C	Baseline		SD PD
D	FollowUp		SD PD
C	FollowUp		SD PD
B	FollowUp		CR PR
B	Baseline		CR PR

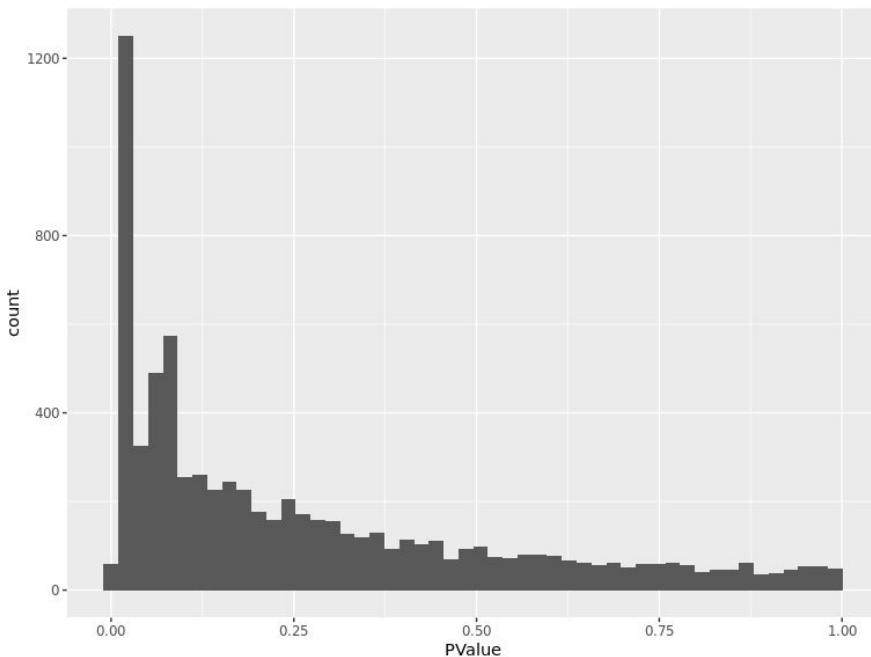
$k = 30$

reduced_dim = "X_scANVI"

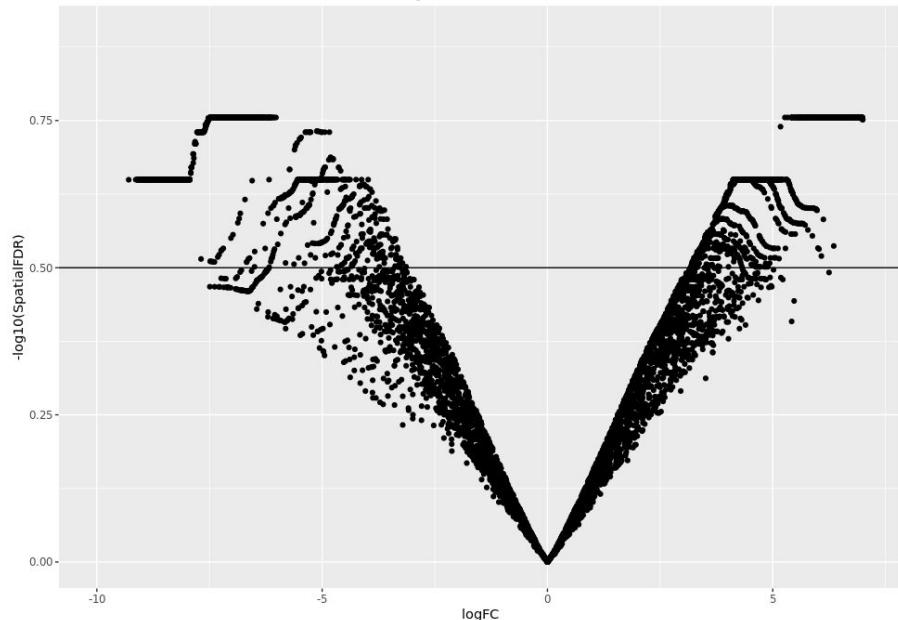


1565/7380 neighborhoods where differentially abundant between BOR responders and non-responders (CR_PR vs SD_PD)

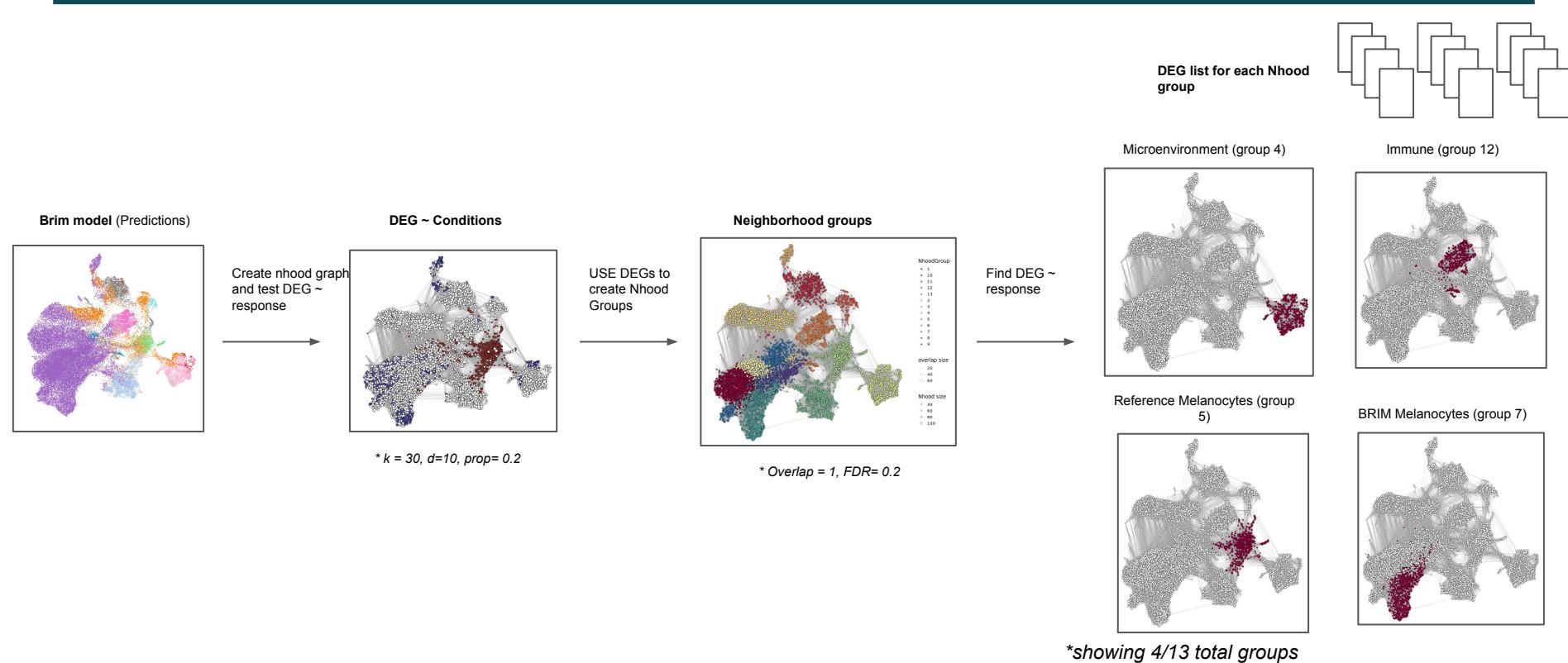
Distribution of uncorrected p values



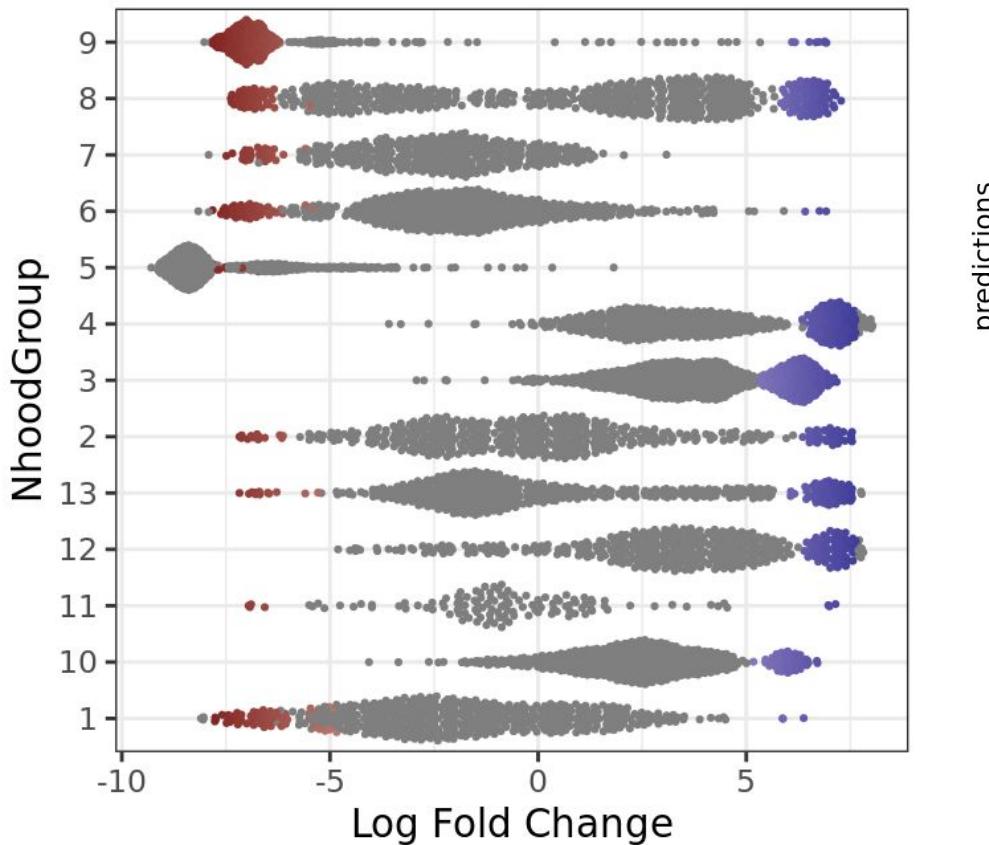
Volcano plot of differentially abundant neighborhoods



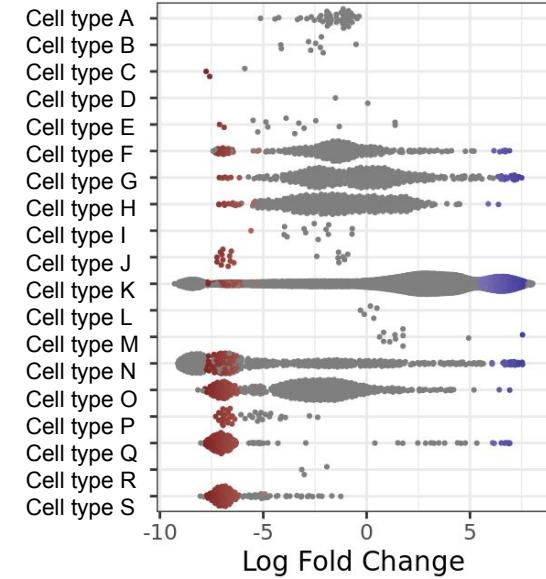
Investigate Differential Gene Expression using Milo



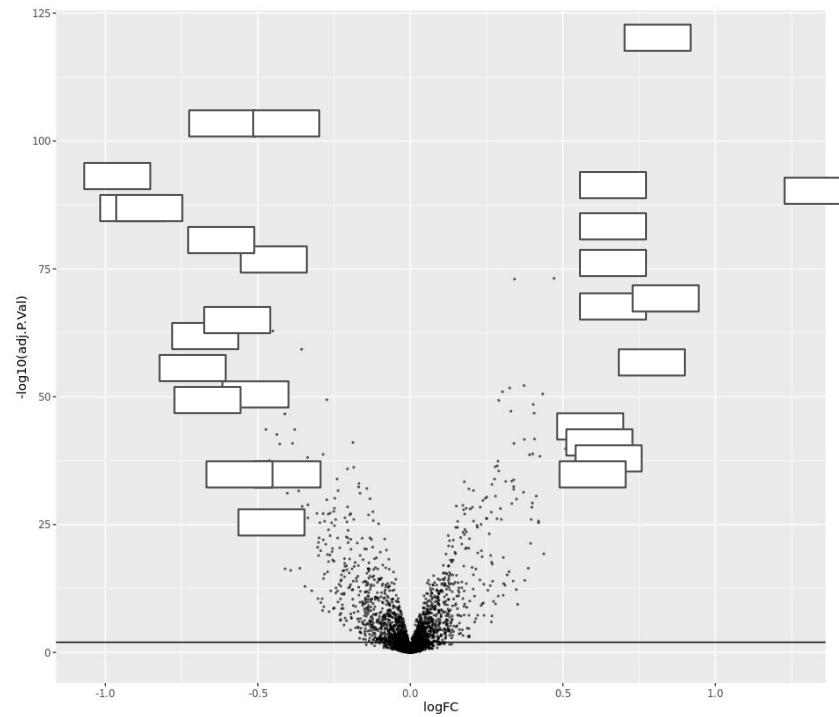
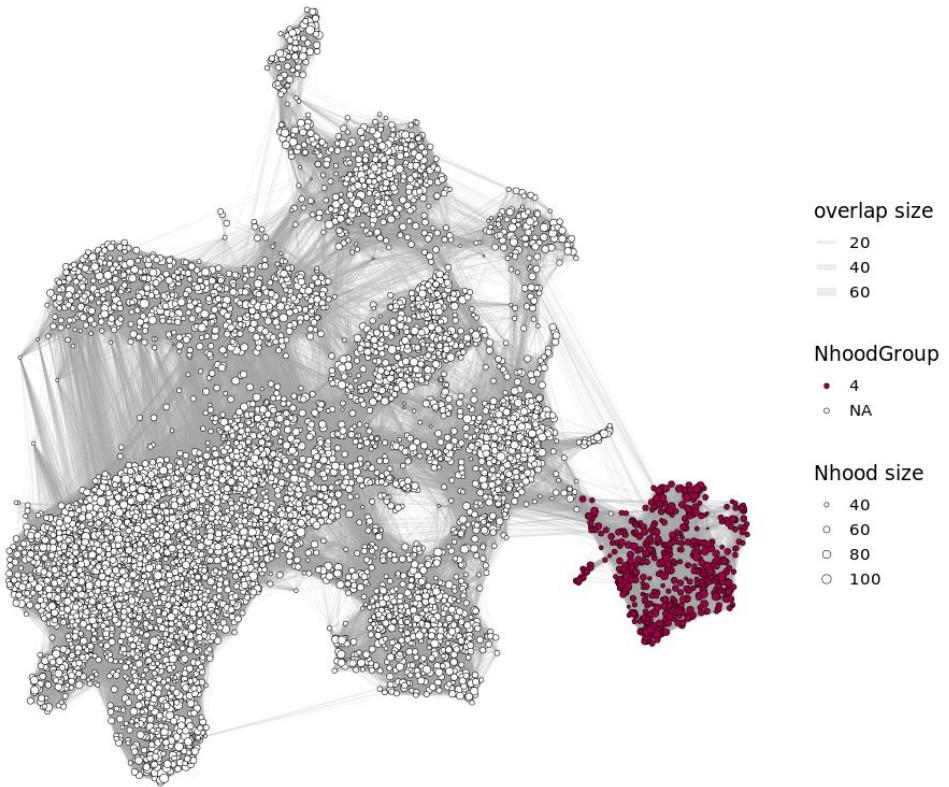
Differential abundance on top 2000 most variable gene with spatial FDR < 0.2



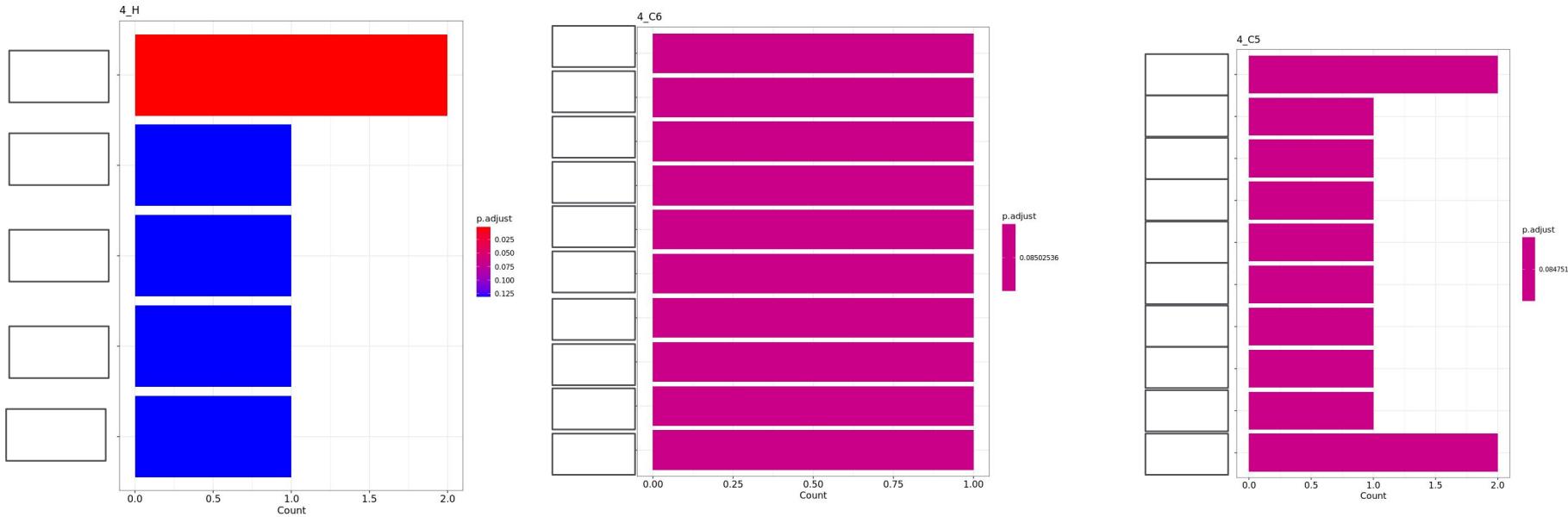
predictions



~ response specific changes in BRIM group 4 endothelial cells



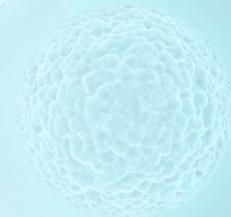
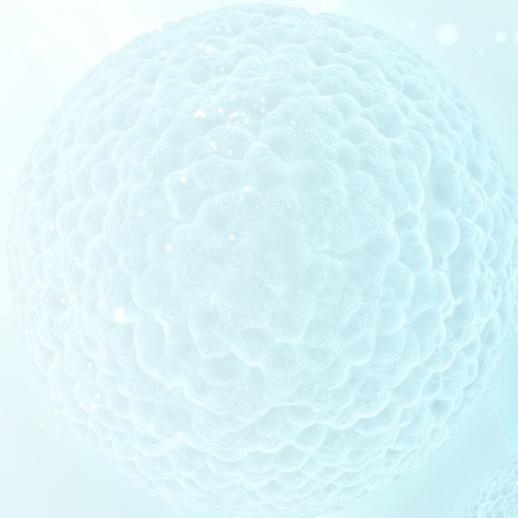
GSEA pathways enriched in endothelial cells ~response





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THANK YOU



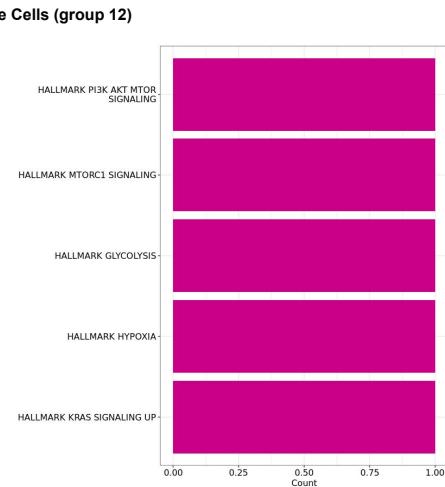
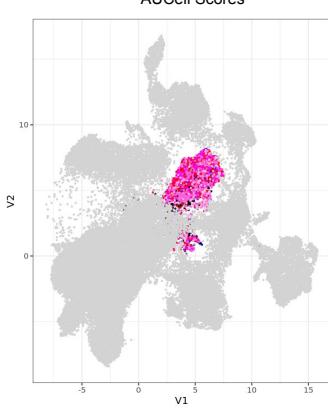
Wrap Up

Conclusions

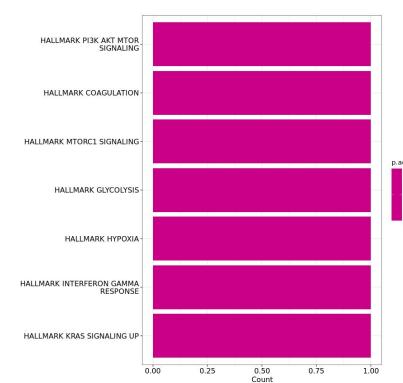
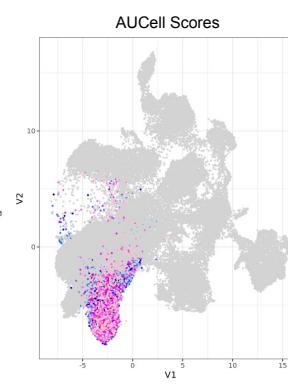
-
- **Identification of pathways associated with response in BRIM samples**
-

Gene Set for Differentially Expressed Genes in Nhood Groups

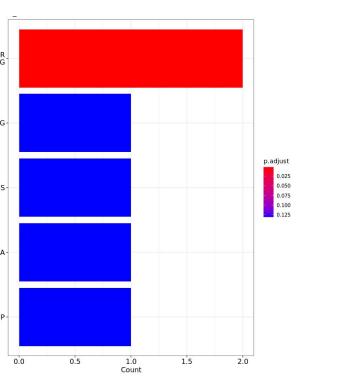
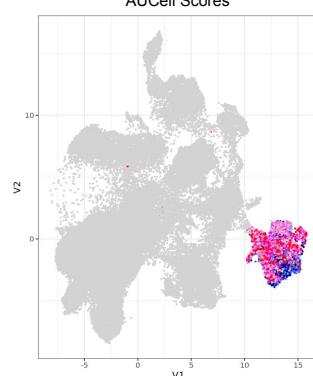
AUCCell Scores
Immune Cells (group 12)



AUCCell Scores
BRIM Melanocytes (group 7)

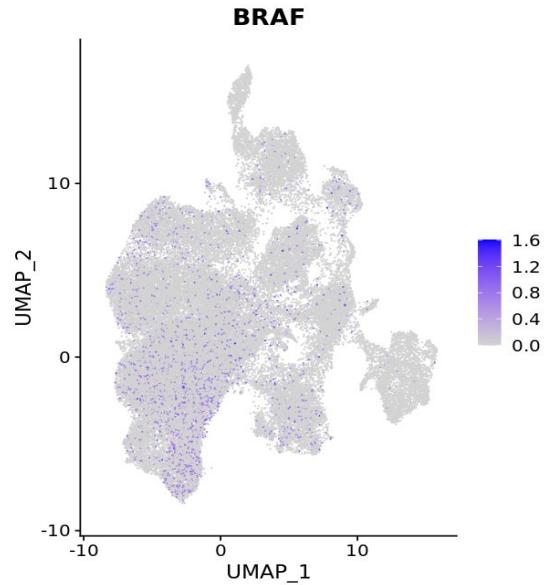


AUCCell Scores
Micro Environment Cells (group 4)

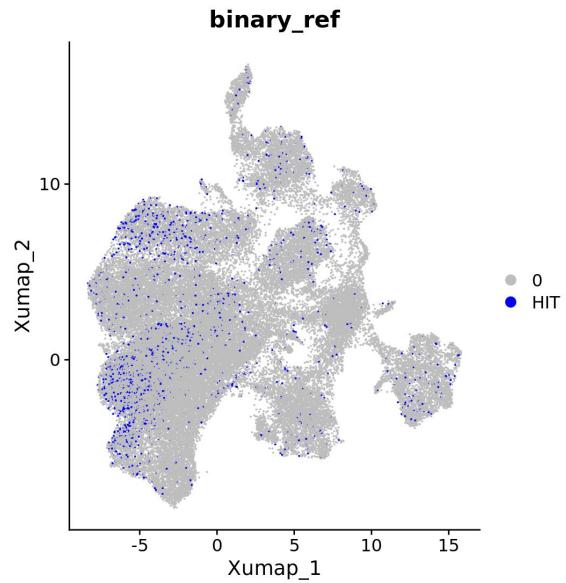


BRAF Analysis

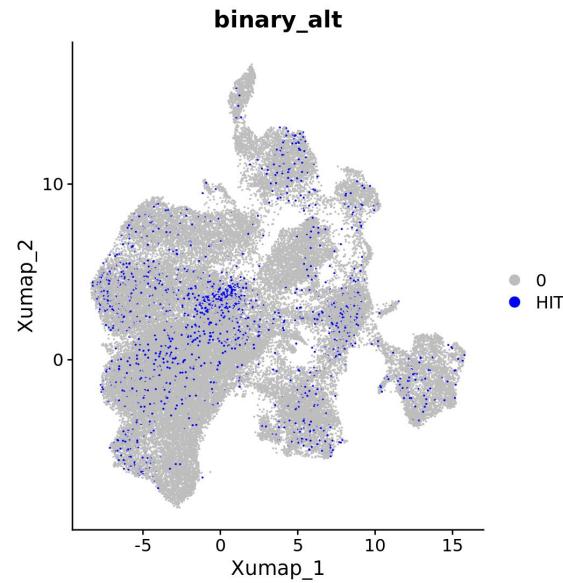
BRAF expression (normalized)



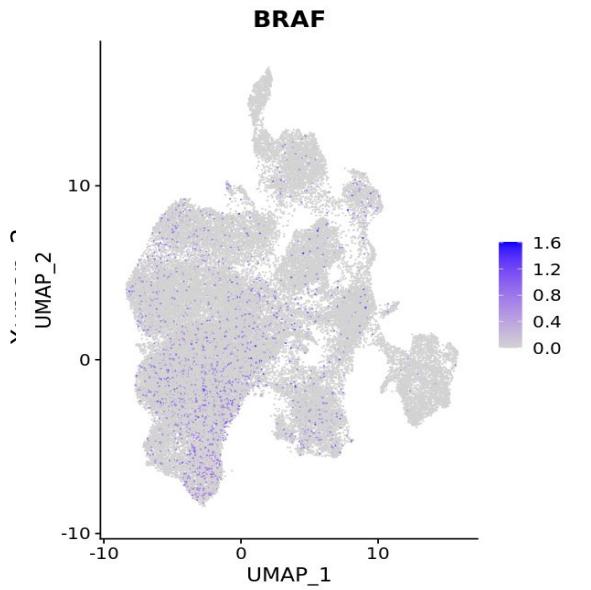
BRAF V600 ref



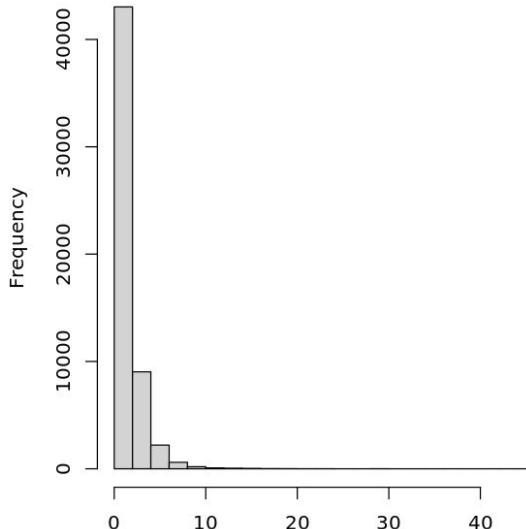
BRAF V600 alt



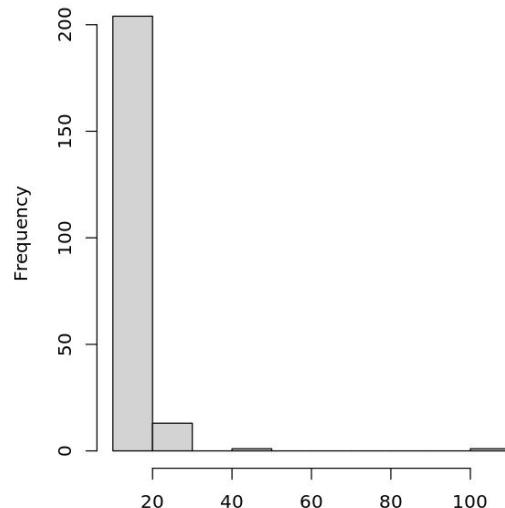
BRAF expression



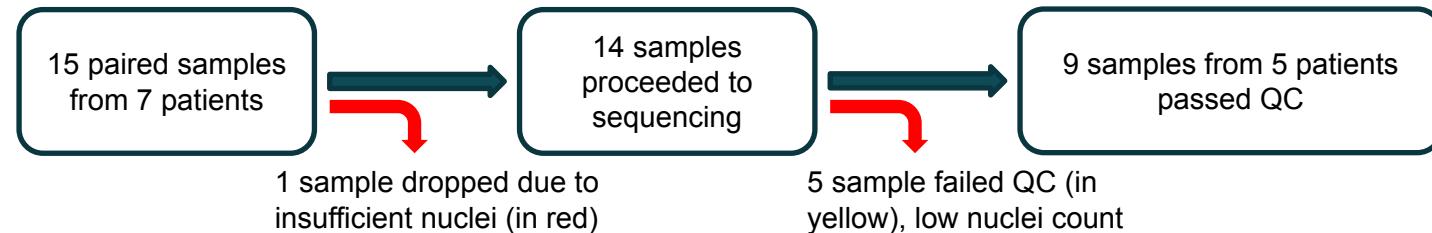
Histogram of BRAF expression



Histogram of BRAF expression
(removing cells w less than 10 counts)



Overview of snRNAseq data generated from BRIM samples

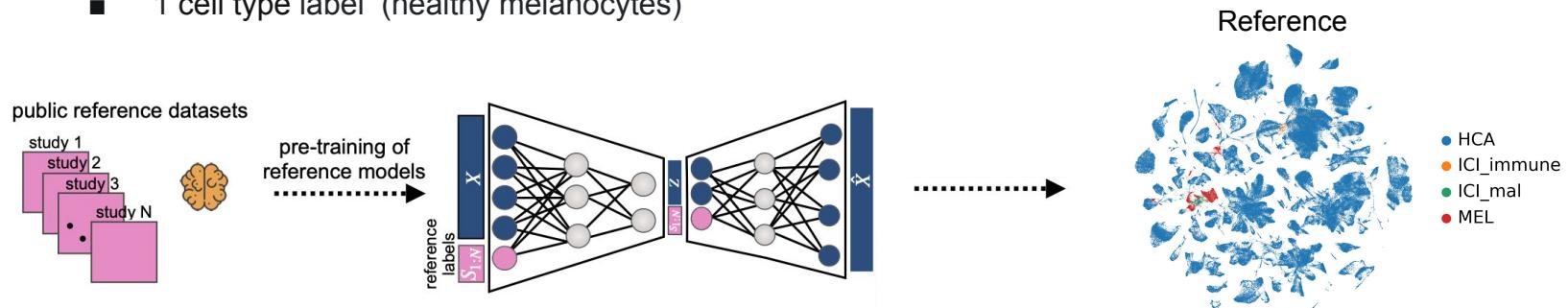


Response	Sample #	Study Id	Patient Id	stage	BRAF mutation	Treatment	BORR	Notes
PD/SD	04240193N	NO25395	1154	Baseline	V600E	Prior BRAFi V(720)+C(60)	PD	Minimal tissue, <4mg
	04240194N	NO25395	1154	C2D15				Minimal tissue, large amount of OCT left
	04240195N	NO25395	1155	Baseline	V600E	V(720)+C(60)	SD	
	04240196N	NO25395	1155	C2D15				Very little tissue found
	05320283N	GO28141	2446	Baseline	V600E	V(960)+C(60)	SD	
	05320282N	GO28141	2446	C2D15				
	04240222N	NO25395	1358	Baseline	V600E	Prior BRAFi, C(60)	SD	
CR/PR	04240217N	NO25395	1358	C2D15				
	03660222N	NO25026	1451	Baseline	V600E	V(960)+C(60)	CR	
	04240208N	NO25395	1451	C2D15				Large amount of OCT left
	04240212N	NO25395	1451	C2D15				
	04240220N	NO25395	1360	Baseline	V600E	V(960)+C(60)	PR	Shave of CNB, large OCT left
	04240221N	NO25395	1360	C2D15				Minimal tissue
	04240201N	NO25395	1056	Baseline	V600E	V(720)+C(60)	PR	
	04240203N	NO25395	1056	C1D14				

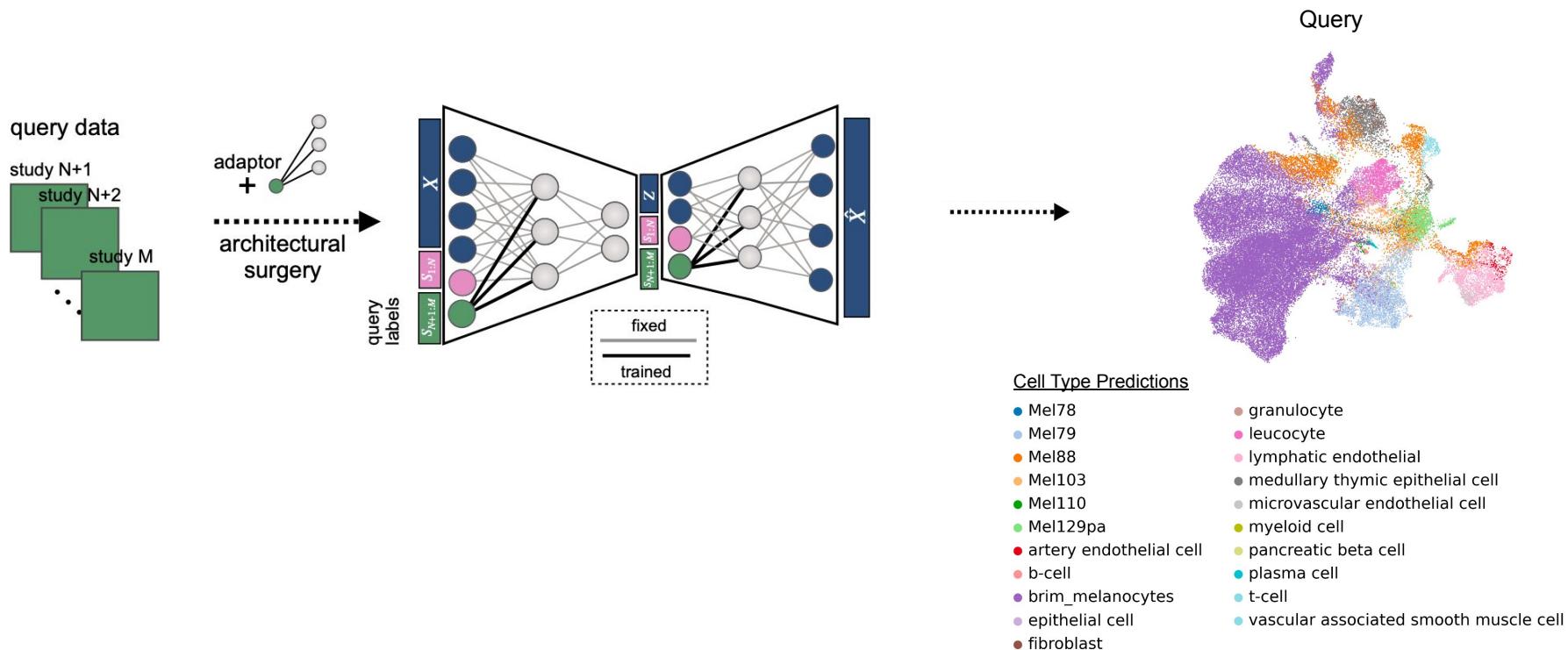
Overview of snRNAseq data generated from BRIM samples

Create a Reference Model

- **Reference Model generated from integration of 4 datasets**
 - HCA: Tabula Sapiens
 - 500,000 cells from 24 organs of 15 normal donors
 - 161 cell type labels
 - ICI_immune: Immune cells of melanoma patients resistant to immune checkpoint inhibitor
 - 5,168 cells from 31 patients
 - 6 cell type labels
 - ICI_mal: Malignant cells melanoma patients resistant to immune checkpoint inhibitor
 - 2,018 cells from 31 patients
 - 1 cell type label (malignant melanocytes)
 - MEL: Healthy human melanocytes
 - 14,370 cells 37 healthy skin samples across multiple anatomical locations from 22 donors
 - 1 cell type label (healthy melanocytes)

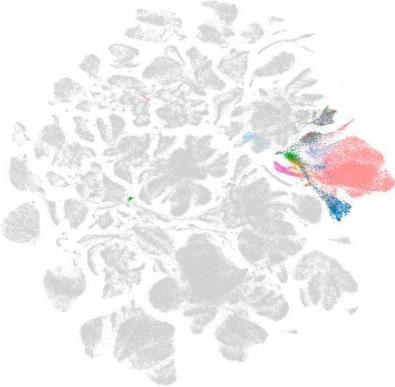


Perform Architectural Surgery using Query cells



Reference and Query annotated dataset used for BRIM sc analysis

predictions

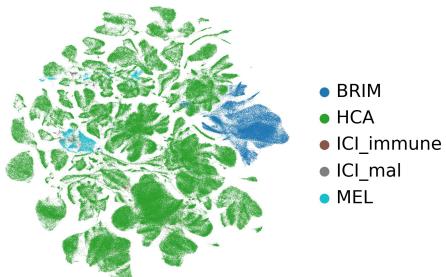


- Mel78
- Mel79
- Mel81
- Mel88
- Mel102
- Mel103
- Mel110
- Mel129pa
- Mel194
- adventitial cell
- artery endothelial cell
- b-cell

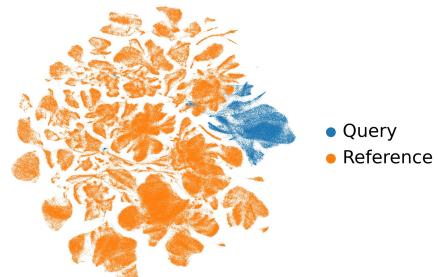
- brim_melanocytes
- cd8b-positive nk t cell
- ciliated cell
- cyc_mel
- endothelial cell of vascular tree
- epithelial cell
- fibroblast
- granulocyte
- hepatocyte
- ionocyte
- leucocyte
- luminal cell of prostate epithelium

- lymphatic endothelial
- medullary thymic epithelial cell
- microvascular endothelial cell
- muscle cell
- myeloid cell
- pancreatic beta cell
- plasma cell
- slow muscle cell
- stromal cell
- t-cell
- vascular associated smooth muscle cell
- NA

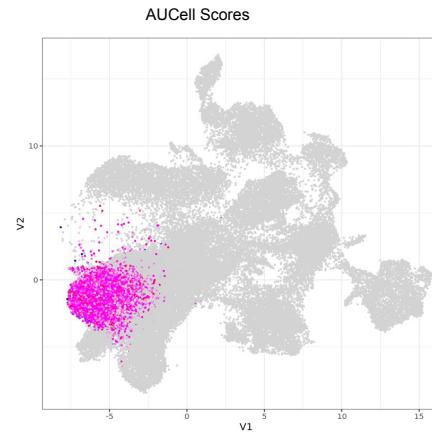
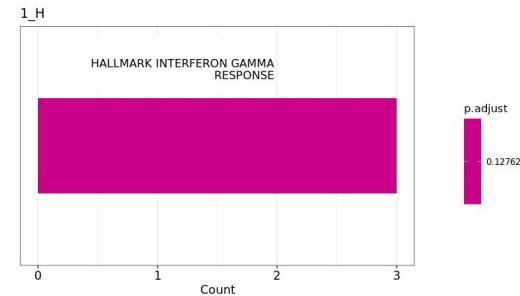
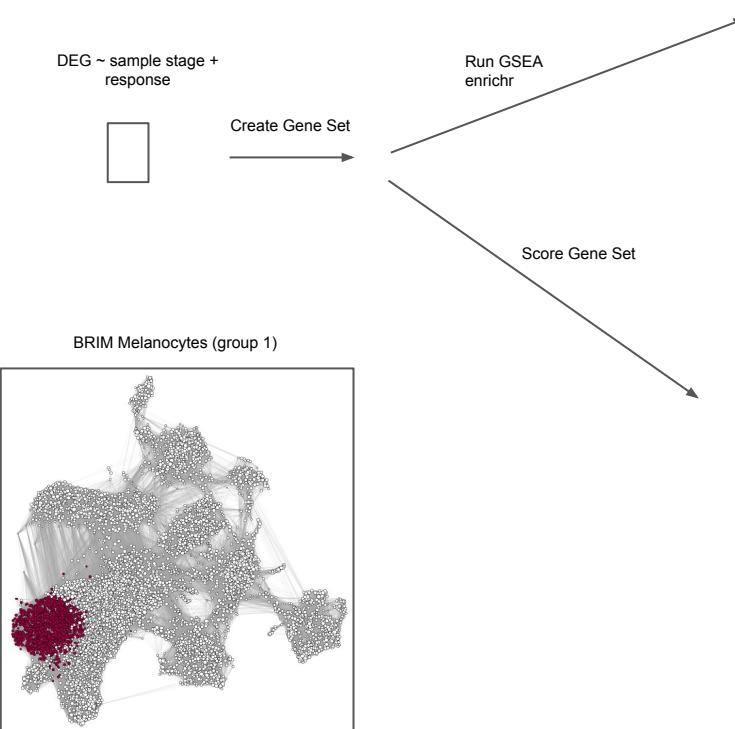
dataset_name



batch



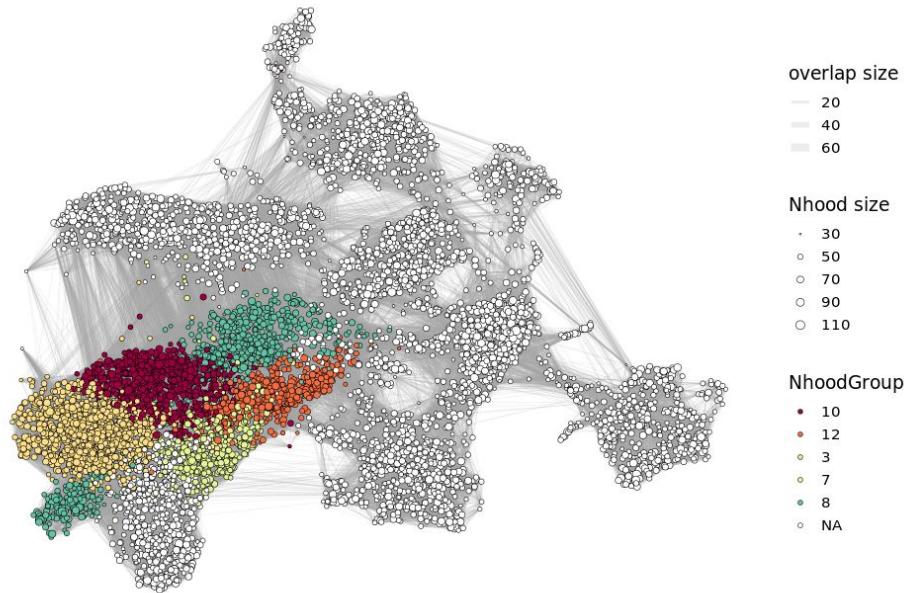
GSEA Analysis Overview



Positive Scores: Magenta
Negative Scores: Blue

* gene set was created by filtering genes that met criteria
adj.P.Val < 0.05 & abs(logFC) > log2(2)

~ sample stage + response specific changes in BRIM melanocyte specific nhood groups



overlap size

— 20
— 40
— 60

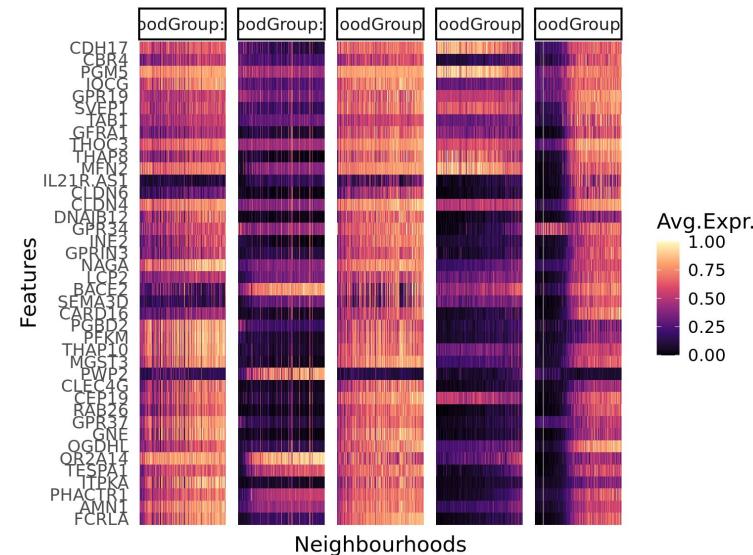
Nhood size

• 30
○ 50
○ 70
○ 90
○ 110

NhoodGroup

• 10
● 12
○ 3
○ 7
● 8
○ NA

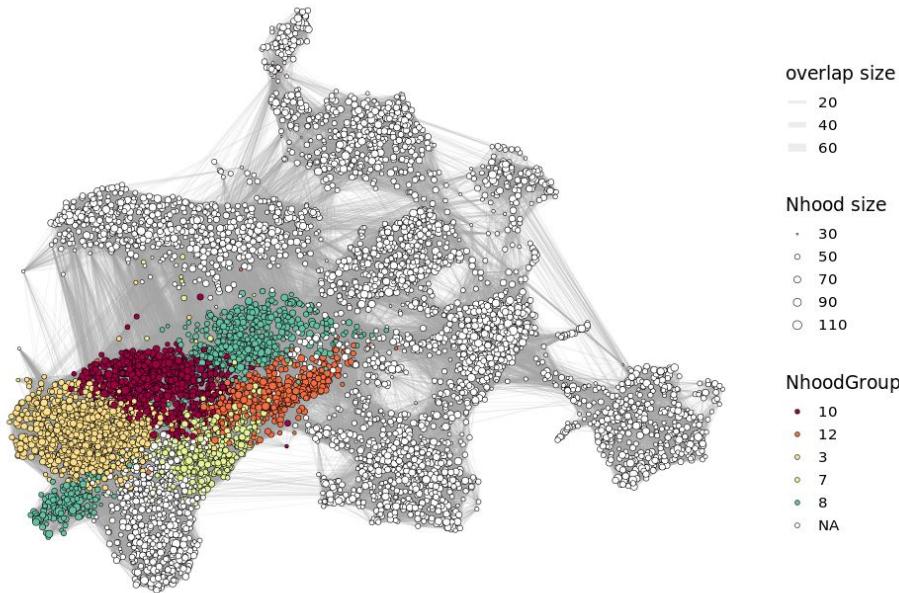
Union of DEG across all BRIM melanocyte nhood



DEG defined as adj.P.Val < 0.0001 & logFC > 1

Radia Johnson

~ sample stage + response specific changes in BRIM melanocyte specific nhood groups



overlap size

20
40
60

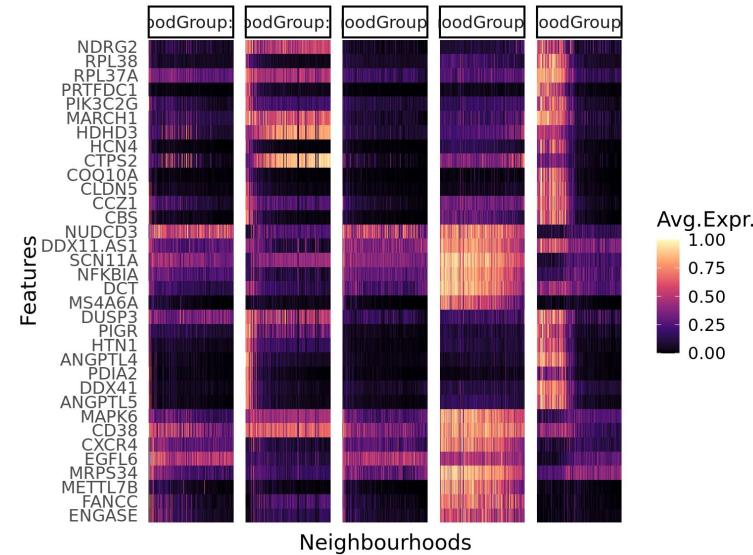
Nhood size

30
50
70
90
110

NhoodGroup

10
12
3
7
8
NA

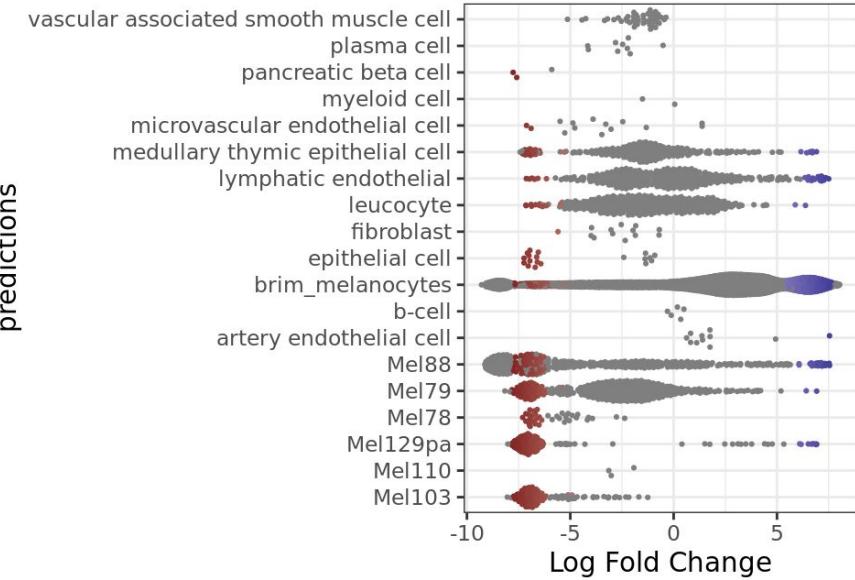
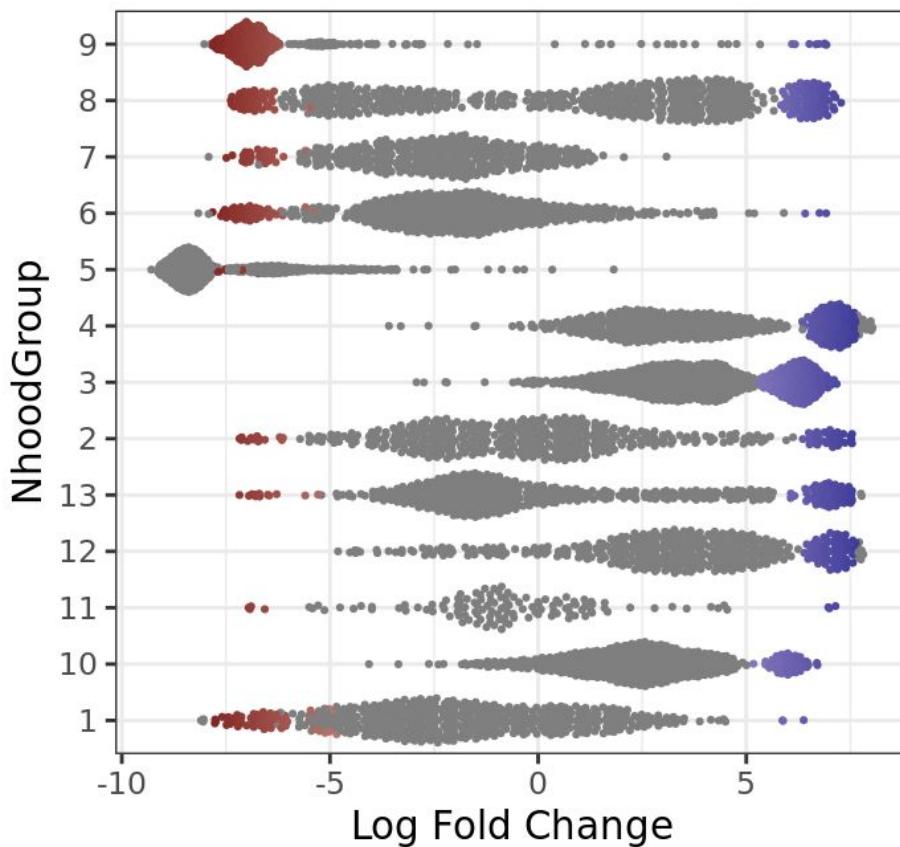
Union of DEG across all BRIM melanocyte nhoods



DEG defined as adj.P.Val < 0.0001 & logFC < -1

Radia Johnson

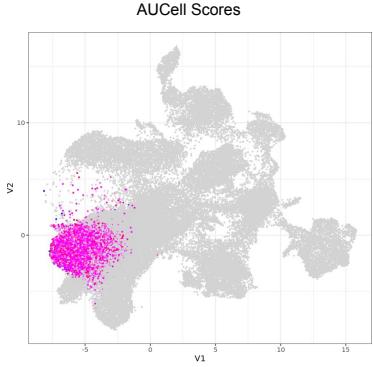
Differential abundance on top 2000 most variable gene with spatial FDR < 0.2



Published melanocyte (ICI_mal) [metadata](#)

Gene Set for Differentially Expressed Genes in Nhood Groups

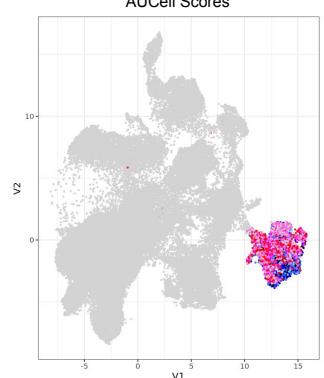
BRIM Melanocytes (group 1)



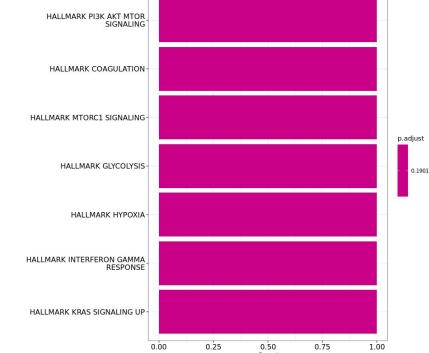
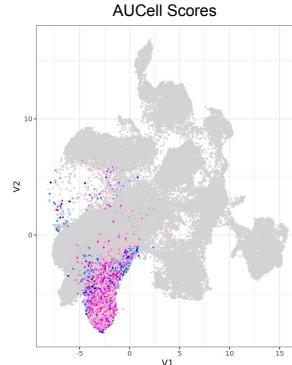
Immune Cells (group 12)



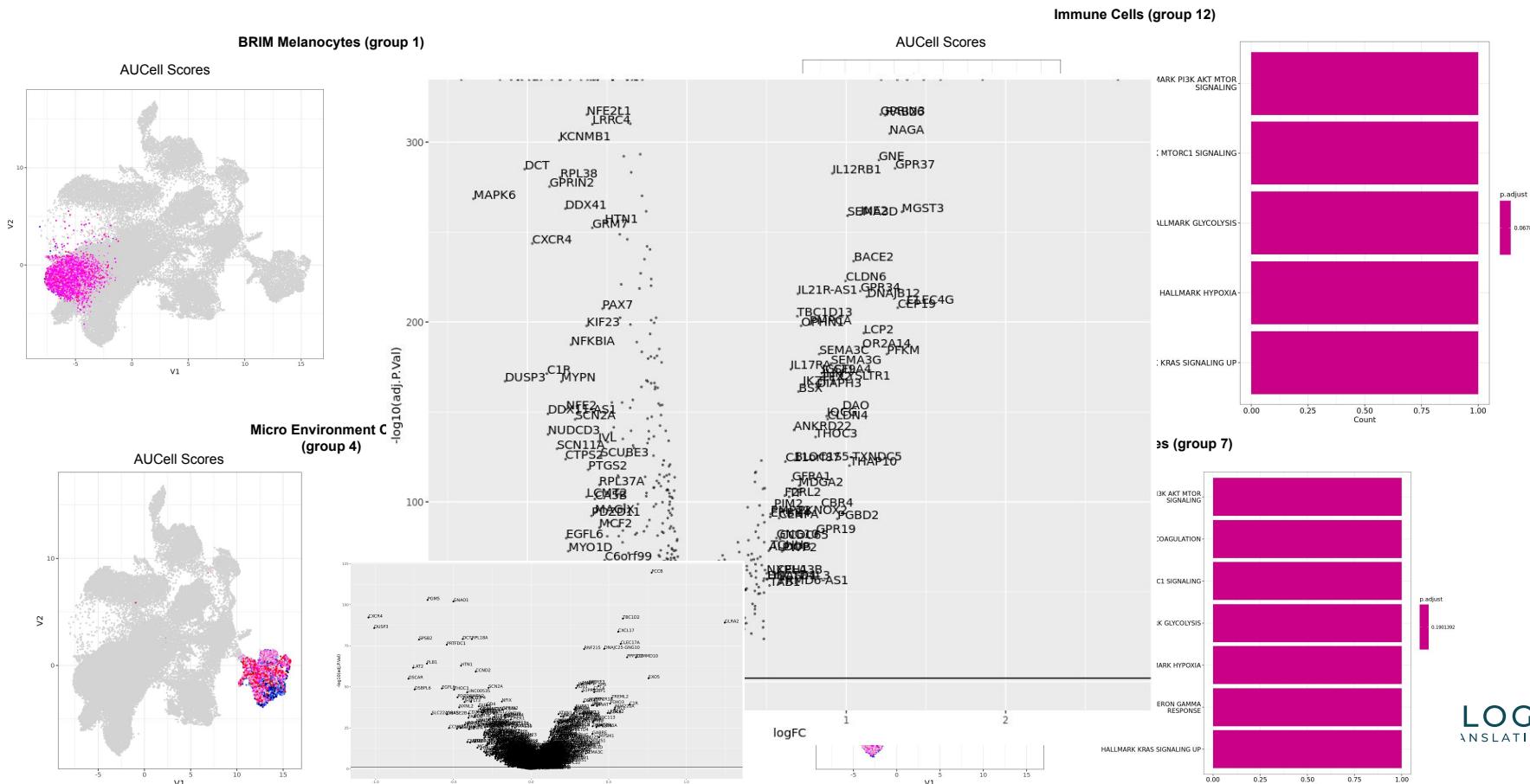
Micro Environment Cells (group 4)



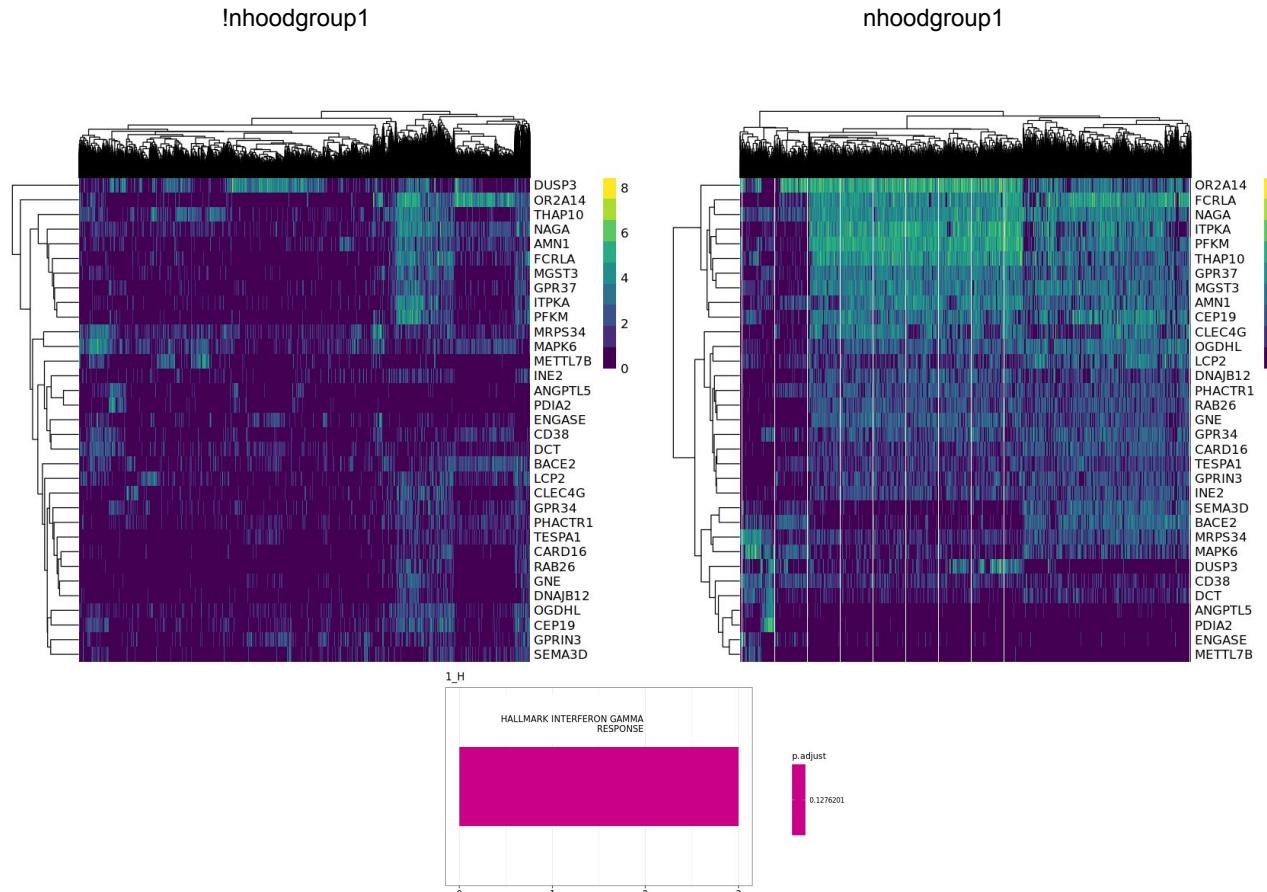
BRIM Melanocytes (group 7)



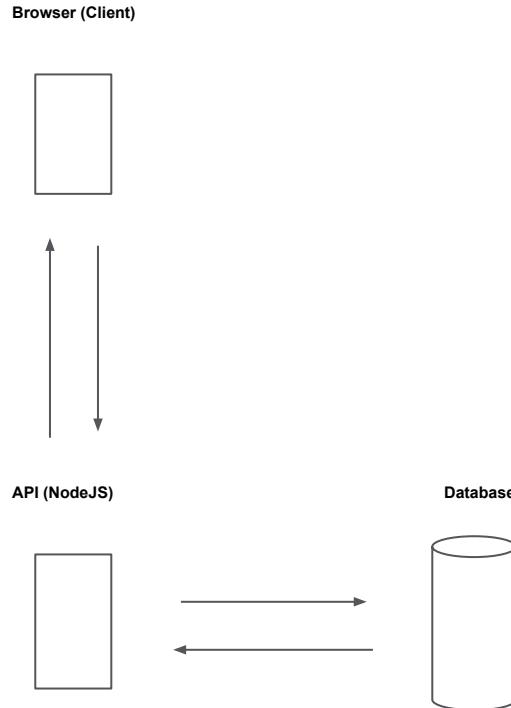
Gene Set for Differentially Expressed Genes in Nhood Groups

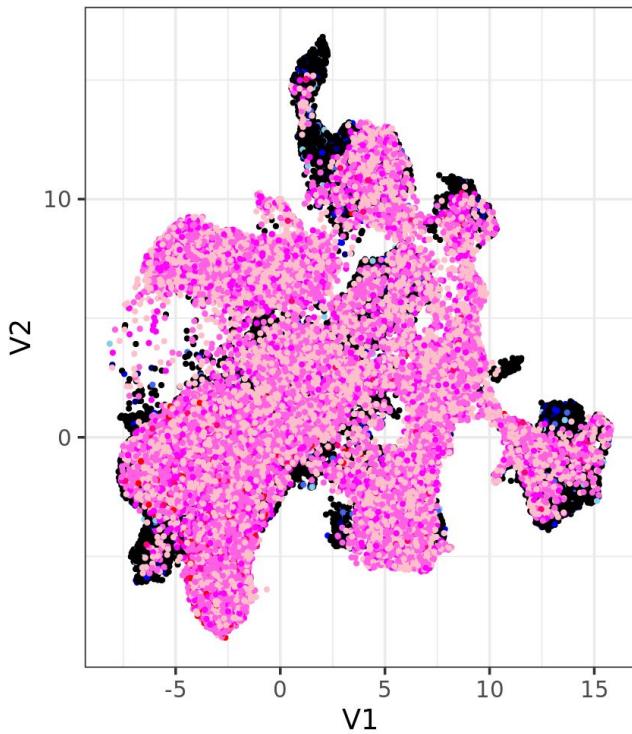


Nhoodgroup1 - looking at gene set estab. Thru ranking

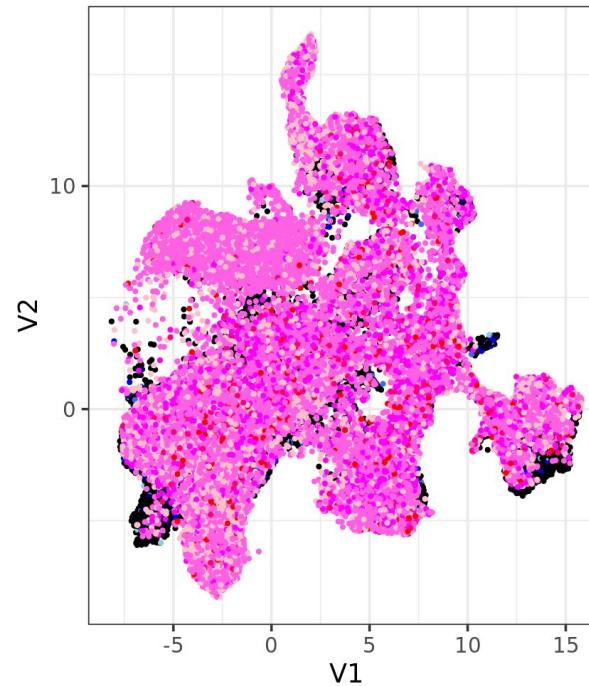


Web App Architecture





Interferon
Gamma



PI3K