Bringing single cell genomics closer to the clinic for patients with leukemia

Scott Furlan, MD (he/his) - 7-29-22
Assistant Professor, FHCRC

Overview and Disclosures

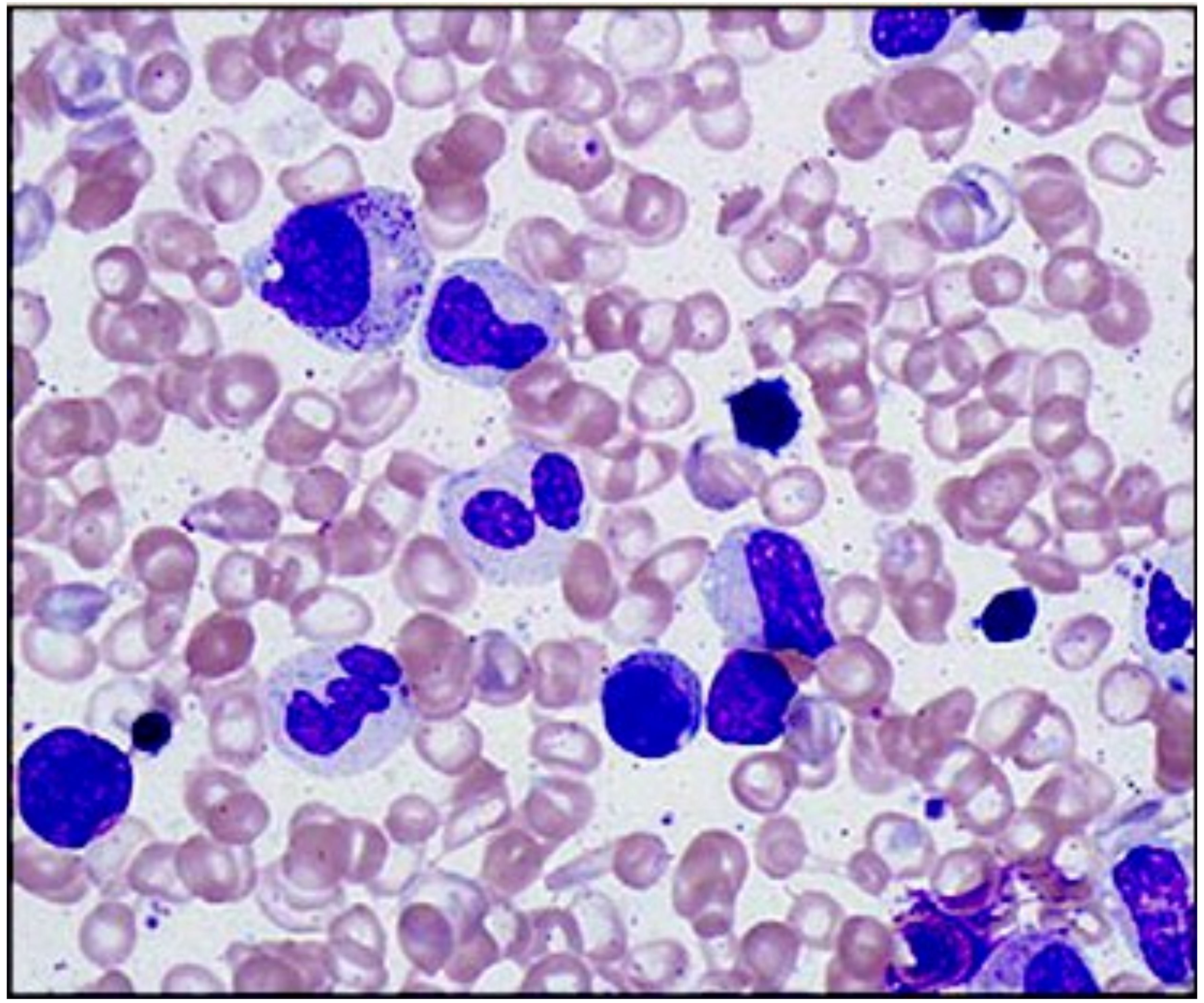
- Introduce the concept of measurable residual disease (MRD) as told from the of one patient
- Provide rationale for the use of single cell genomics to potentially improve MRD assessment after transplant
- Share our preliminary data using single cell RNA seq in patients with relapsed leukemia
  - Highlight novel molecular and computational approaches
- Broader applicability to understanding the biology of acute leukemia and mechanisms of relapse

No Conflicts of Interest or relevant disclosures!

Thank you __

A bedside to bench approach to a clinical conundrum

- 12 yo with a history of Myelodysplastic Syndrome diagnosed in late 2018
- Evolved to Acute Myeloid Leukemia (AML) shortly thereafter
- Matched Unrelated Bone Marrow Transplant in April 2019
- Two years later (April 2021), patient developed low blood counts on routine monitoring -> AML



Nguyen P.L. Hematol Oncol Clin North Am. 2009; 23: 675-691

A bedside to bench approach to a clinical conundrum

- After relapse, underwent reinduction chemotherapy and was being considered for a second transplant using cord blood.
- Flow cytometry 1-2%
- But his TP53mut (R248Q), IDH1mut (R132C) > 10% VAF.

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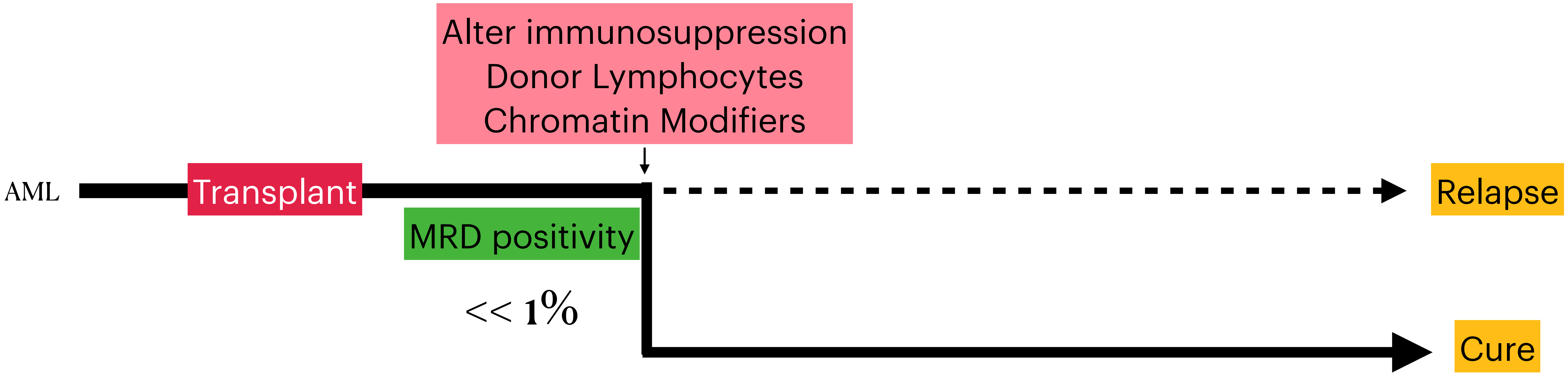

Alter immunosuppression
Donor Lymphocytes
Chromatin Modifiers

AML

Transplant

MRD positivity

<< 1%

Relapse

Cure

Graphic adapted from: Austin A, Byrne, M. *Current Opinion in Hematology.* 2021;28(6):380-388.

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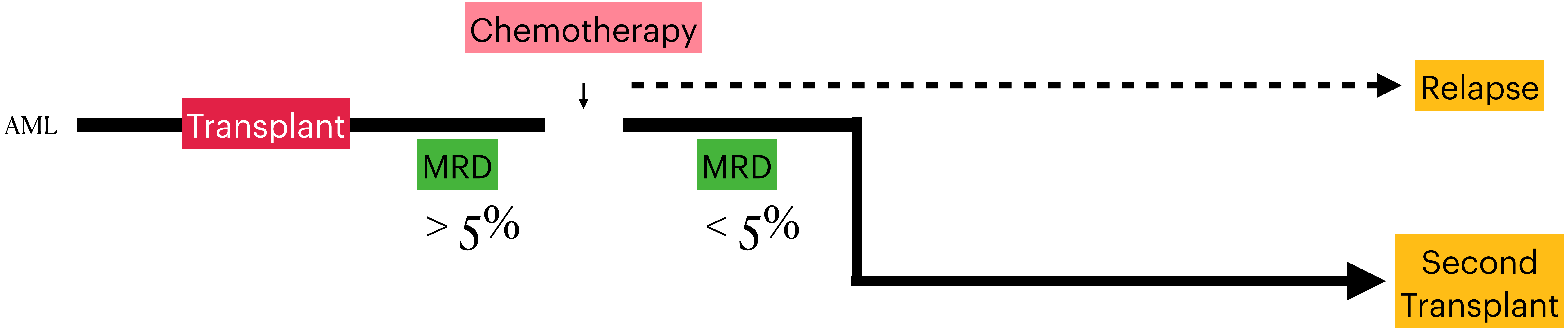

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What is the current status of MRD testing?

PCR

NGS

MFC

Chimerism Testing

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  - High sensitivity, but are limited in their applicability (A priori)

The 'long tail' of acute leukemia is a problem for molecular assays

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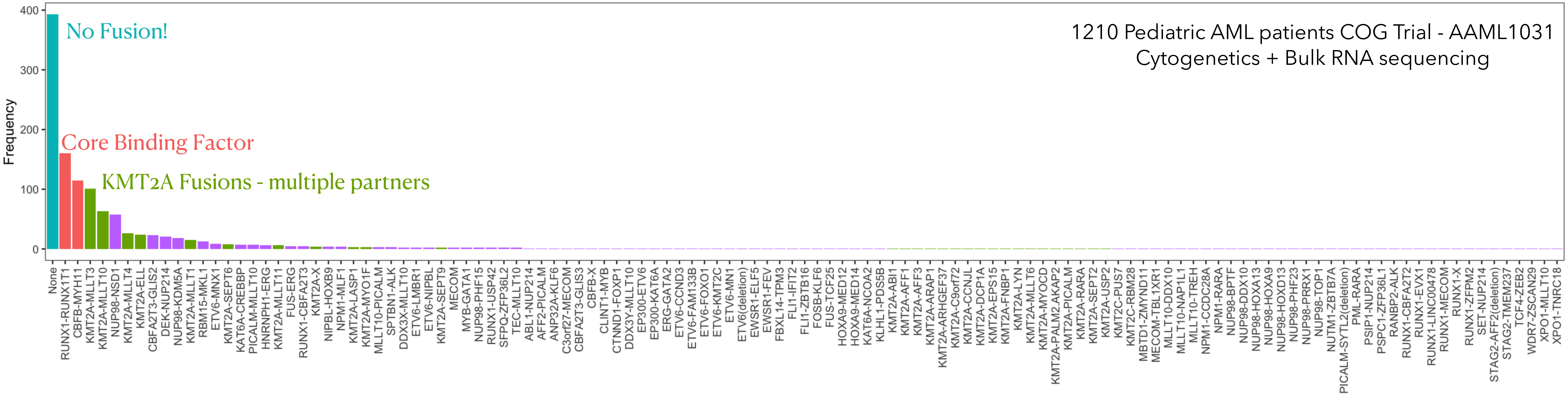
No Fusion!

Core Binding Factor

KMT2A Fusions - multiple partners

1210 Pediatric AML patients COG Trial - AAML1031
Cytogenetics + Bulk RNA sequencing

The 'long tail' of acute leukemia is a problem for molecular assays

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- NGS Bulk NGS: Mutations
  - Similarly limited, less sensitive

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- Single cell methods - Multiparameter flow cytometry (MFC):
  - More broadly applicable
  - Limited in sensitivity
  - Challenging to standardize and difficult to interpret

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Chimerism Testing

- Not currently sensitive enough (+/- 5%)

How can we improve?

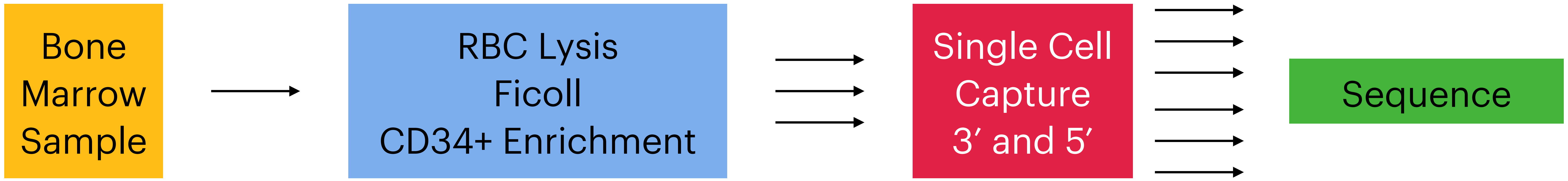
PCR	✓ Upcoming Work	FUSIONS
NGS	✓ Ongoing Work	MUTATIONS
MFC	✓ Gene Expression	MASSIVE PARAMETER SPACE
Chimerism Testing	✓ Novel Computational Approaches	NATURAL VARIATION

SINGLE CELL RNA SEQUENCING MAY BE ABLE TO MEET THIS NEED!

Deliverable:

More CONFIDENT assessment of MRD

Overview of our first experiment



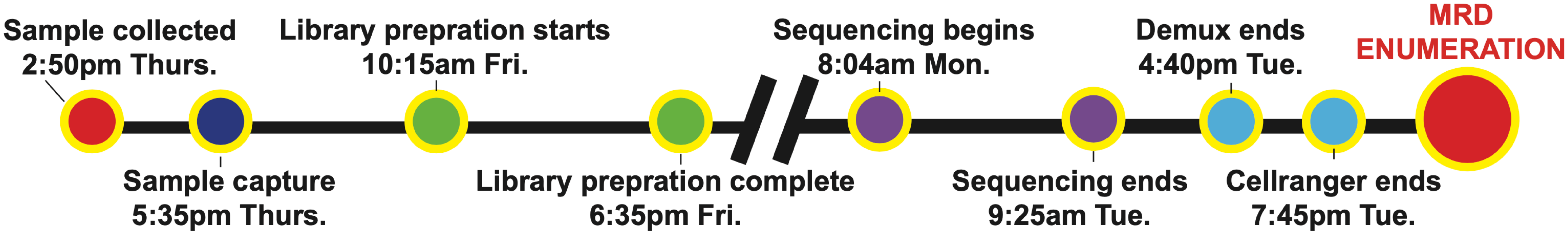
Bone Marrow Sample

RBC Lysis
Ficoll
CD34+ Enrichment

Single Cell Capture
3′ and 5′

Sequence

A note on feasibility



Sample collected
2:50pm Thurs.

Sample capture
5:35pm Thurs.

Library prepration starts
10:15am Fri.

Library prepration complete
6:35pm Fri.

Sequencing begins
8:04am Mon.

Sequencing ends
9:25am Tue.

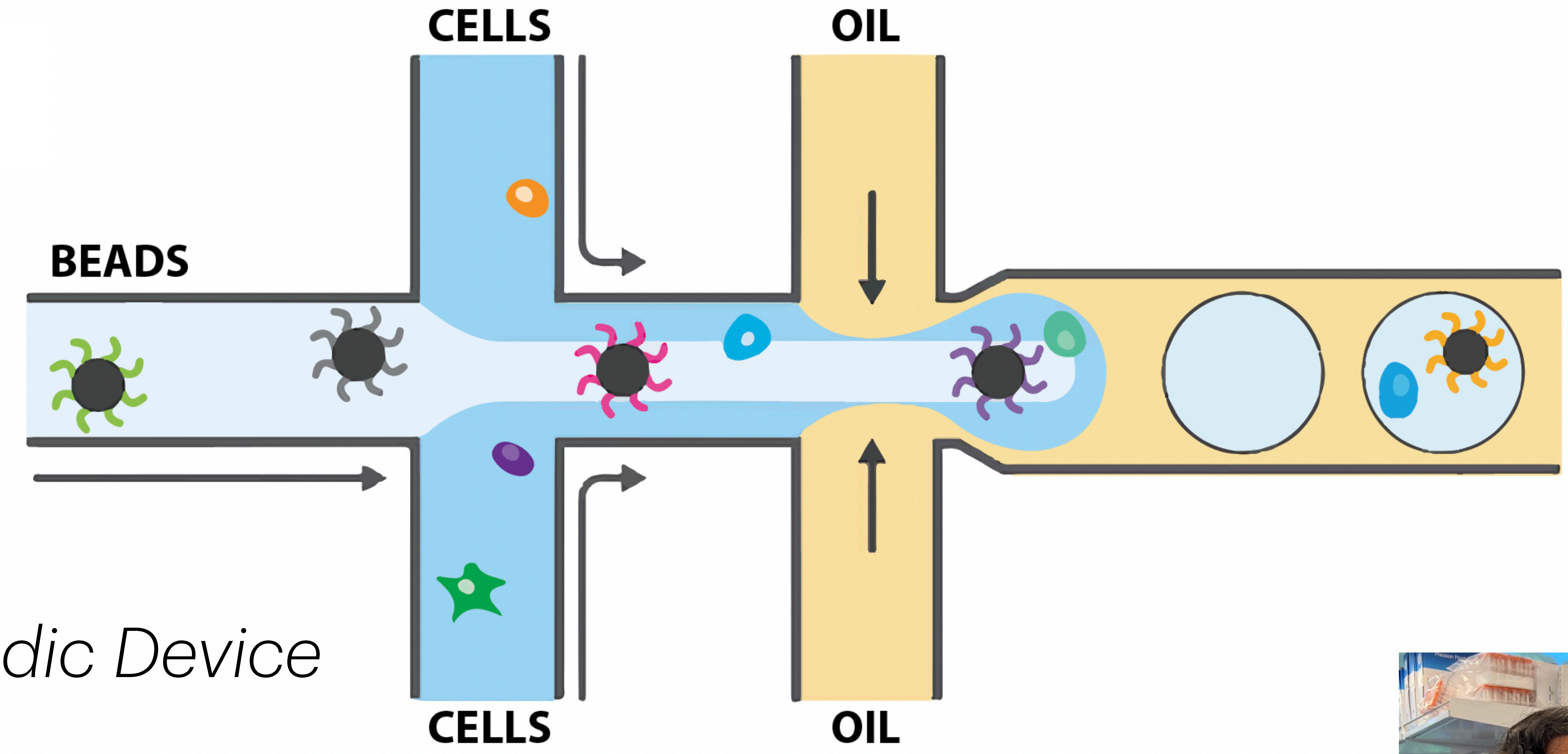
Demux ends
4:40pm Tue.

Cellranger ends
7:45pm Tue.

MRD
ENUMERATION

droplet partitioning single-cell RNA sequencing

cell capture



Microfluidic Device

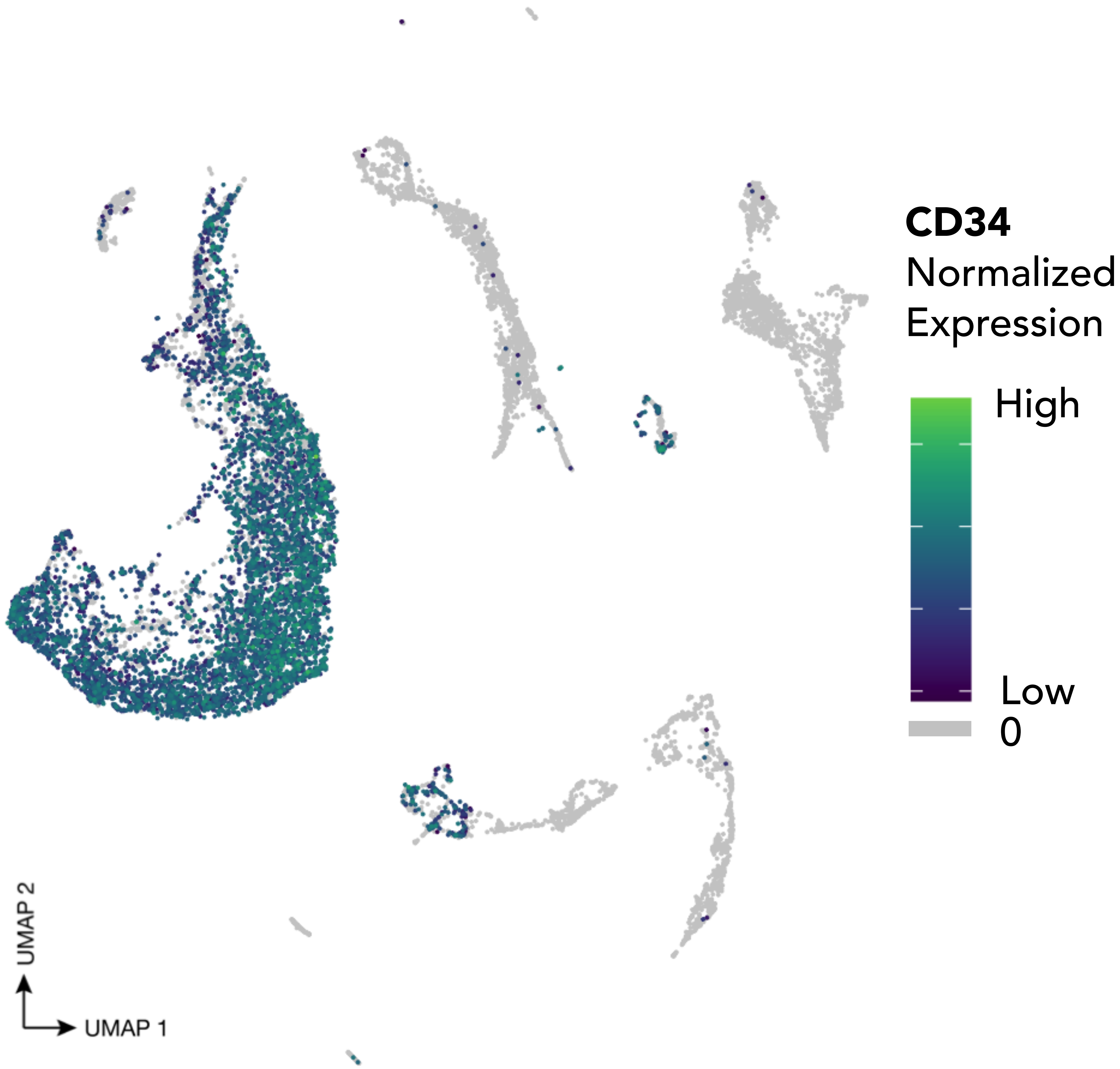
Shruti Bhise MS

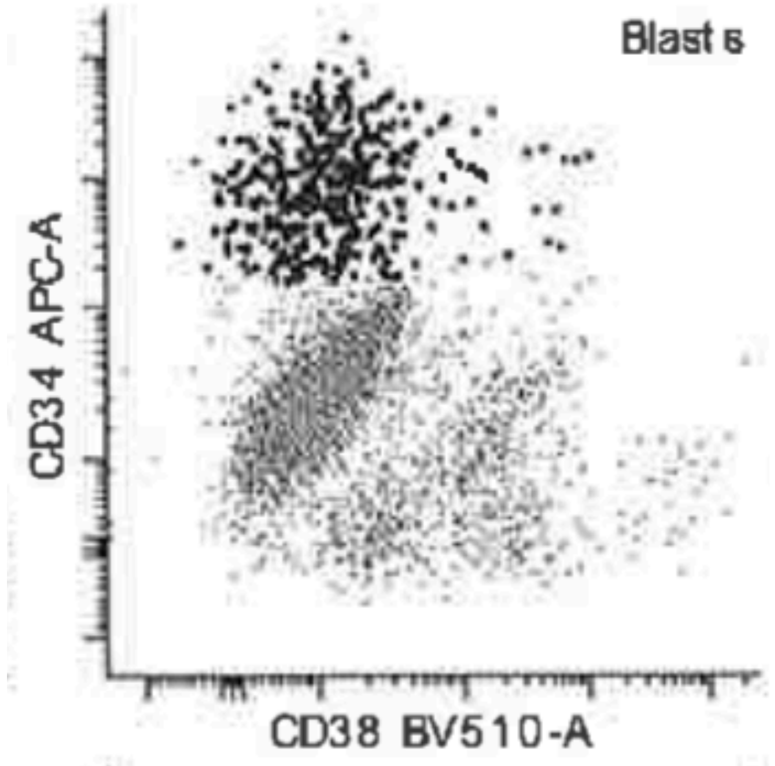
Sami Kanaan PhD

Higher than expected numbers of cells expressing CD34

RBC Lysis
Ficoll
CD34+ Enrichment

3′ Data only





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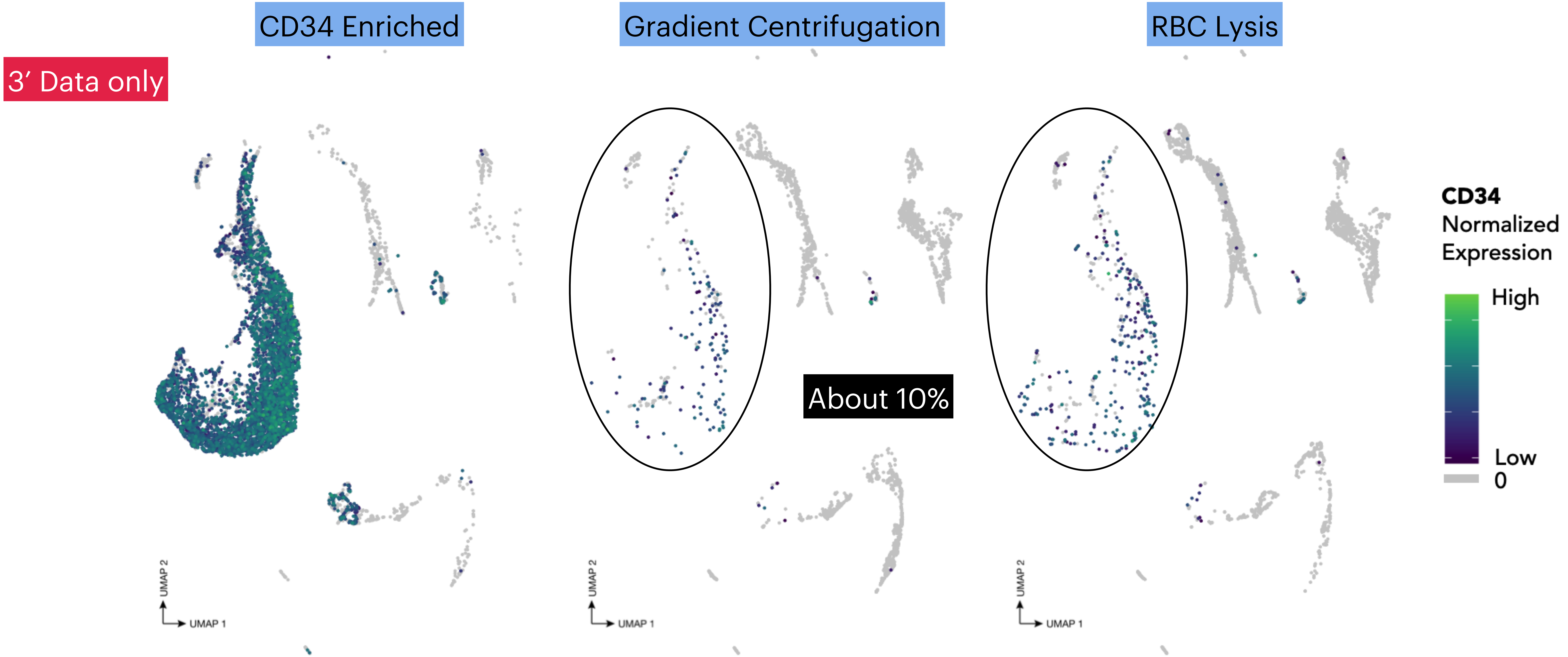
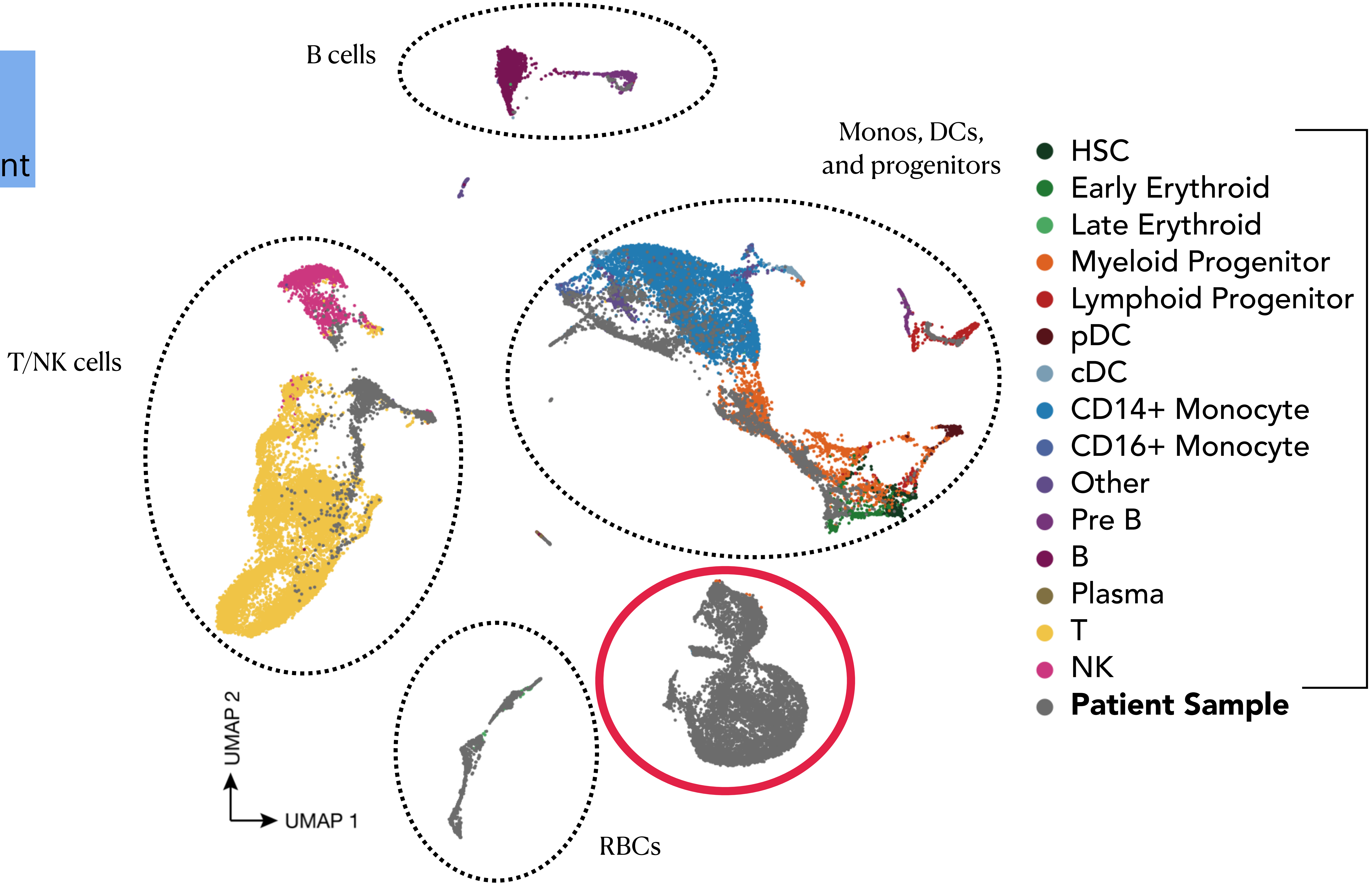


Co-embedding patient cells with healthy atlas

RBC Lysis
Ficoll
CD34+ Enrichment

3′ Data only

Seurat
RPCA
Integration



GSE139369
(Atlas of Healthy
Marrow, Cord, PBMC)

Granja et al.
Nature Biotech 2019

Annotation of cell types

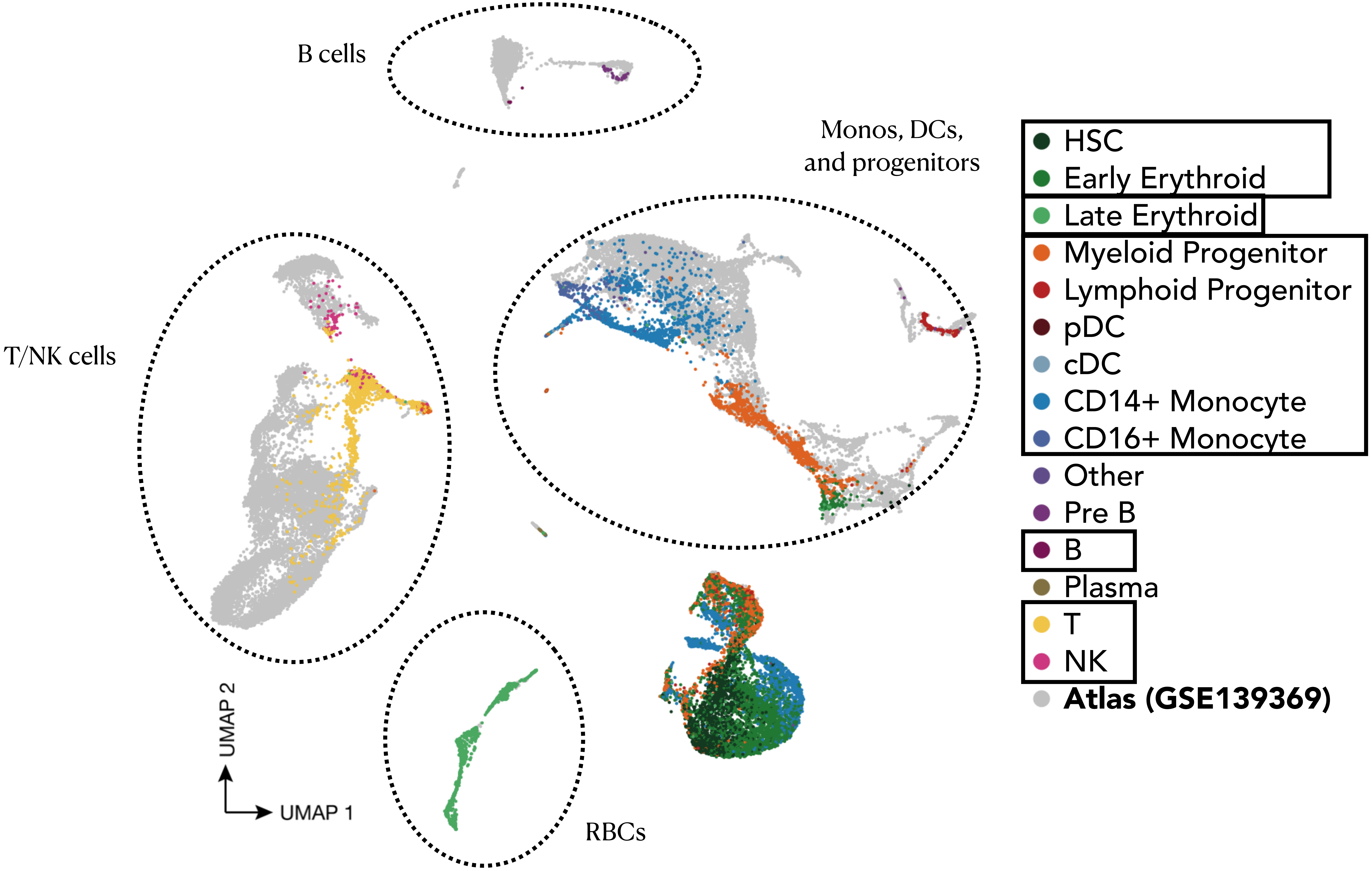
RBC Lysis
Ficoll
CD34+ Enrichment

3′ Data only

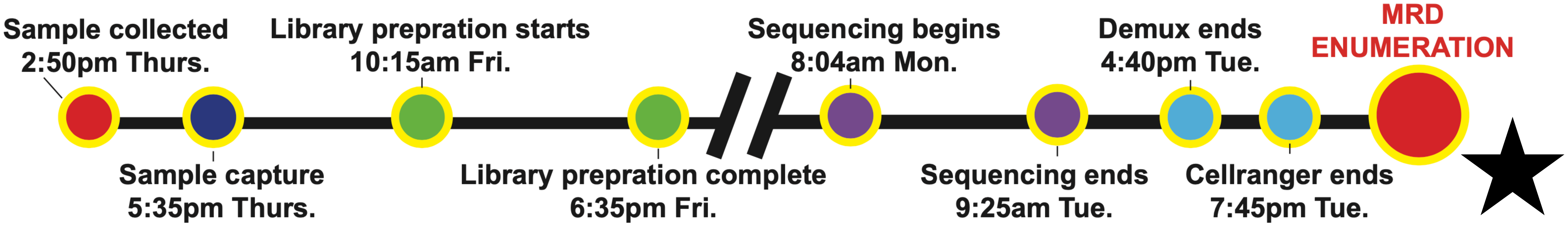
UMAP
Transform

Atlas (GSE139369)

UMAP transform



Celltype classification needs to be quick (and accurate)



Motivation for a new cell classifier

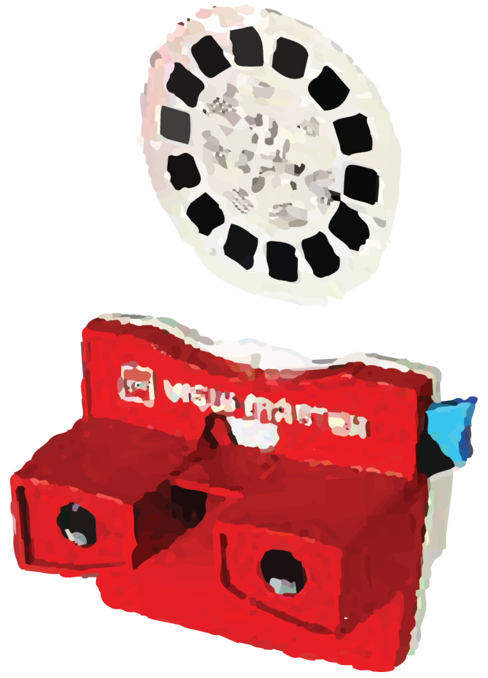
- Wish list:
  - R interactive session
    - One line of code
  - Fast
  - Modular
  - UMI count-based (not have to embed)

OpenCL™ CPU and GPU

ArrayFire

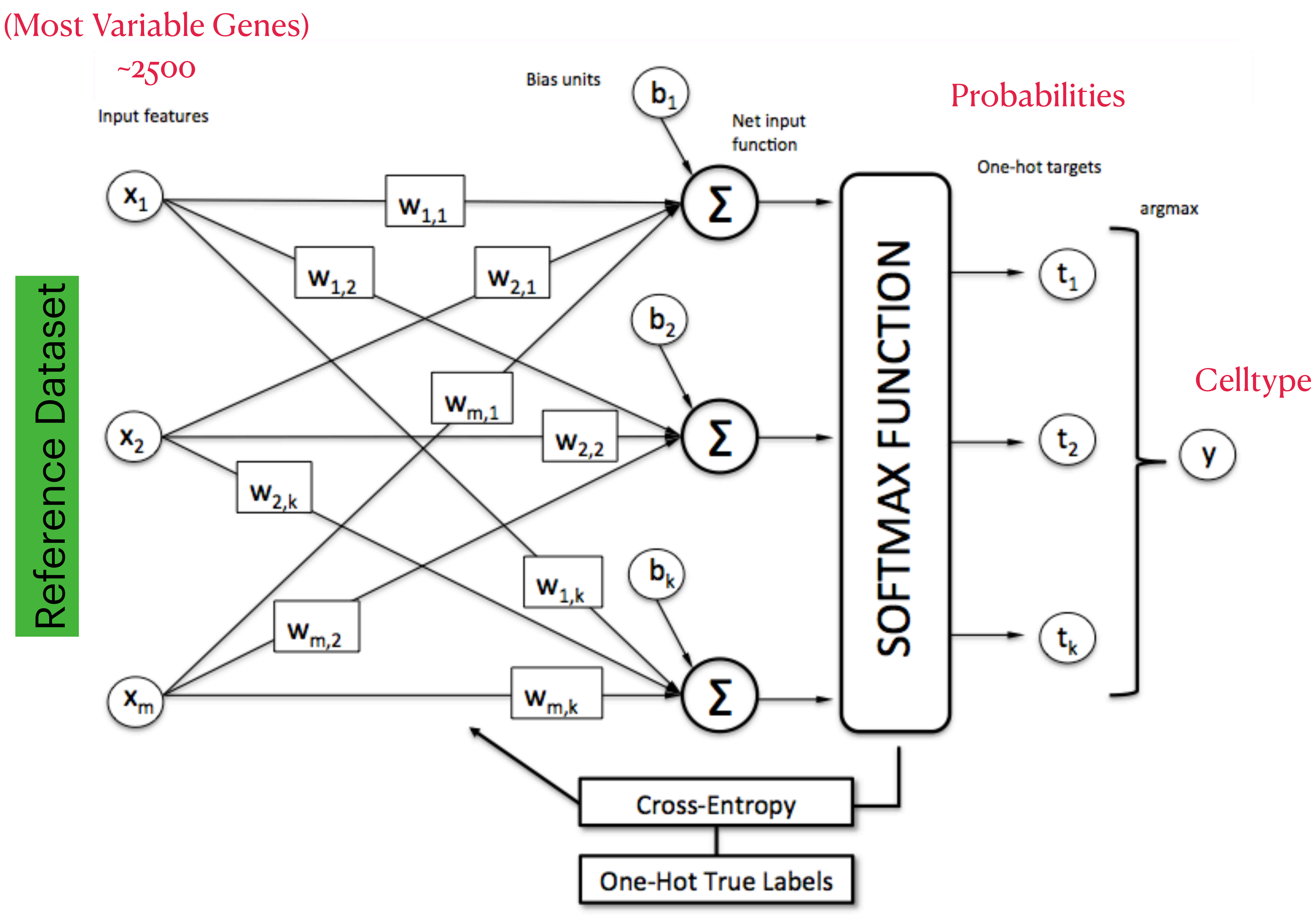
RcppArrayFire

Kazuki Fukui and Ralf Stubner

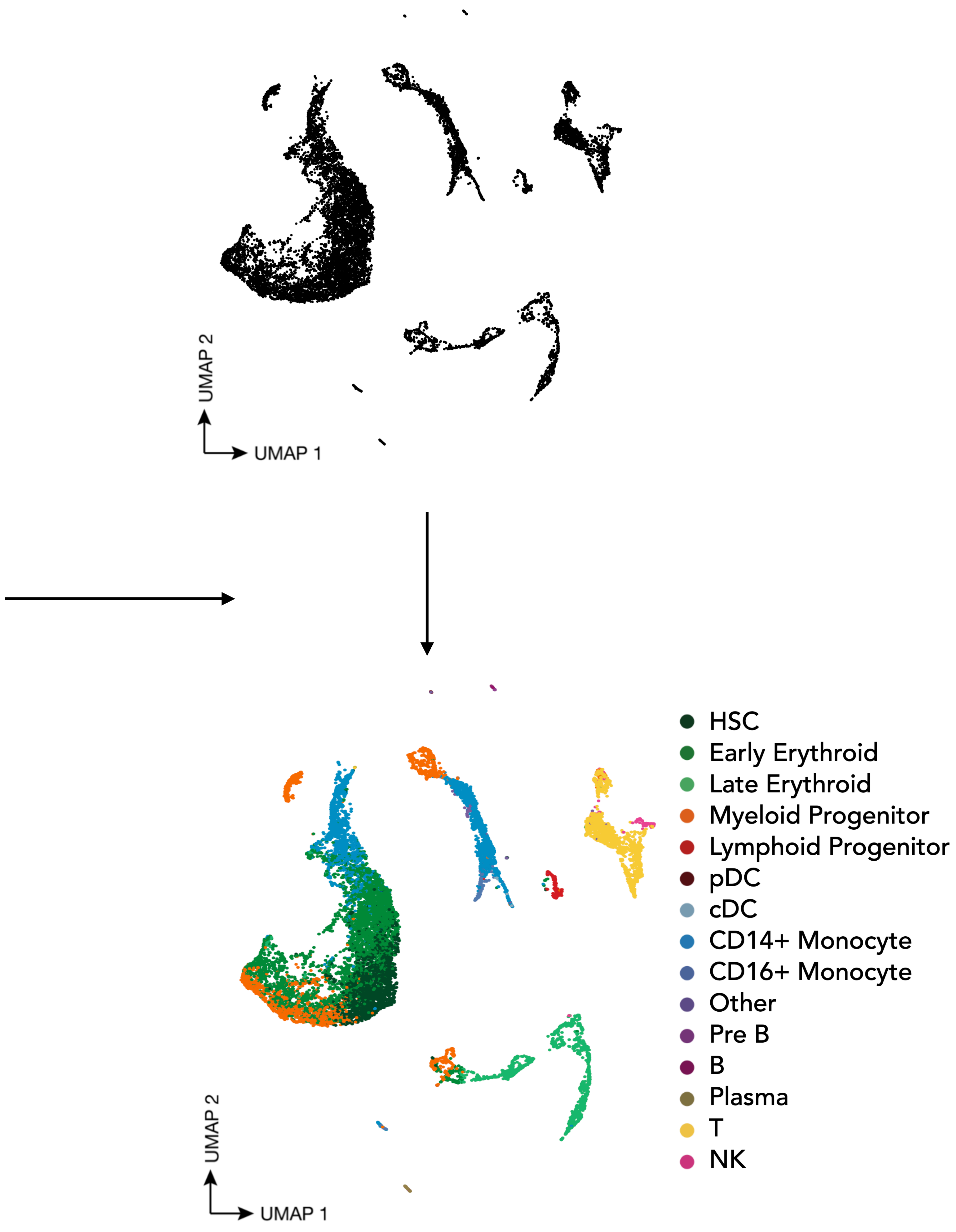


Viewmaster

Viewmaster - Softmax Regression

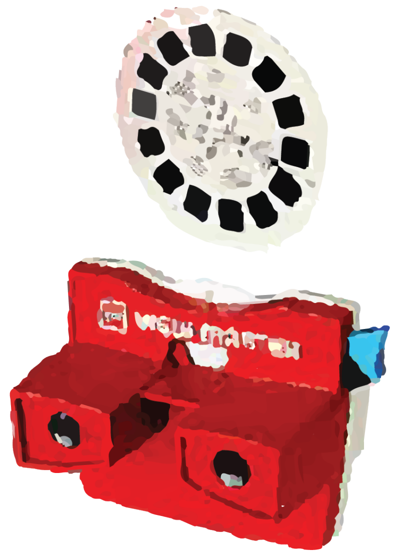


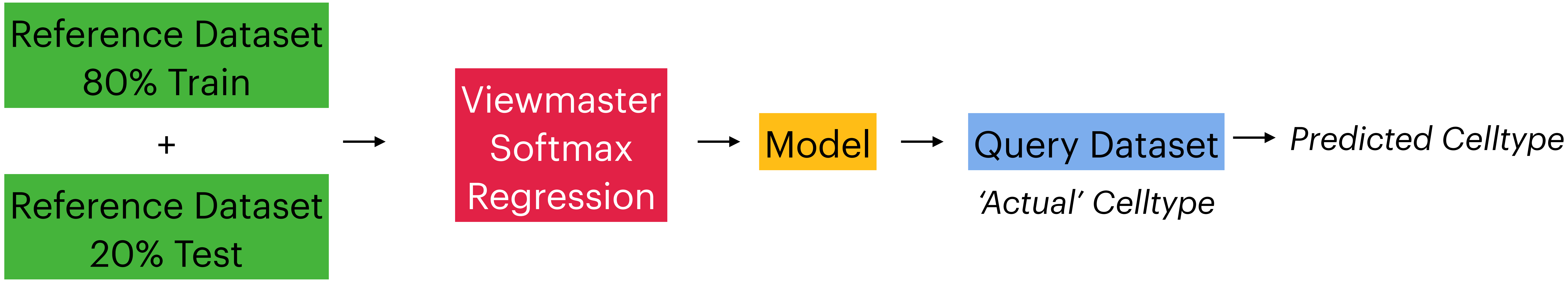
Softmax Regression



https://www.kdnuggets.com/2016/07/softmax-regression-related-logistic-regression.html

Benchmarking Viewmaster





Reference Dataset 80% Train

+

Reference Dataset 20% Test

→

Viewmaster Softmax Regression

→

Model

→

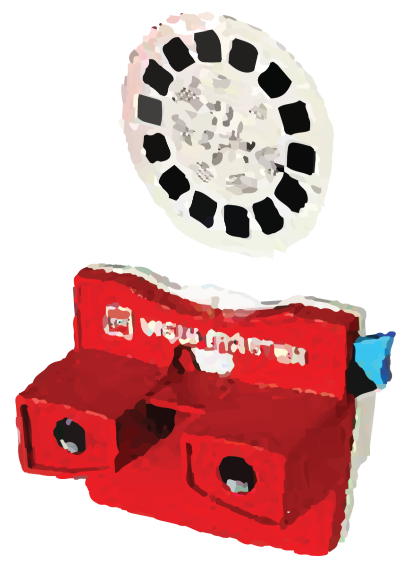
Query Dataset

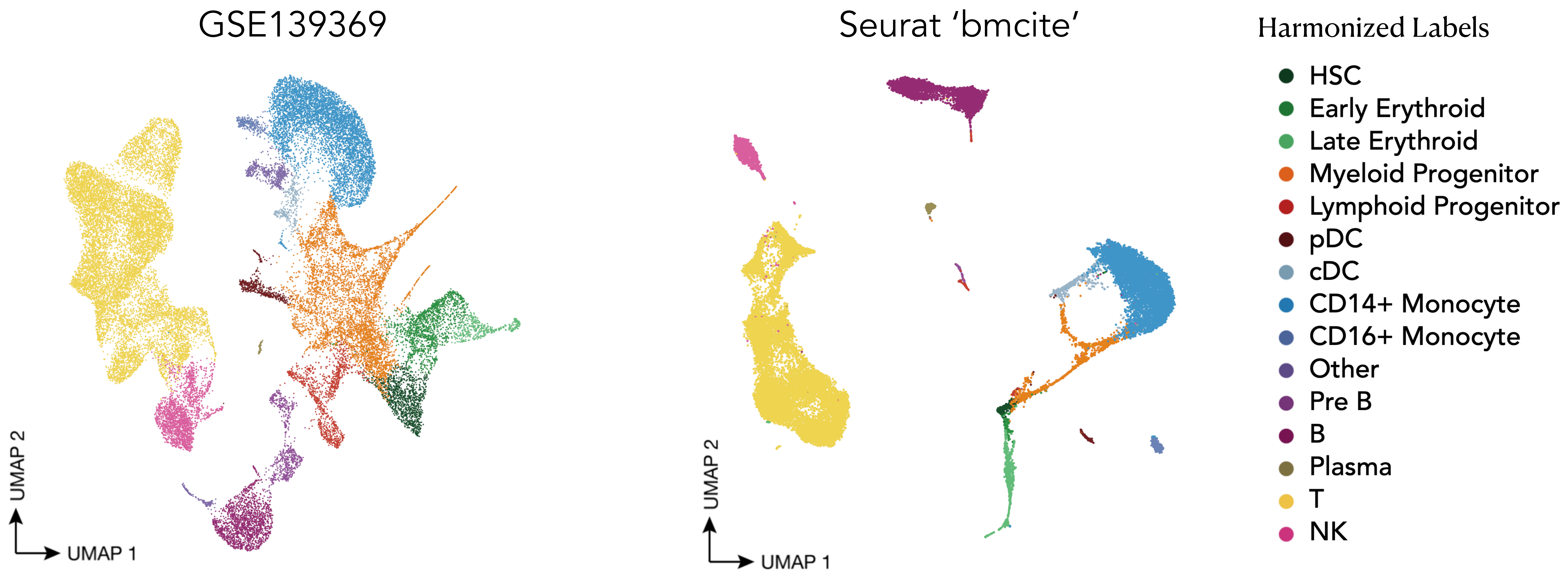
→

Predicted Celltype

'Actual' Celltype

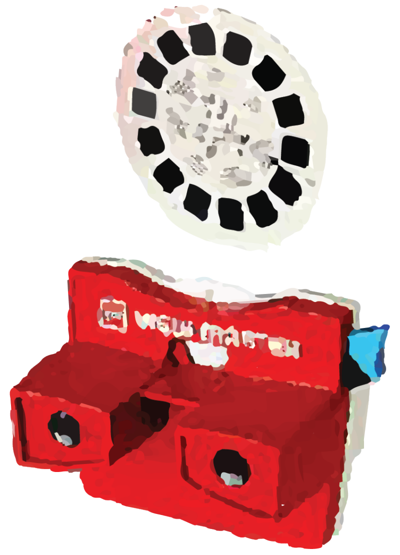
Benchmarking Viewmaster





Granja et al. Nature Biotech 2019

Viewmaster



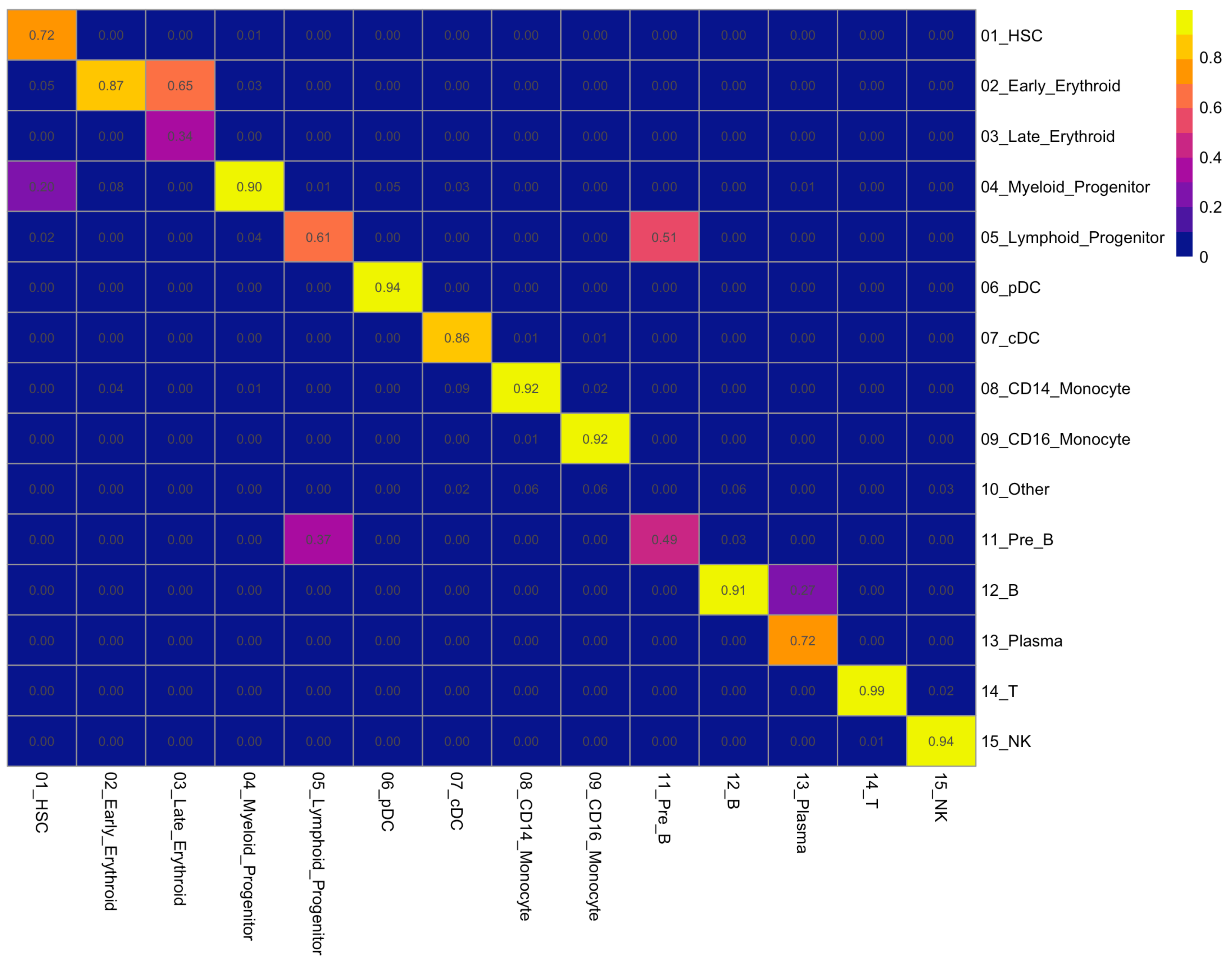
GSE139369 Reference Dataset

Seurat ‘bmcite’ Query Dataset

```
Accuracy on training data: 98.98
Accuracy on testing data: 98.60
```

```
  user  system elapsed 
 7.432   3.325  29.724 
```

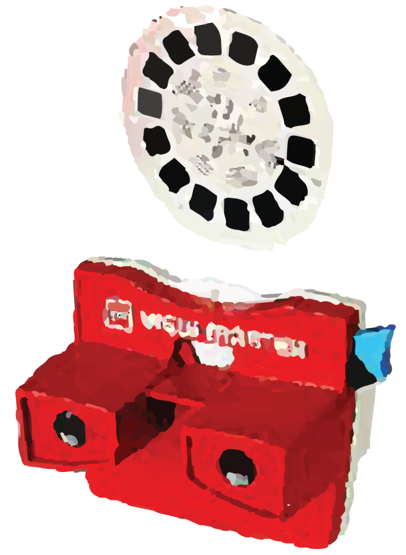
'Actual' Celltype

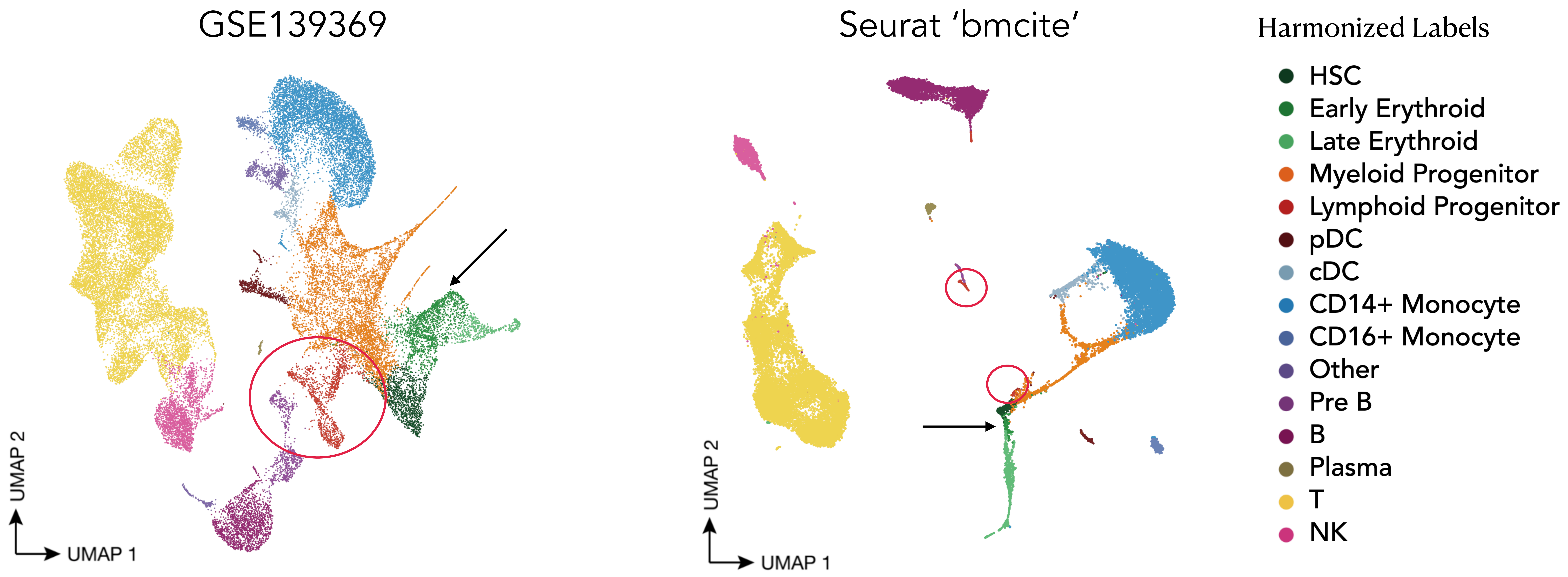


Predicted Celltype

	01_HSC	02_Early_Erythroid	03_Late_Erythroid	04_Myeloid_Progenitor	05_Lymphoid_Progenitor	06_pDC	07_cDC	08_CD14_Monocyte	09_CD16_Monocyte	11_Pre_B	12_B	13_Plasma	14_T	15_NK
01_HSC	0.72	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
02_Early_Erythroid	0.05	0.87	0.65	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
03_Late_Erythroid	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
04_Myeloid_Progenitor	0.20	0.08	0.00	0.90	0.01	0.05	0.03	0.00	0.00	0.00	0.00	0.01	0.00	0.00
05_Lymphoid_Progenitor	0.02	0.00	0.00	0.04	0.61	0.00	0.00	0.00	0.00	0.51	0.00	0.00	0.00	0.00
06_pDC	0.00	0.00	0.00	0.00	0.00	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
07_cDC	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.01	0.01	0.00	0.00	0.00	0.00	0.00
08_CD14_Monocyte	0.00	0.04	0.00	0.01	0.00	0.00	0.09	0.92	0.02	0.00	0.00	0.00	0.00	0.00
09_CD16_Monocyte	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.92	0.00	0.00	0.00	0.00	0.00
10_Other	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.06	0.00	0.06	0.00	0.00	0.03
11_Pre_B	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.00	0.49	0.03	0.00	0.00	0.00
12_B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.91	0.27	0.00	0.00
13_Plasma	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00	0.00
14_T	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.02
15_NK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.94

Rare cell types may confound softmax regression

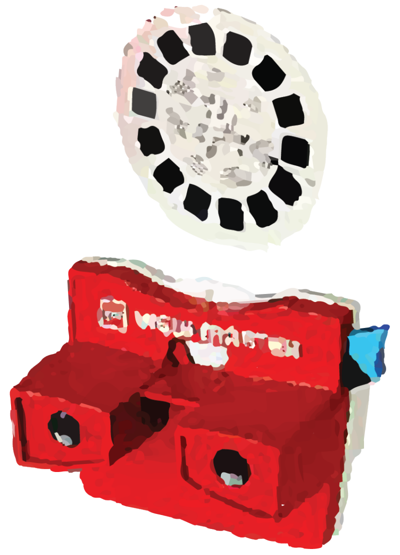



GSE139369

Seurat ‘bmcite’

Granja et al. Nature Biotech 2019

Viewmaster



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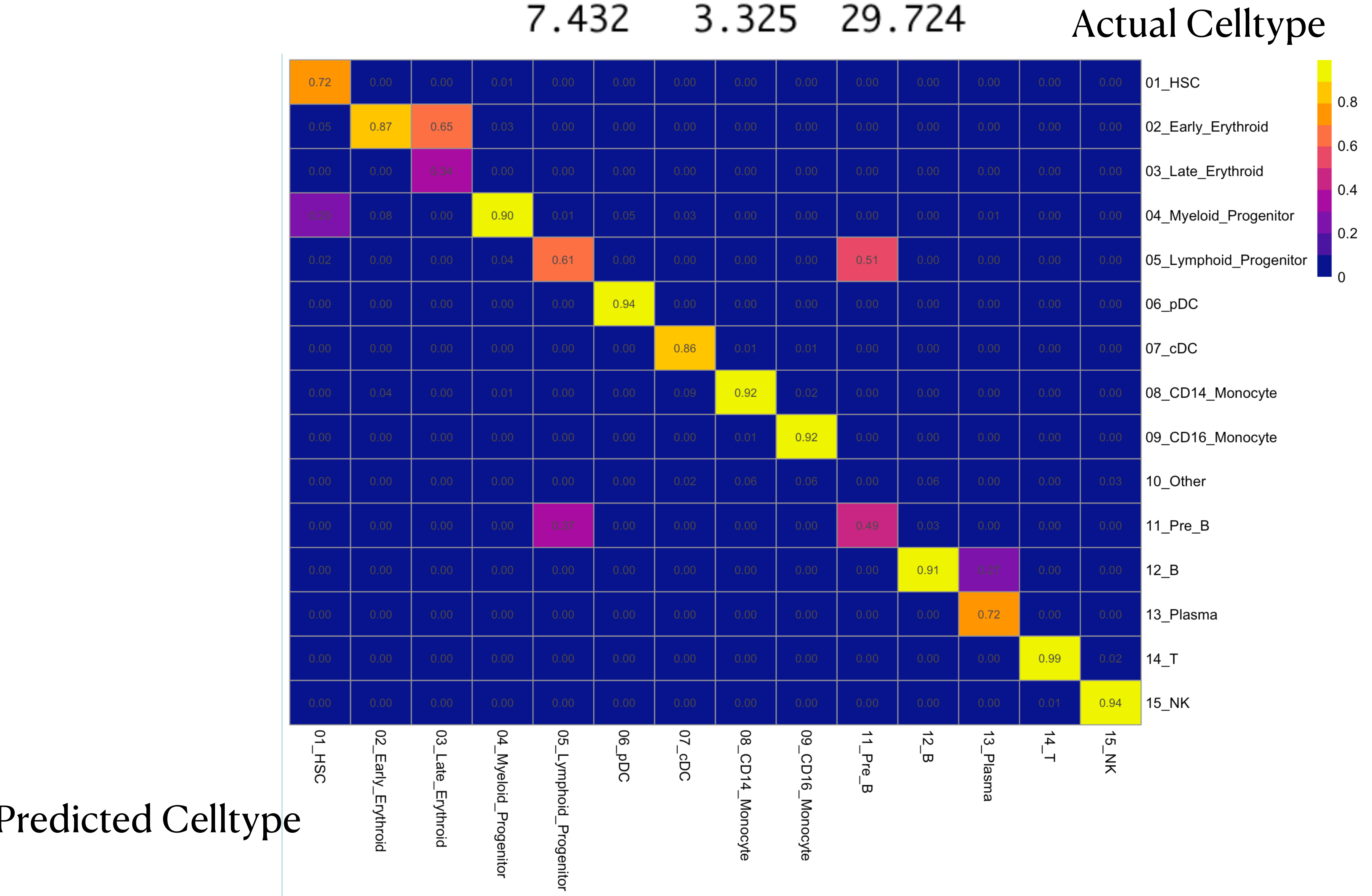

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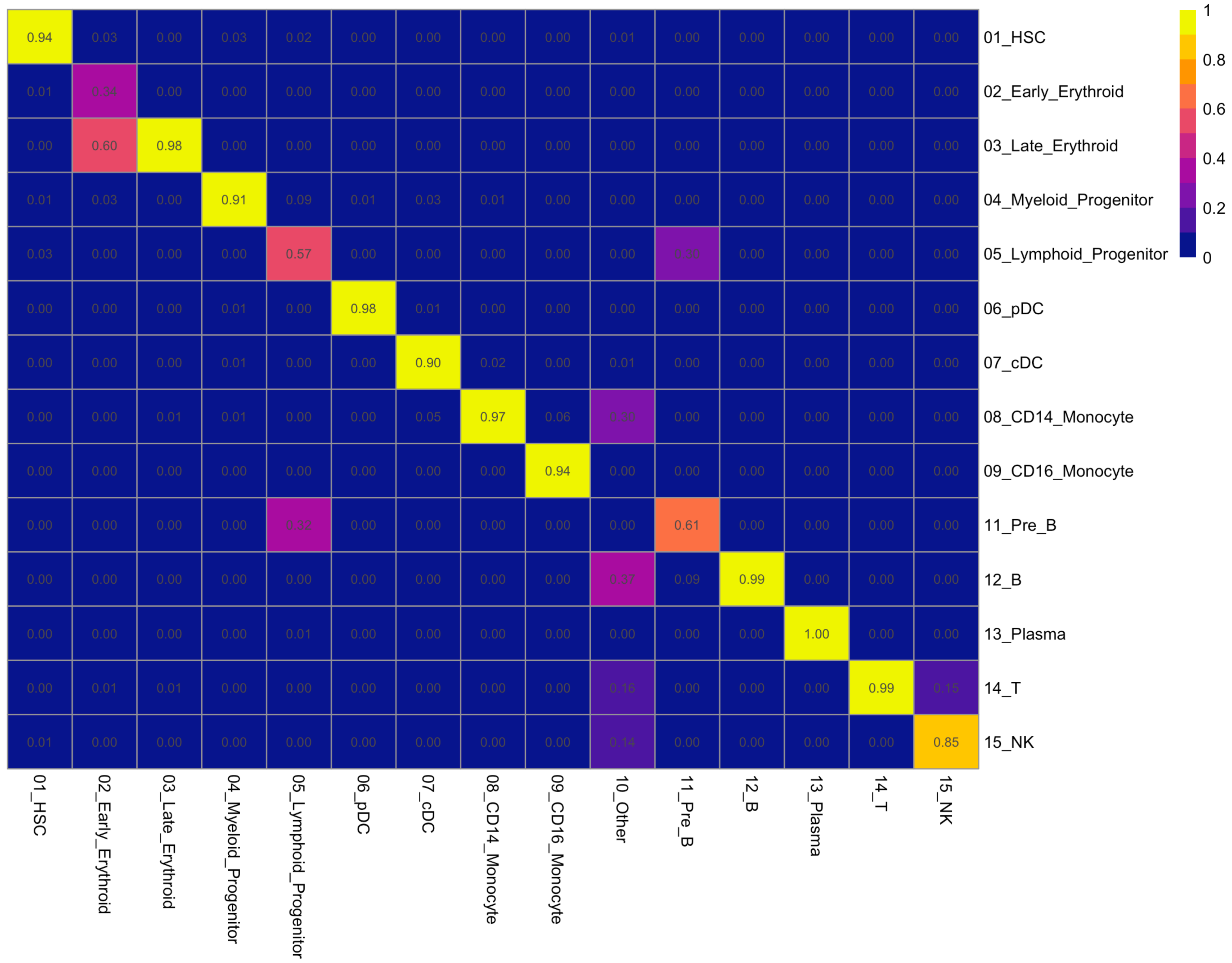
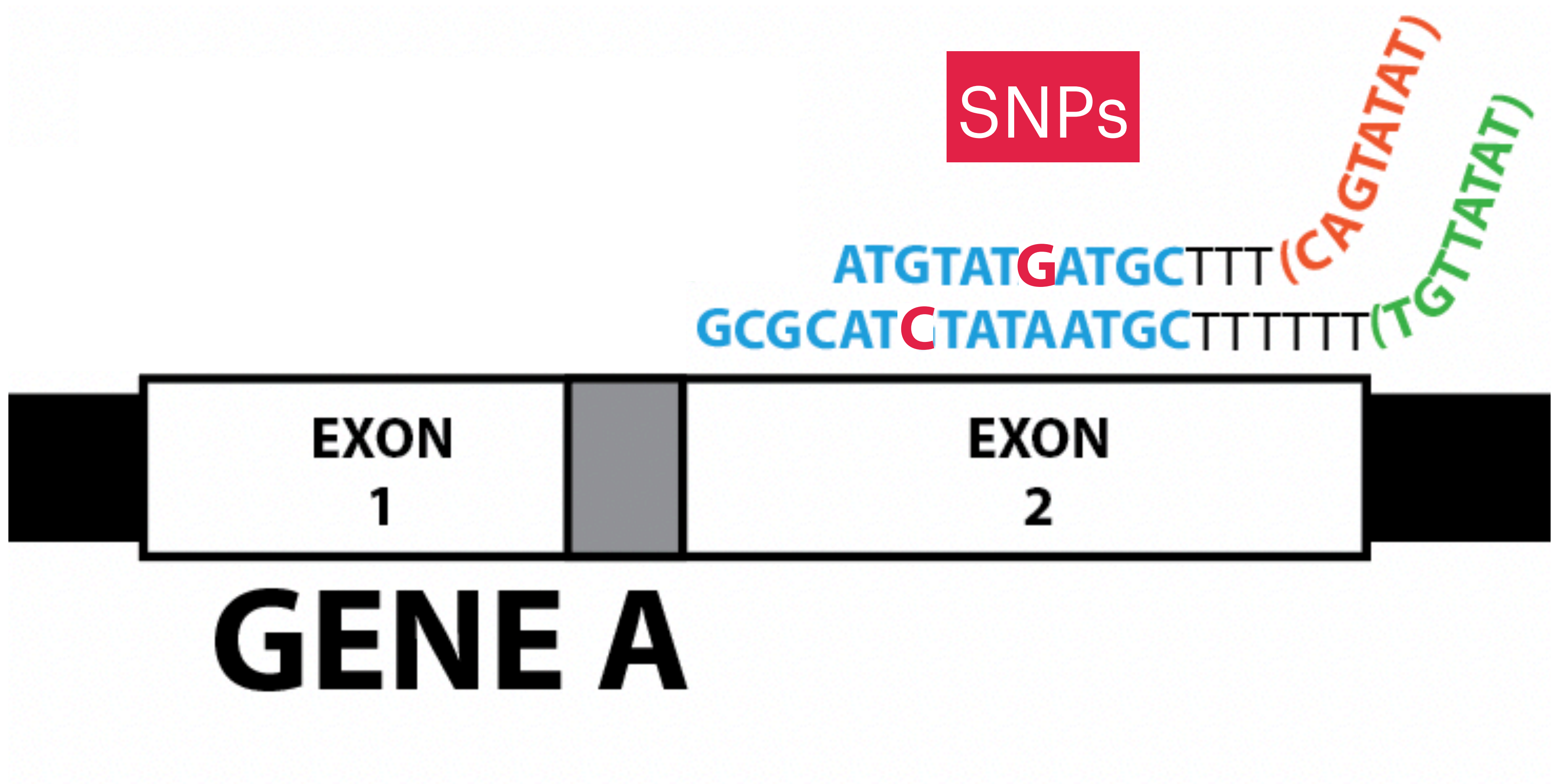


Olivia Waltner BA

Can we leverage natural genetic variation to improve leukemia detection ?



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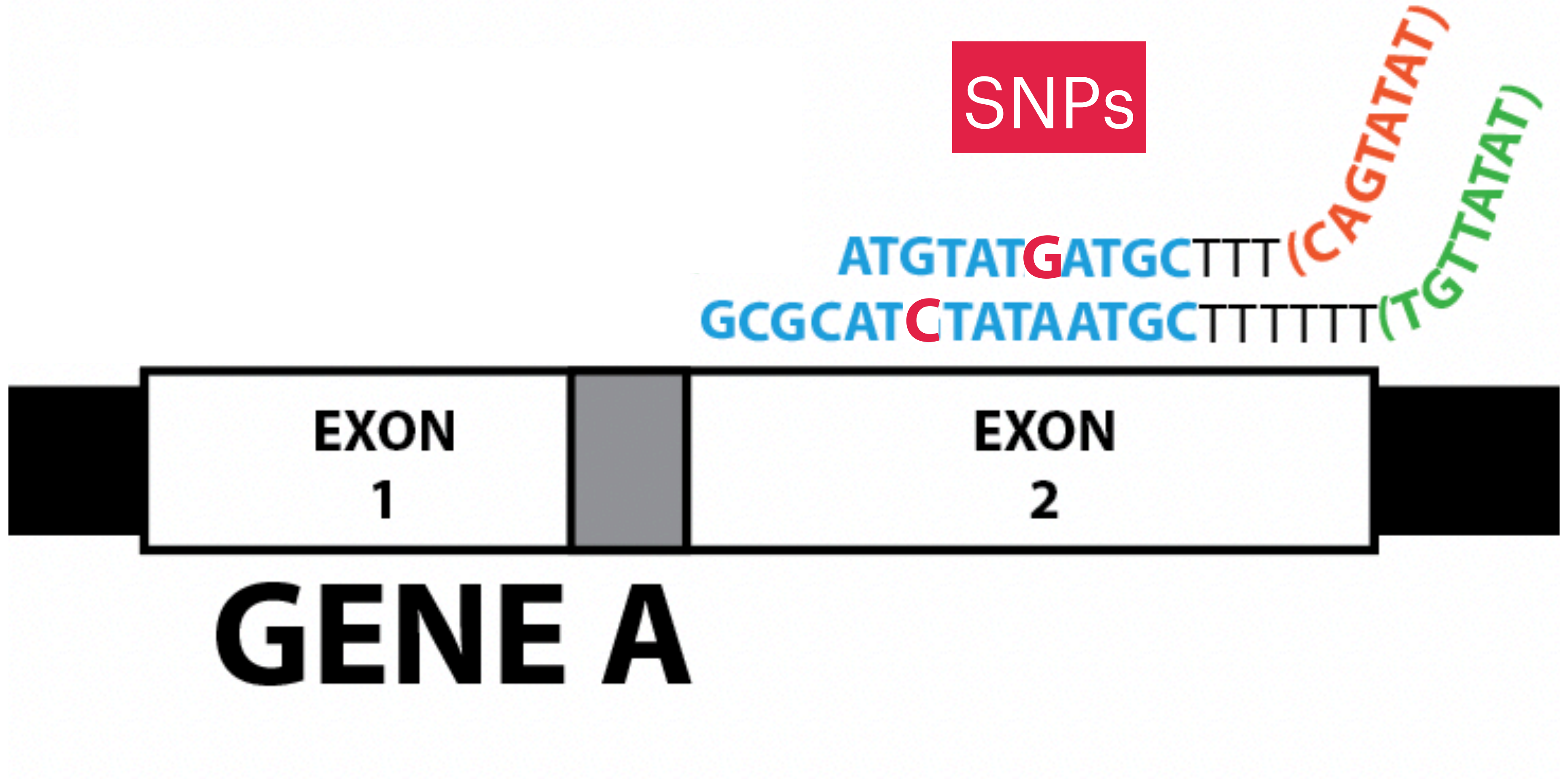
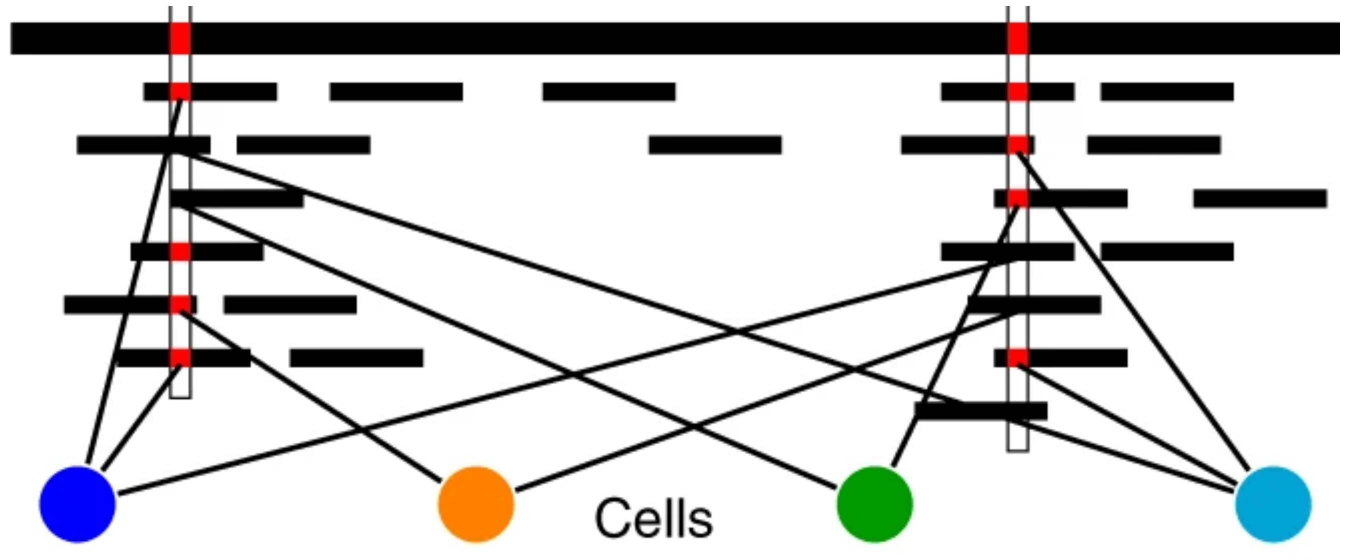
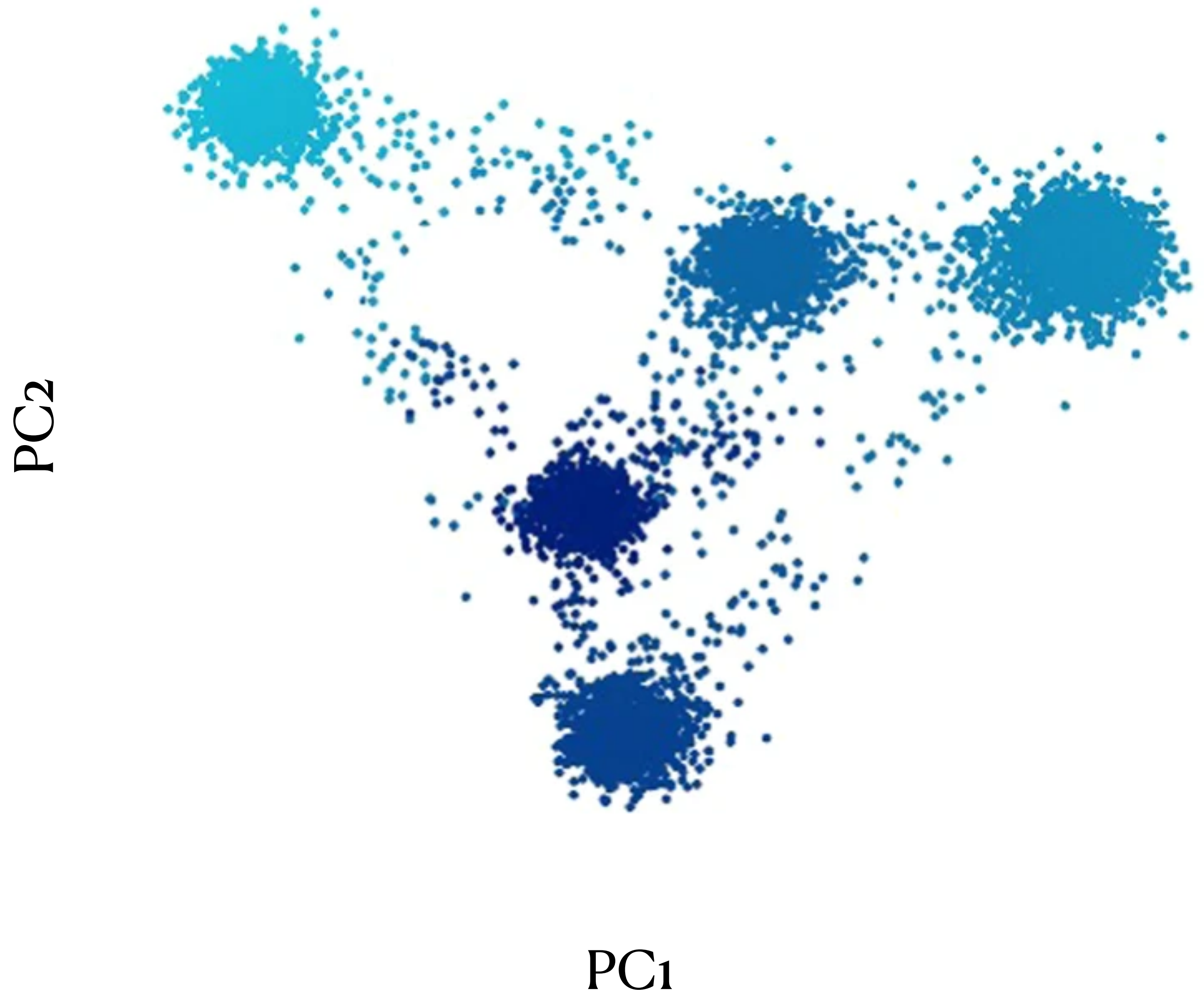
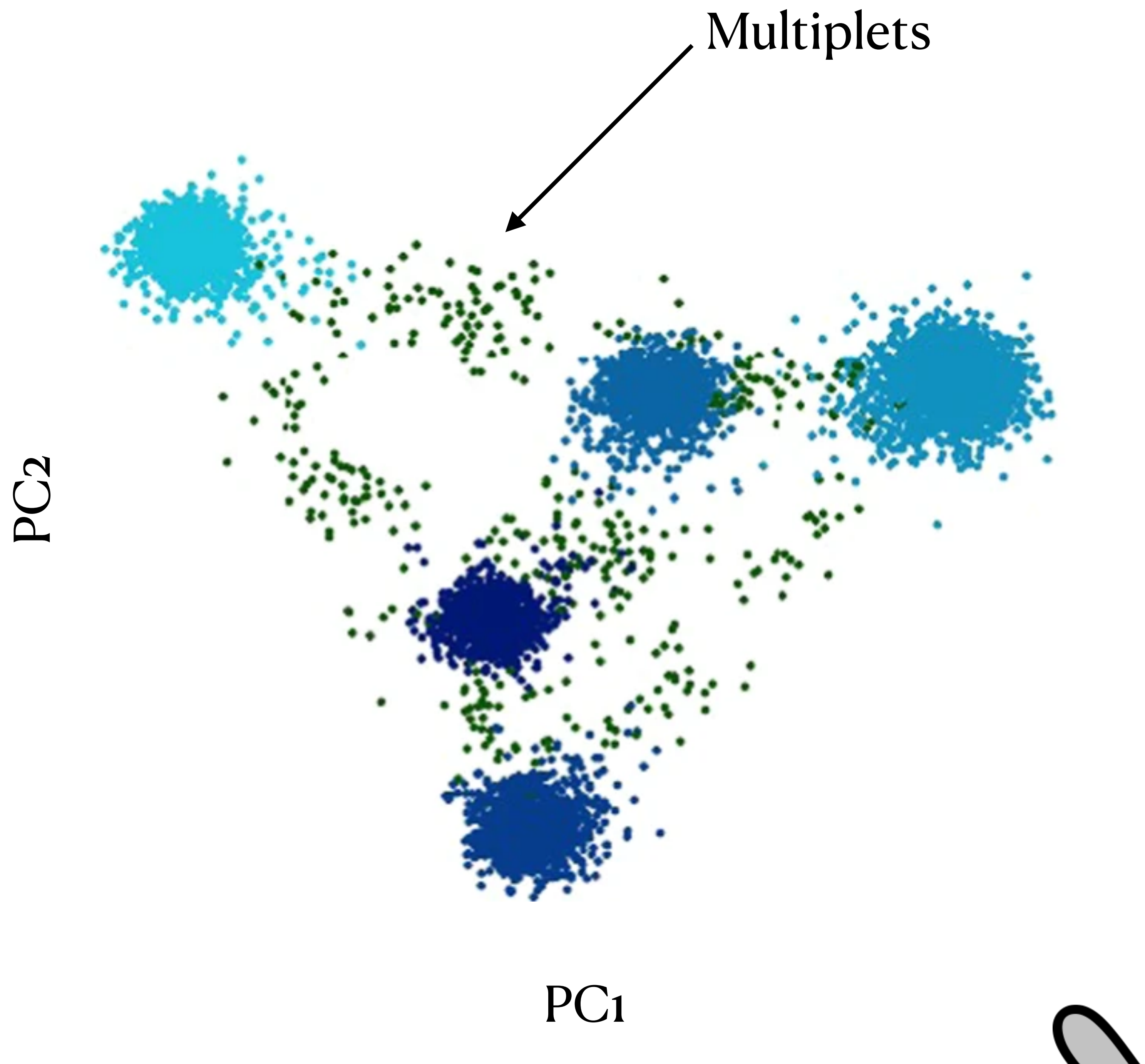


Imperative to pair this with cell type

Souporcell genetically demultiplexes scRNAseq data

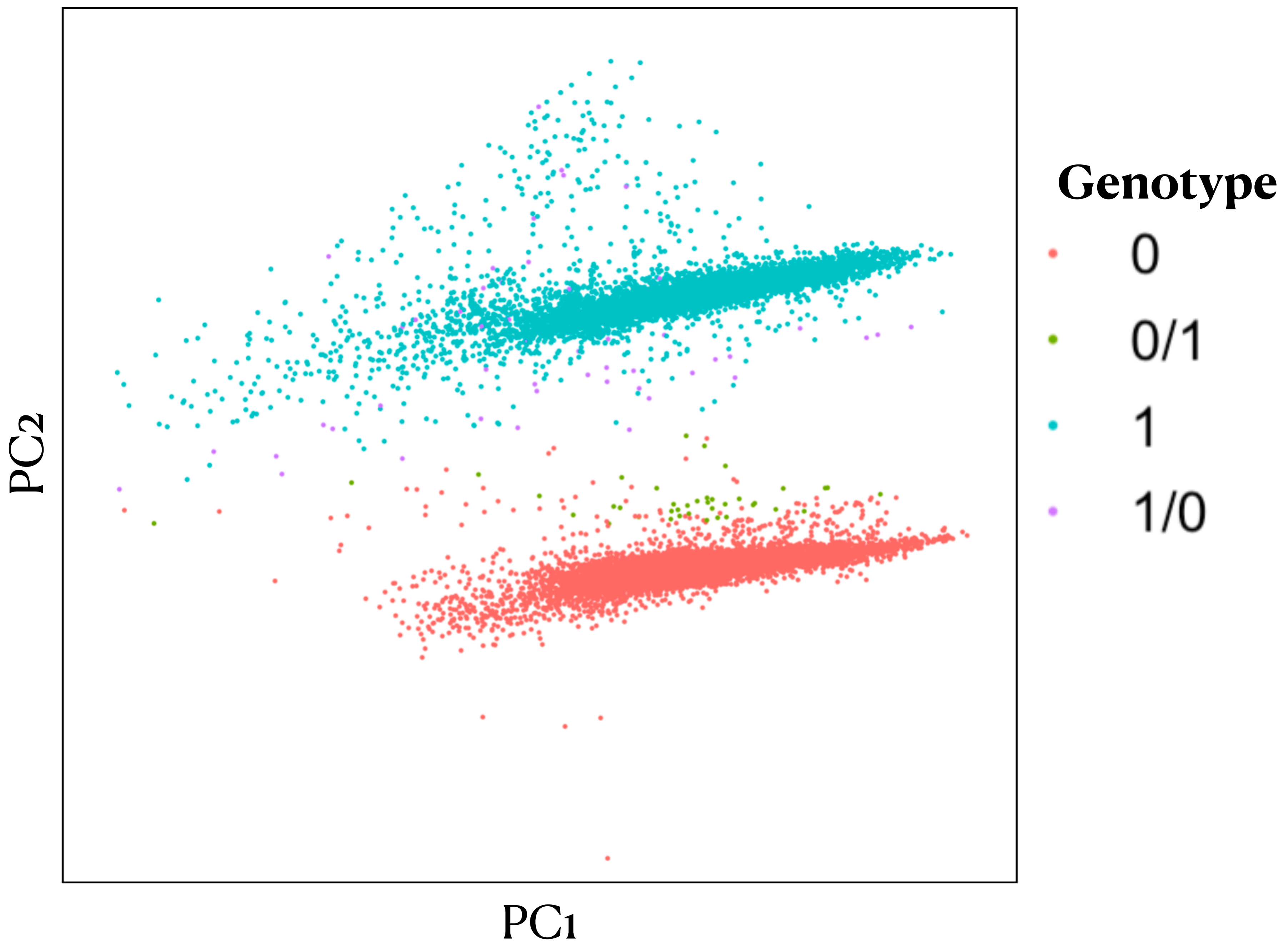




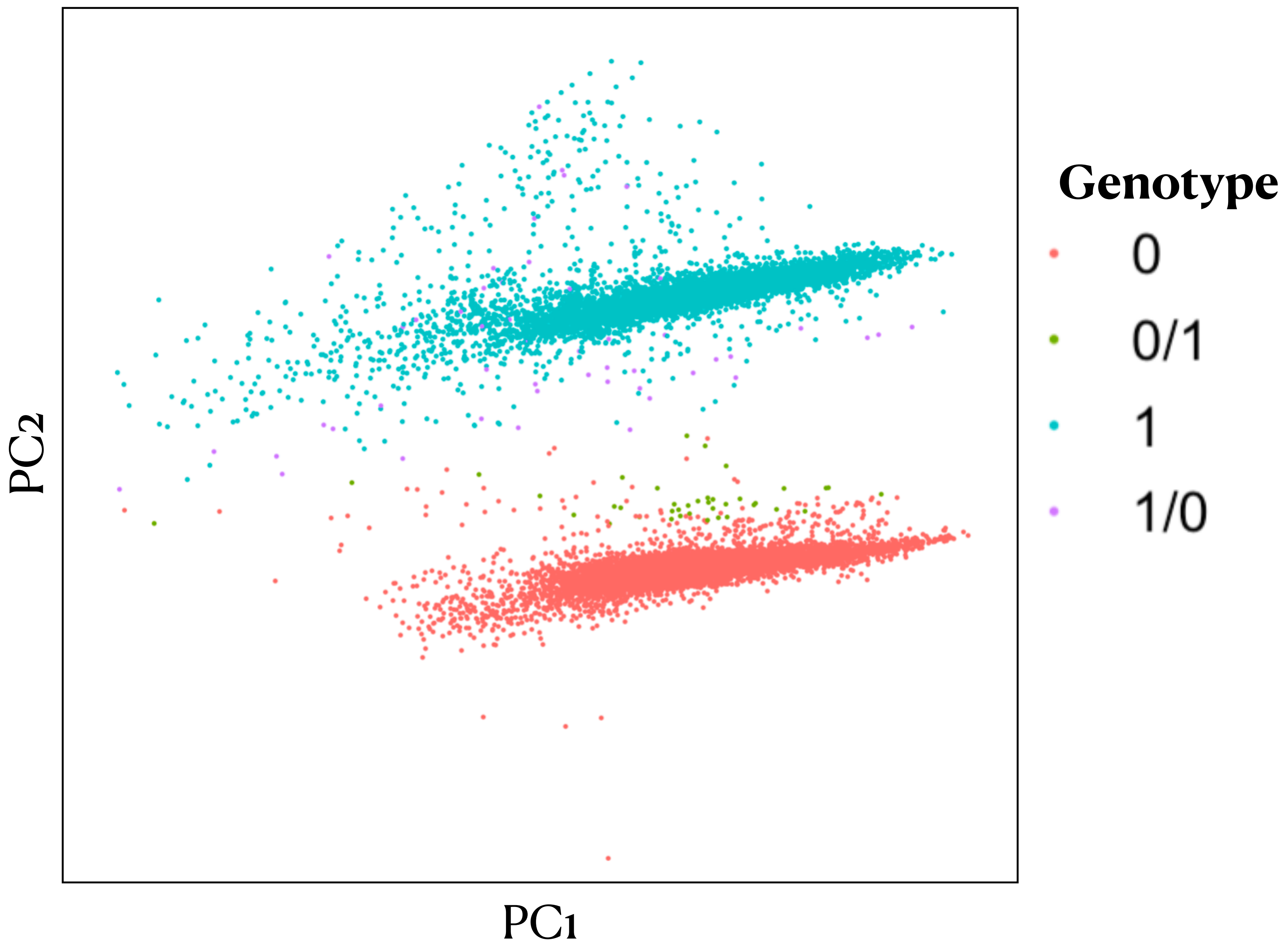


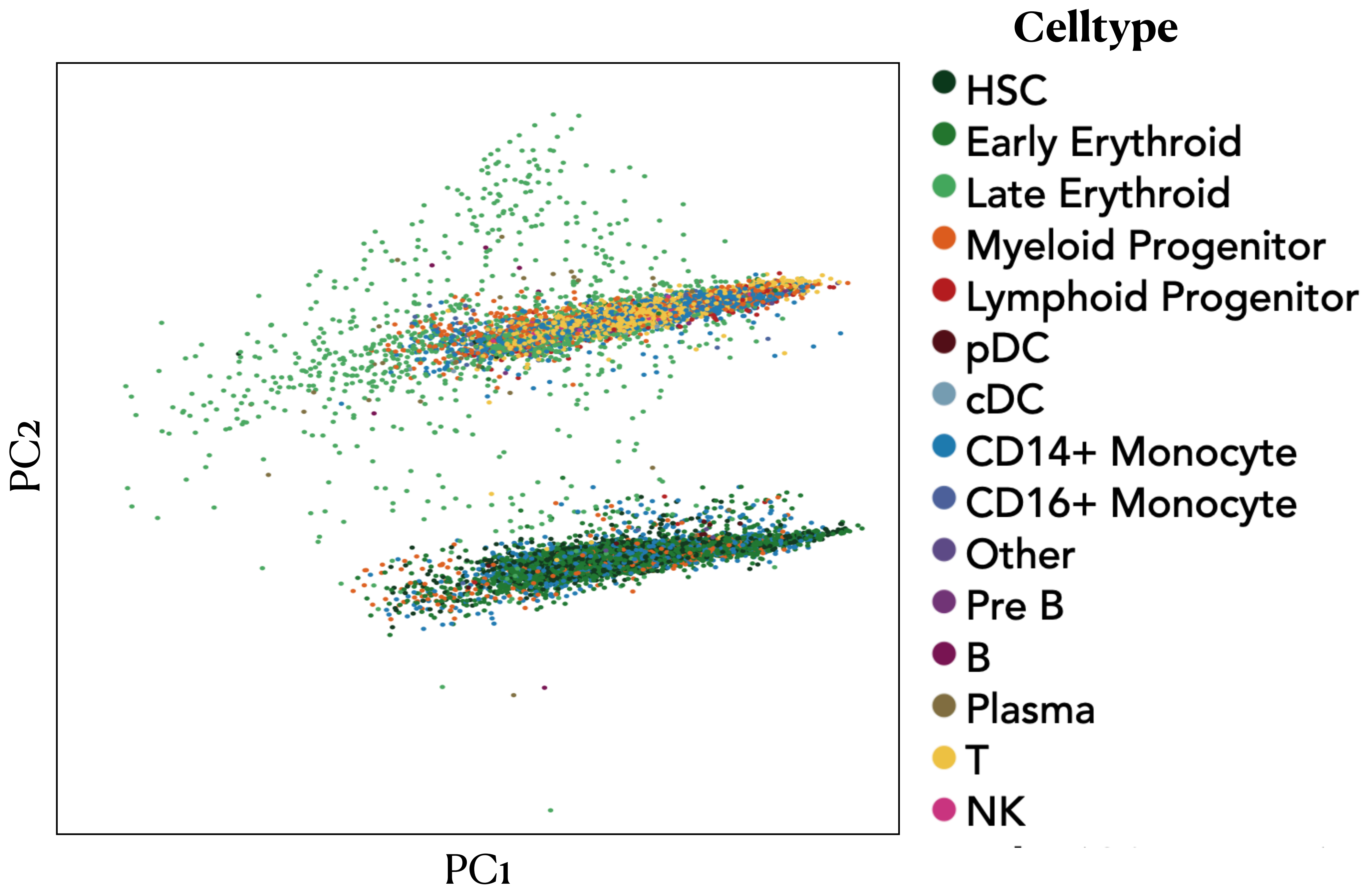
Heaton H, Talman AM, Knights A, et al. *Nat Methods*. 2020;17(6):615-620.

Souporcell identifies two genotypes in our sample



Souporcell struggles with RBCs





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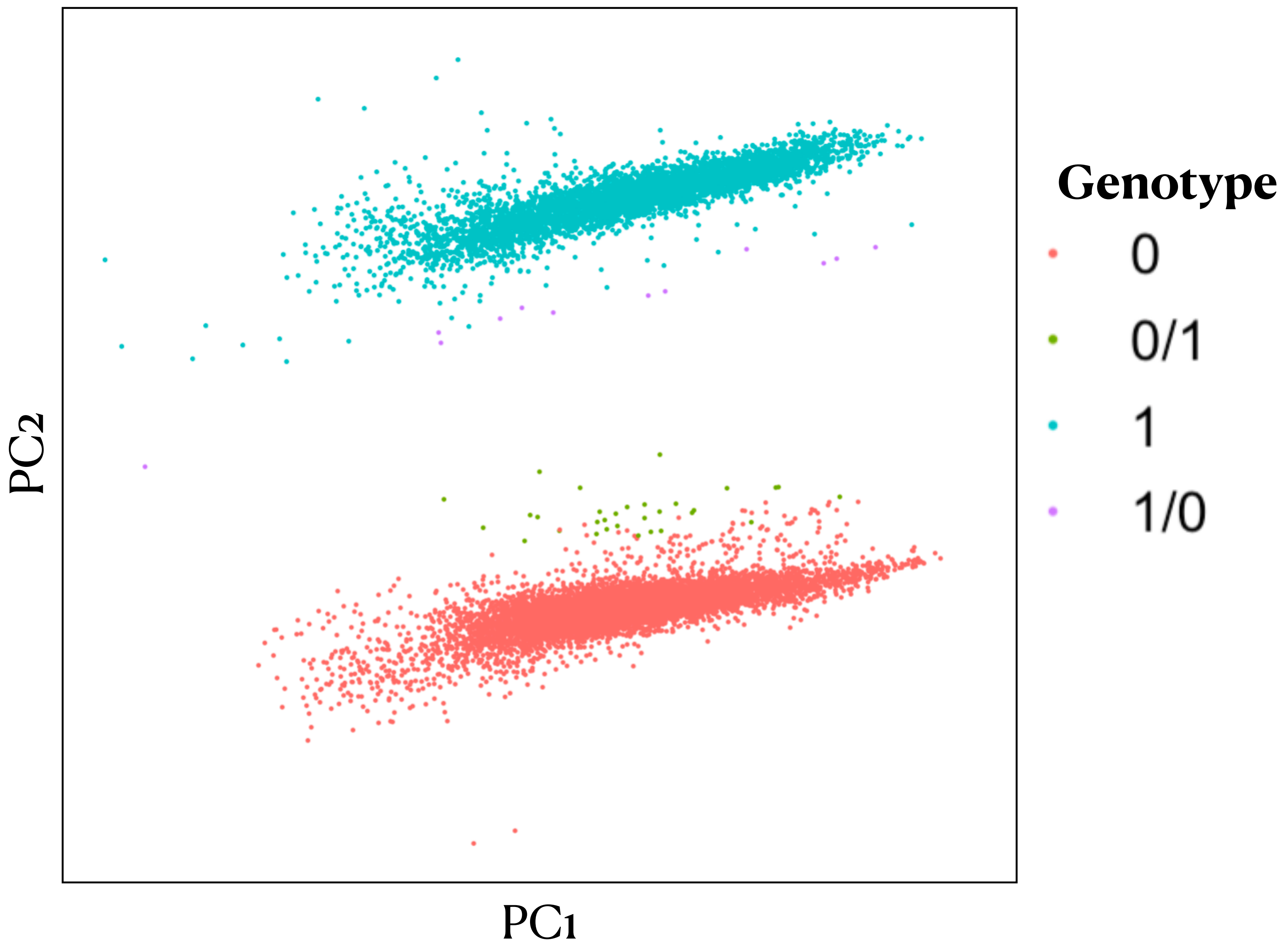
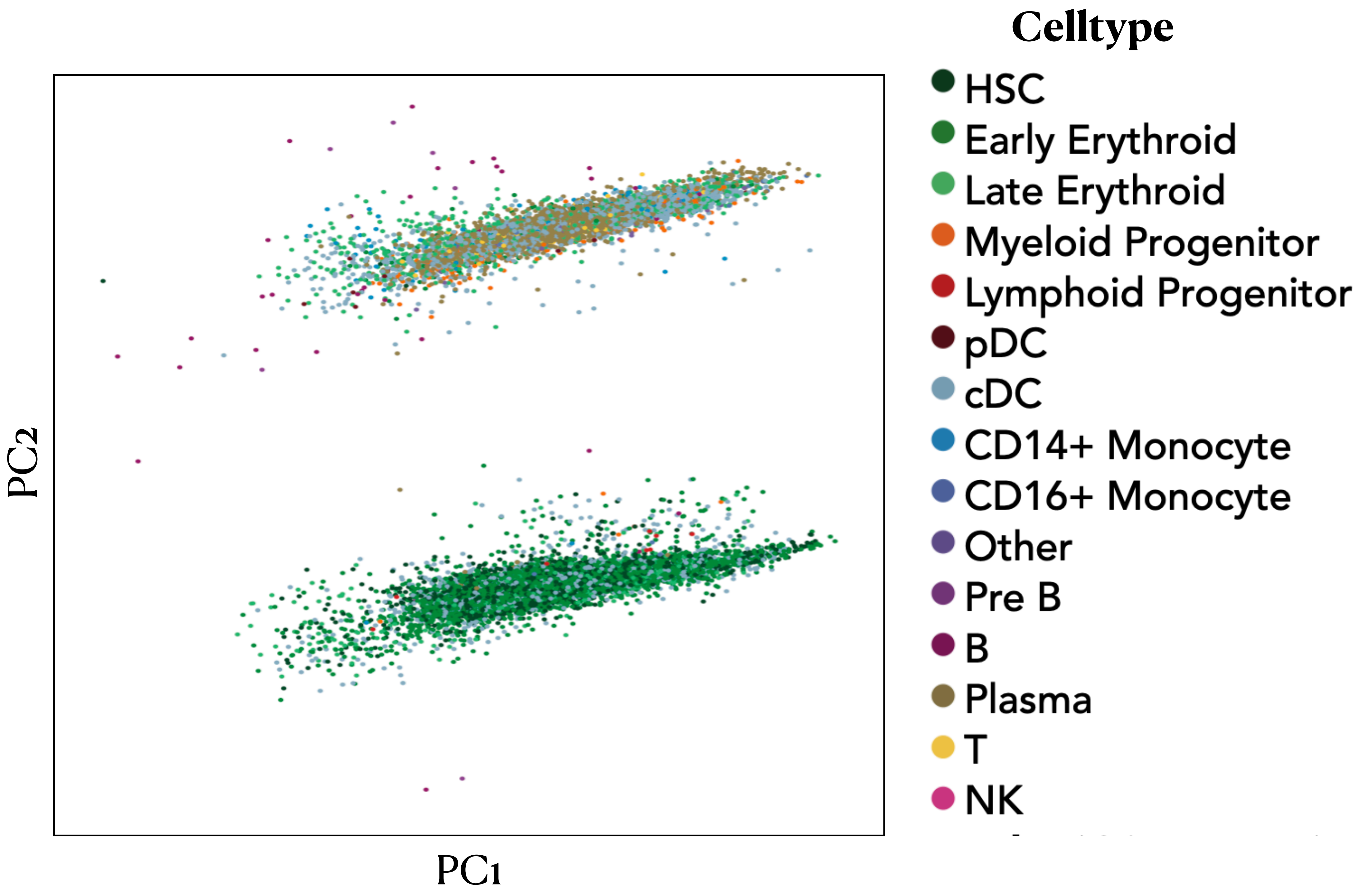
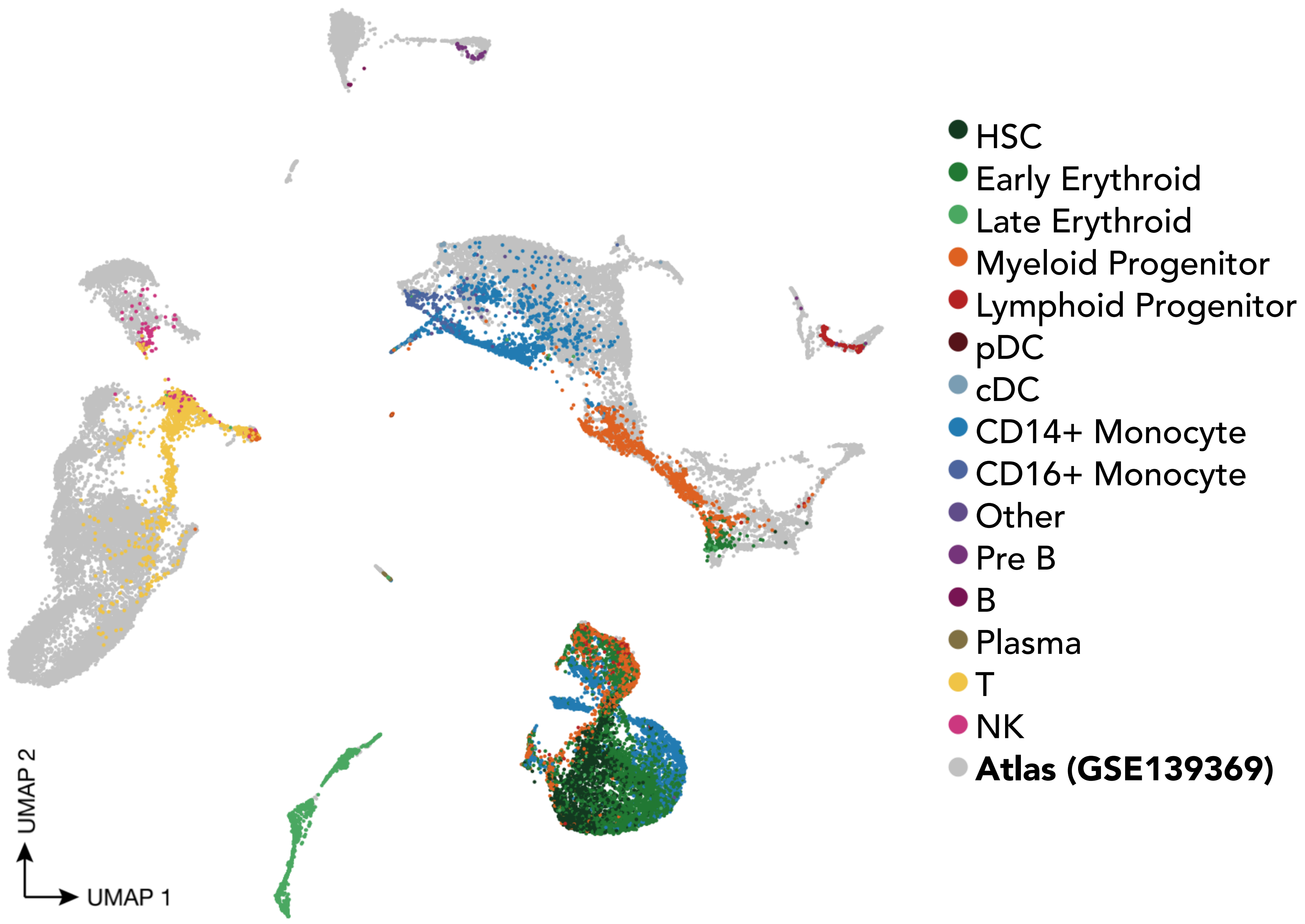
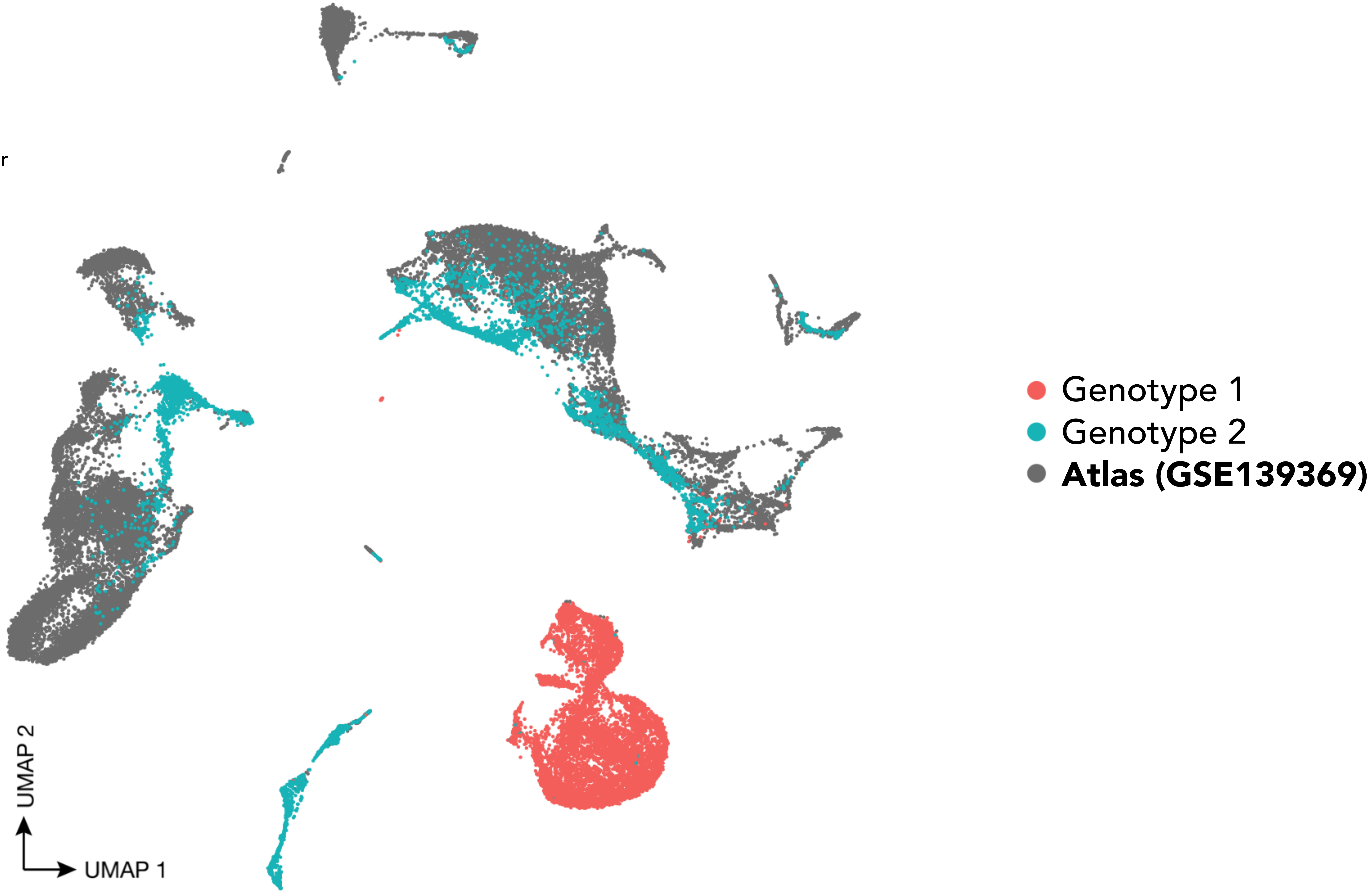




Convincing evidence for relapse using souporcell





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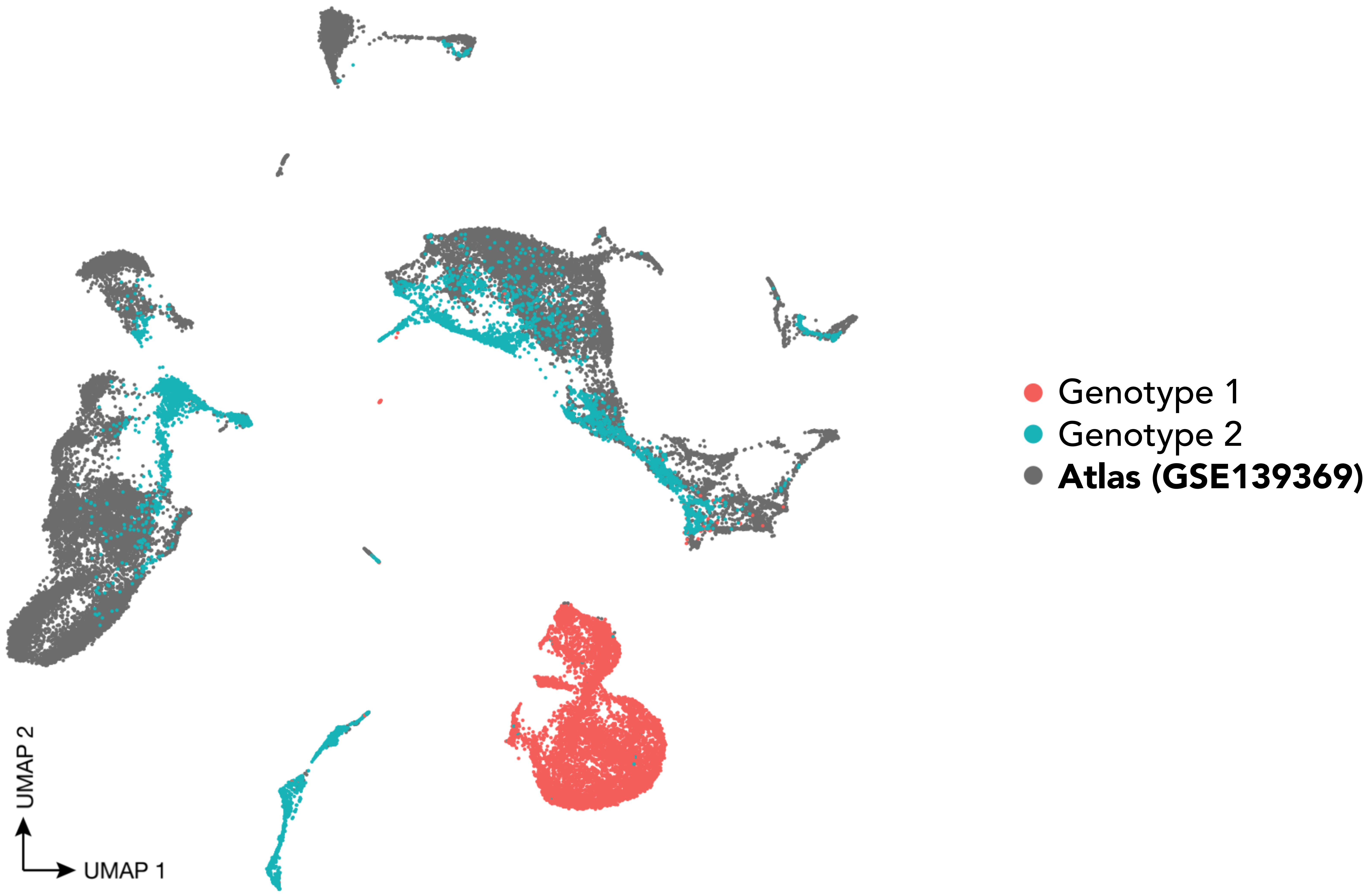
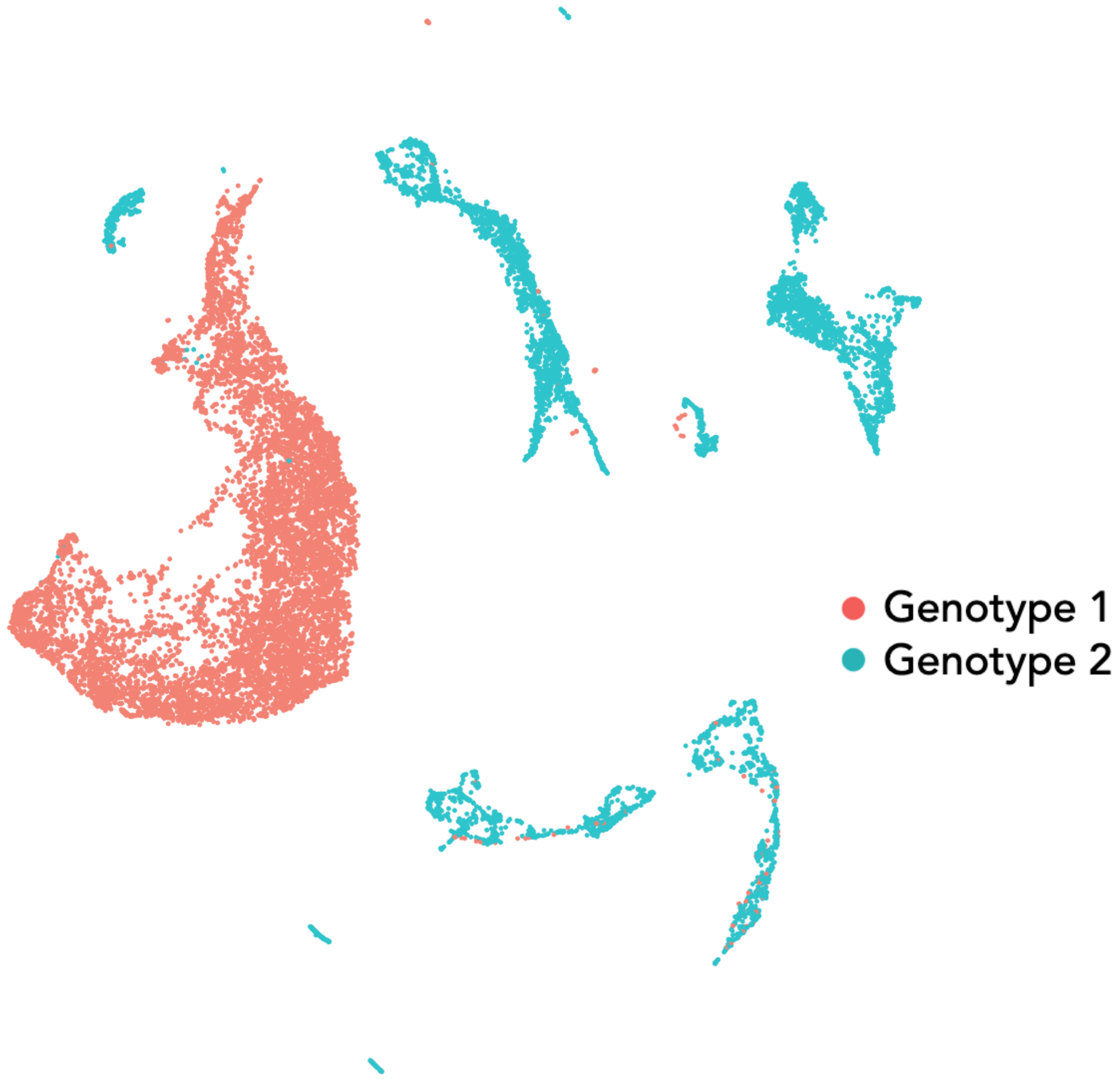
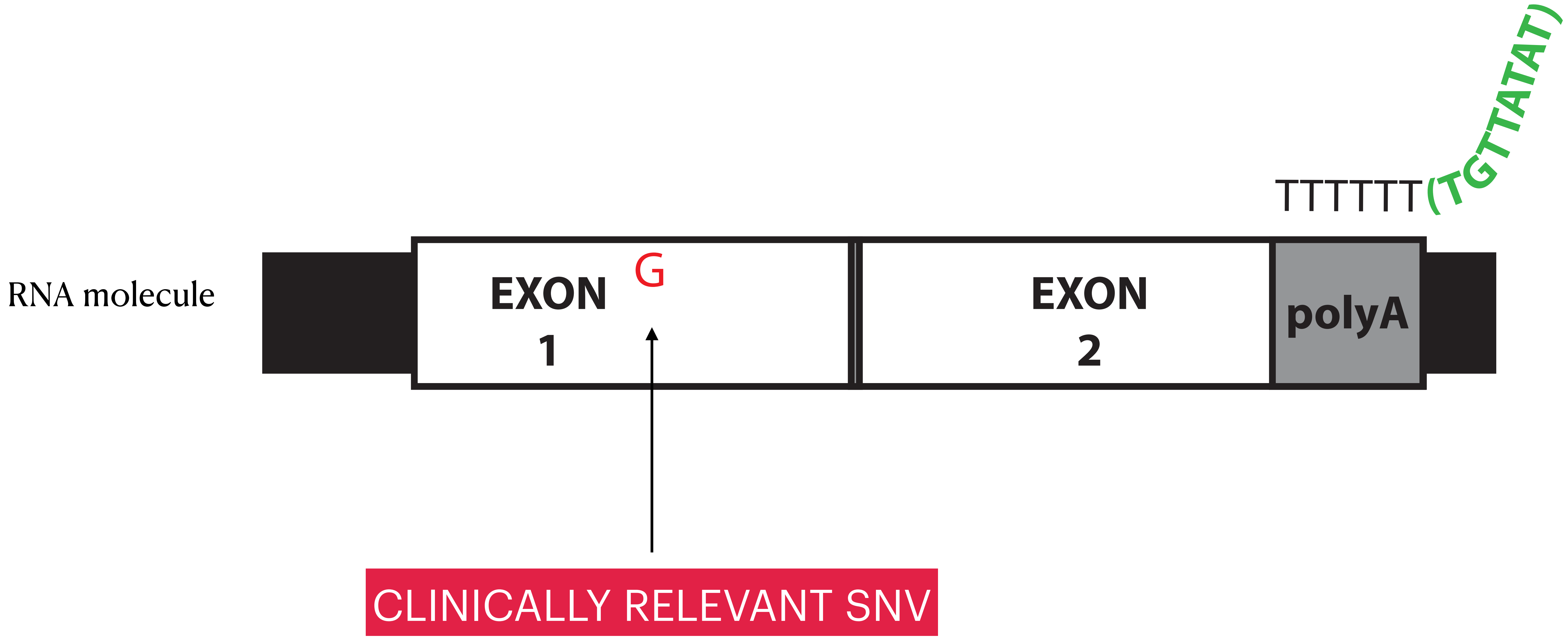


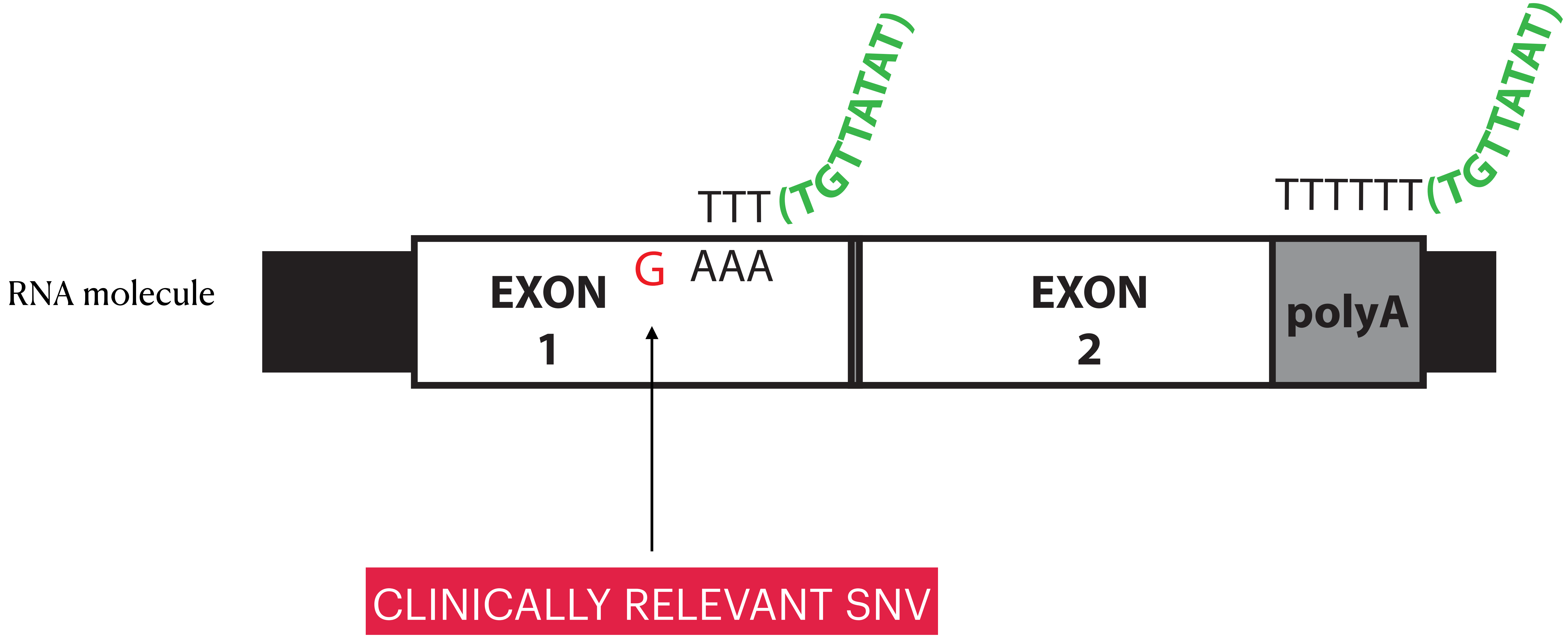


Can we detect AML mutations in scRNAseq data?



CLINICALLY RELEVANT SNV

Can we detect AML mutations in scRNAseq data?



cb_sniffer

Petti et. al. Nature Communications 2019

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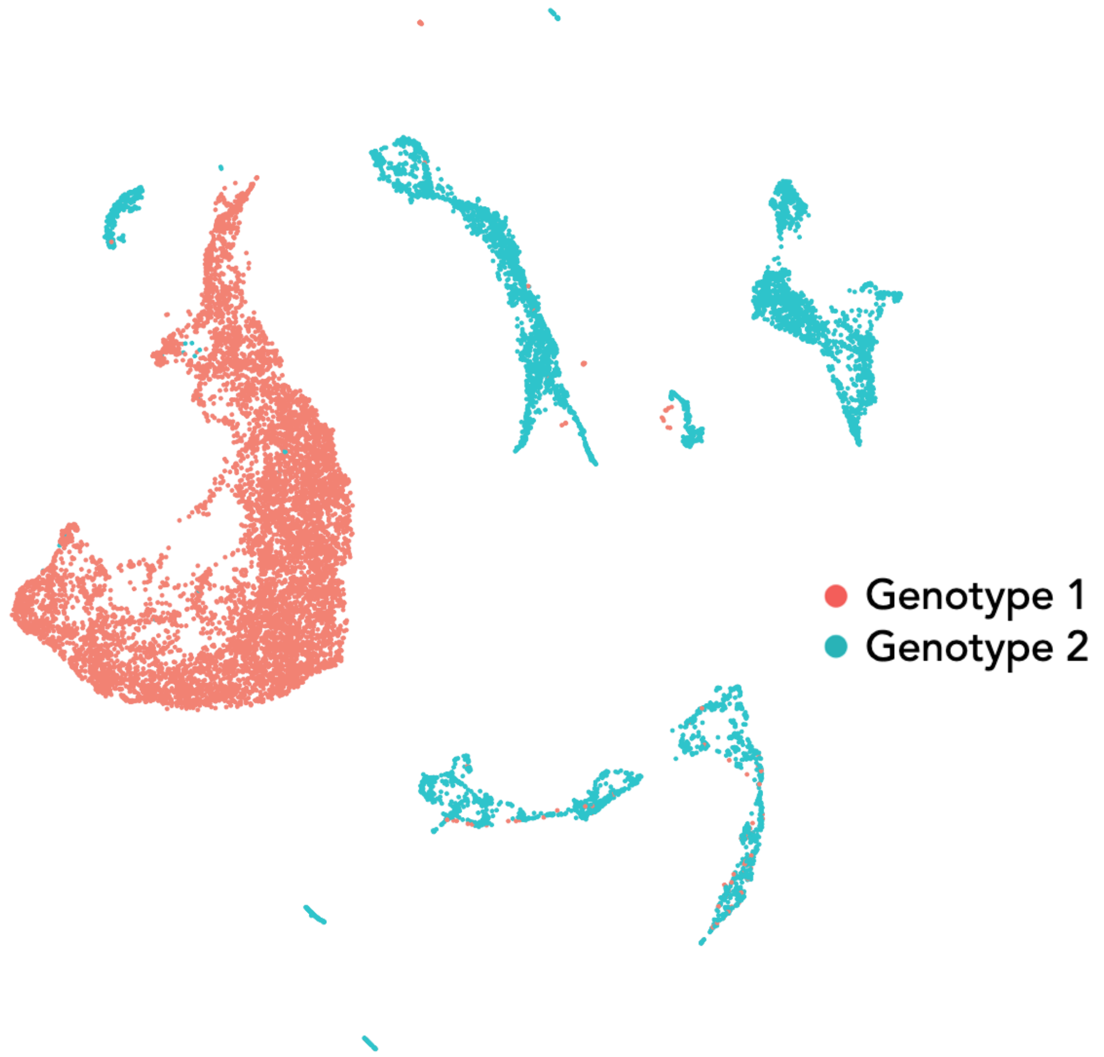
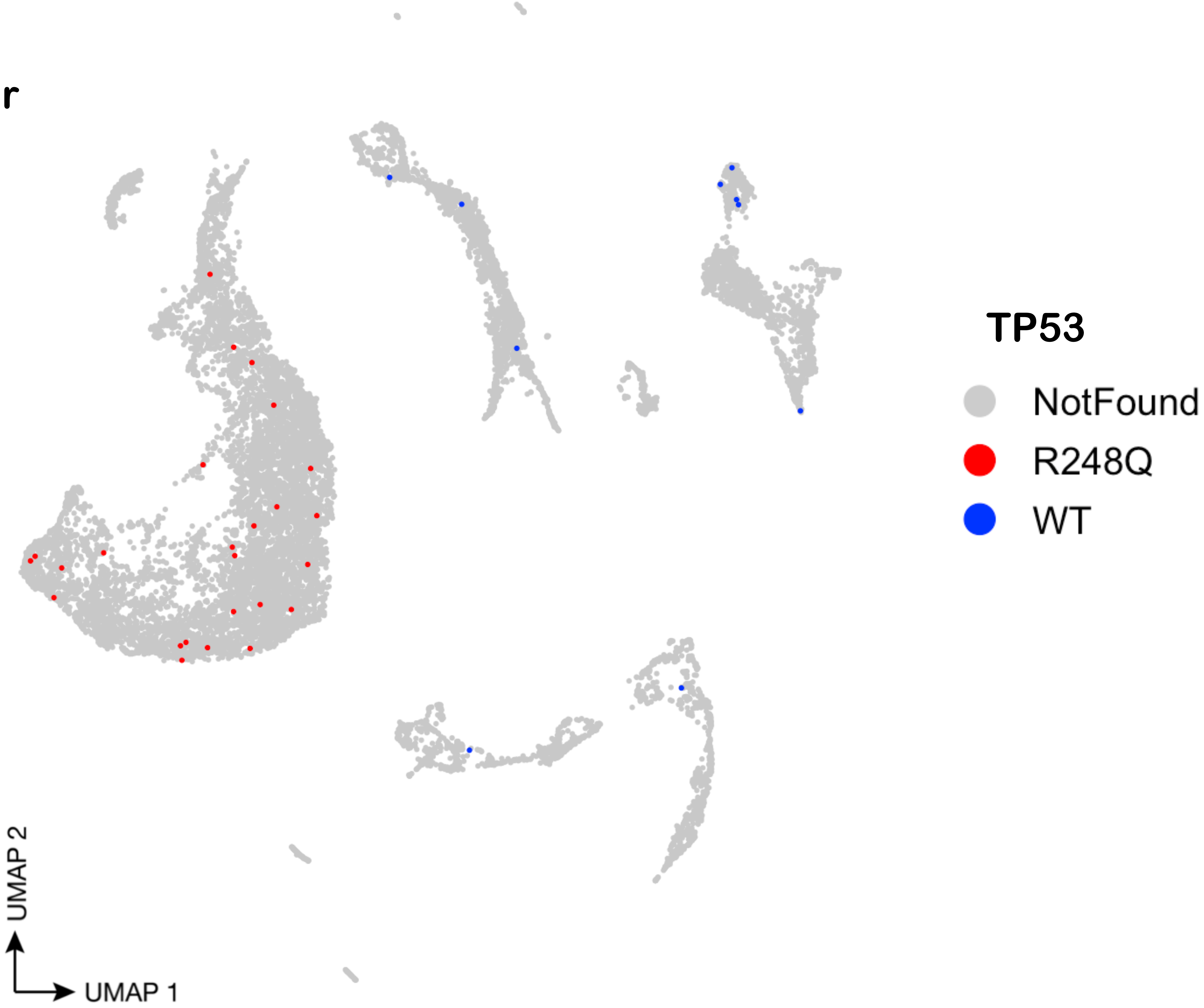
Petti et. al. Nature Communications 2019

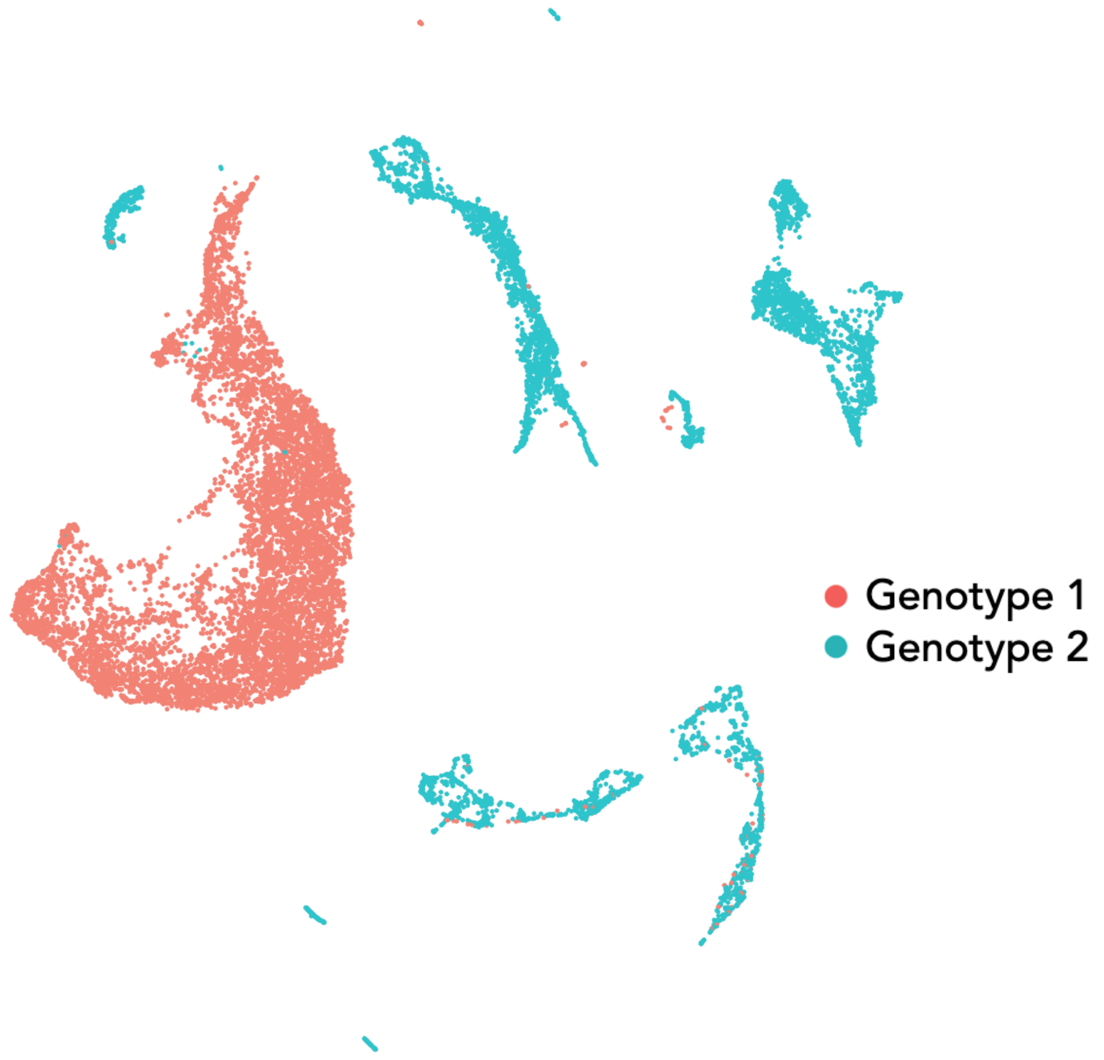


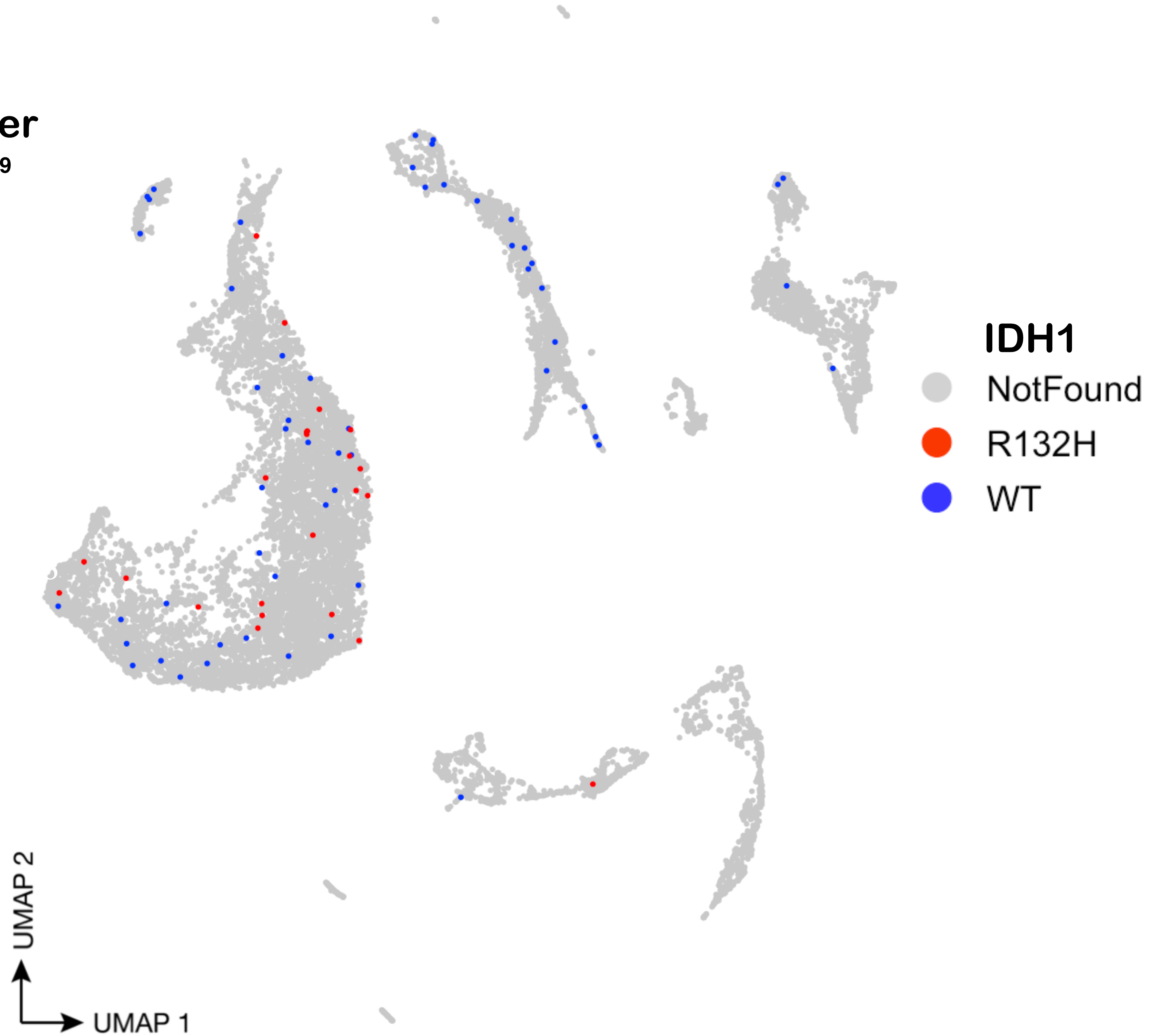


Not sufficient coverage for the study of clonal heterogeneity

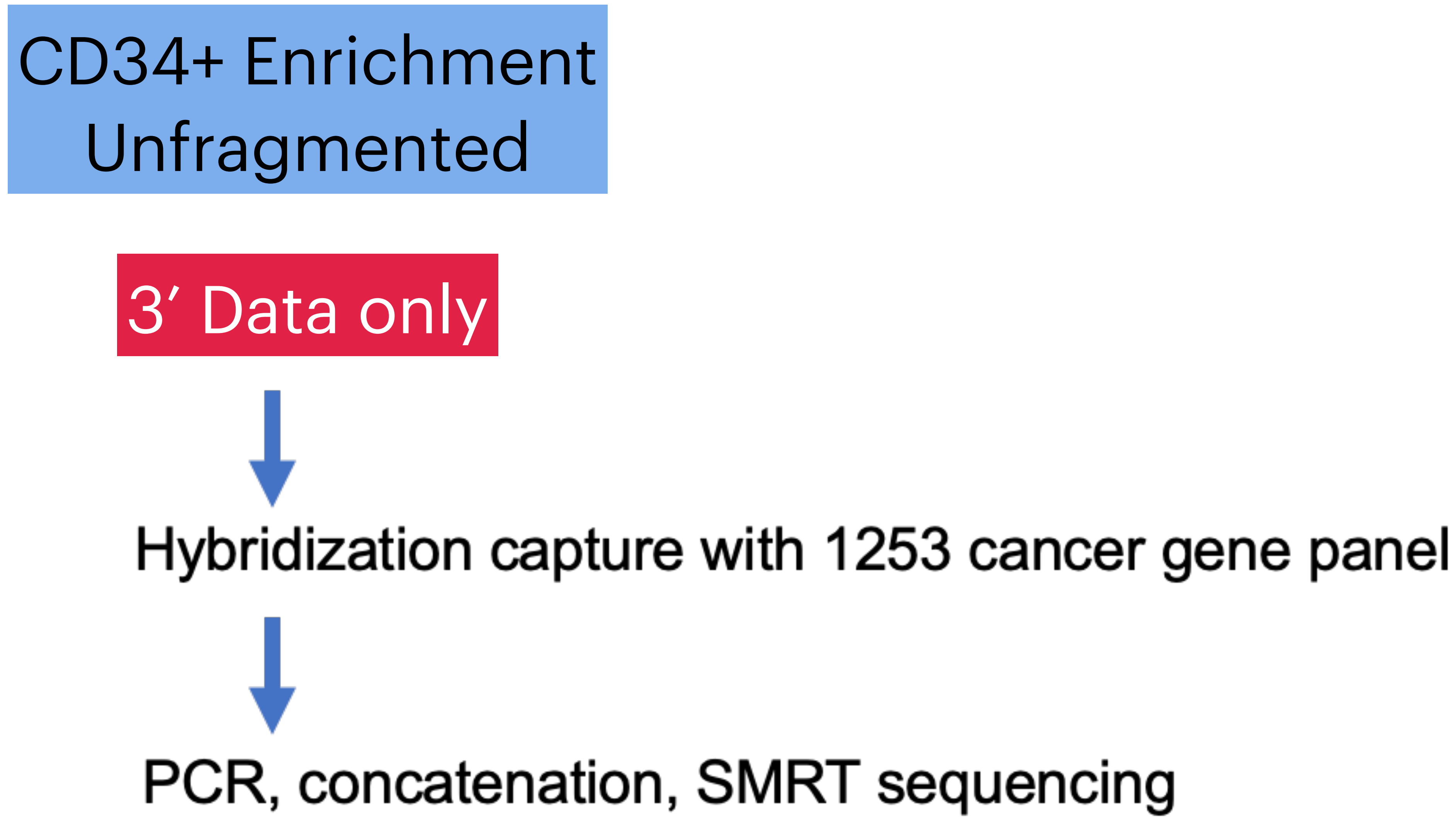
cb_sniffer

Petti et. al. Nature Communications 2019





Can we augment coverage?



CD34+ Enrichment
Unfragmented

3′ Data only

Hybridization capture with 1253 cancer gene panel

PCR, concatenation, SMRT sequencing

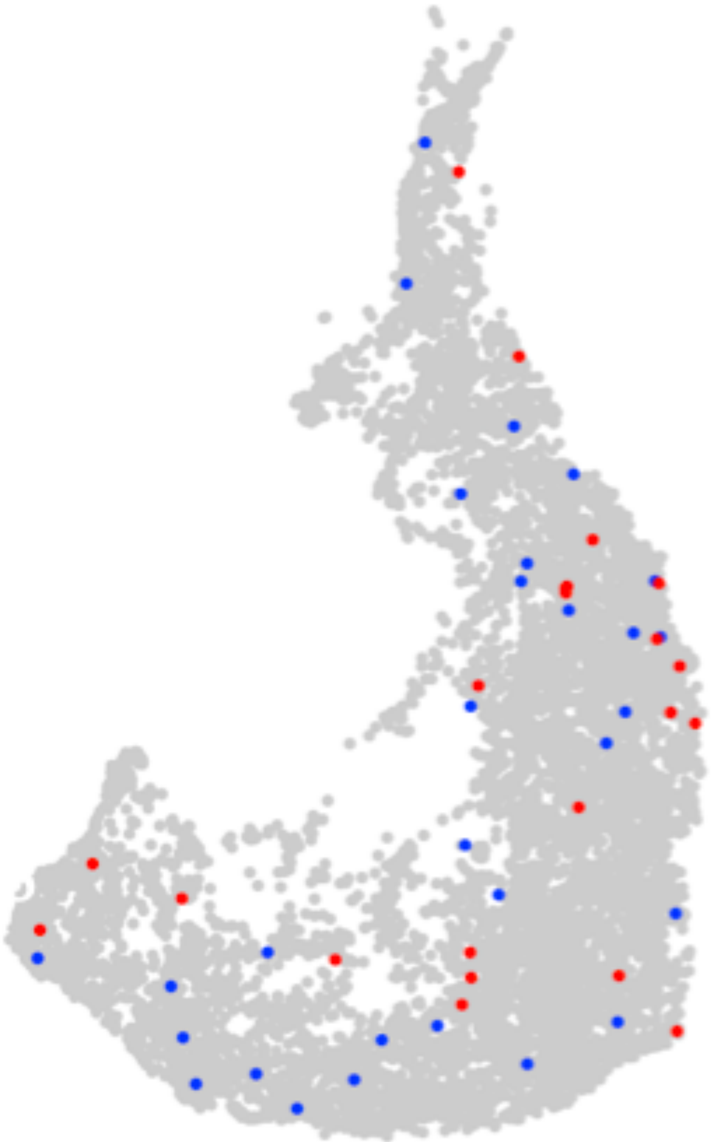
PacBio

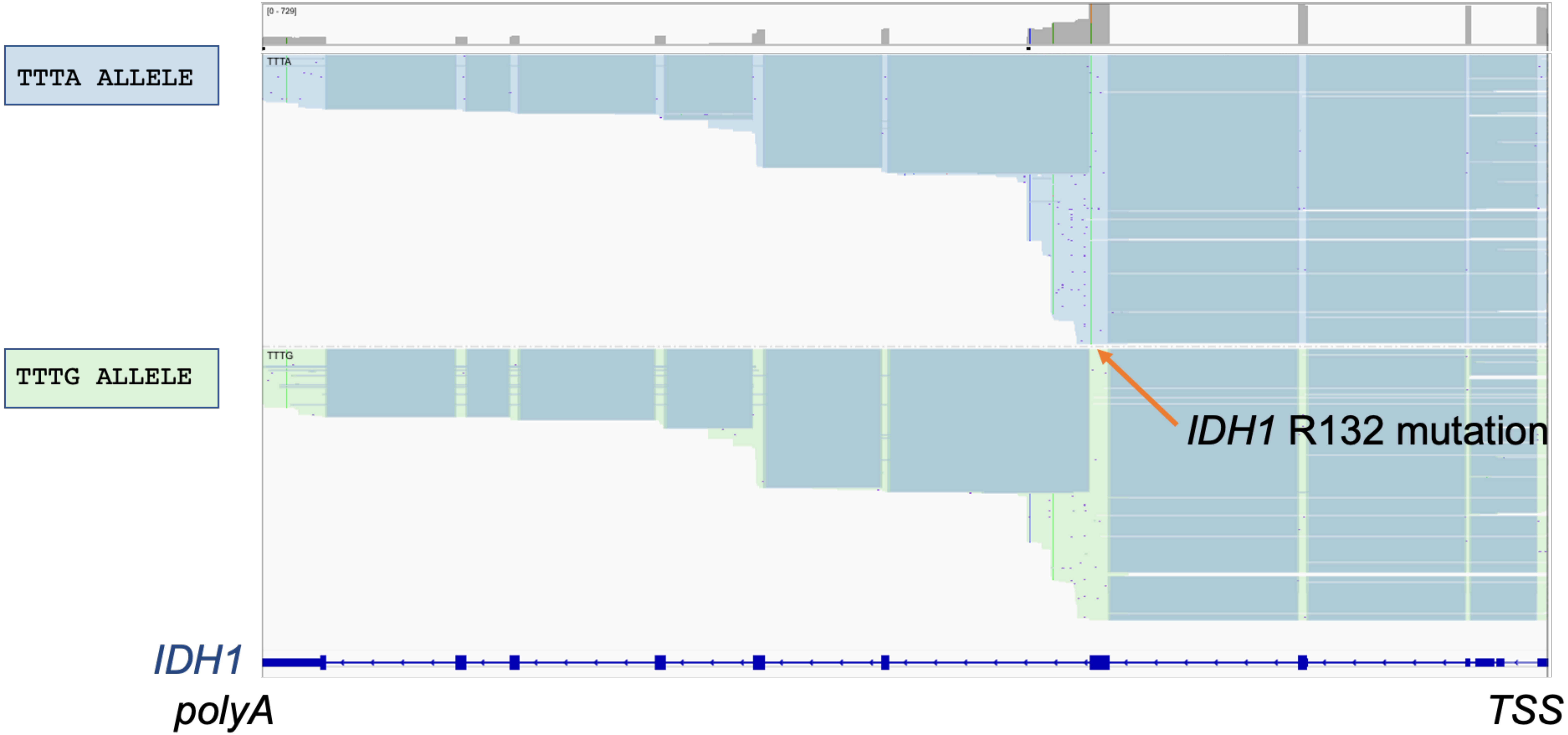
Jason Underwood PhD

We can dramatically increase coverage using IsoSeq

CD34+ Enrichment
Unfragmented

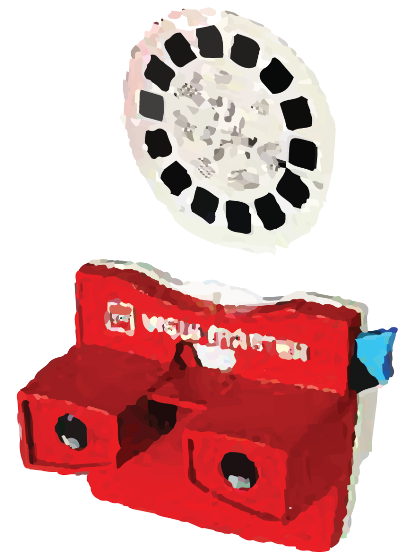
3′ Data only



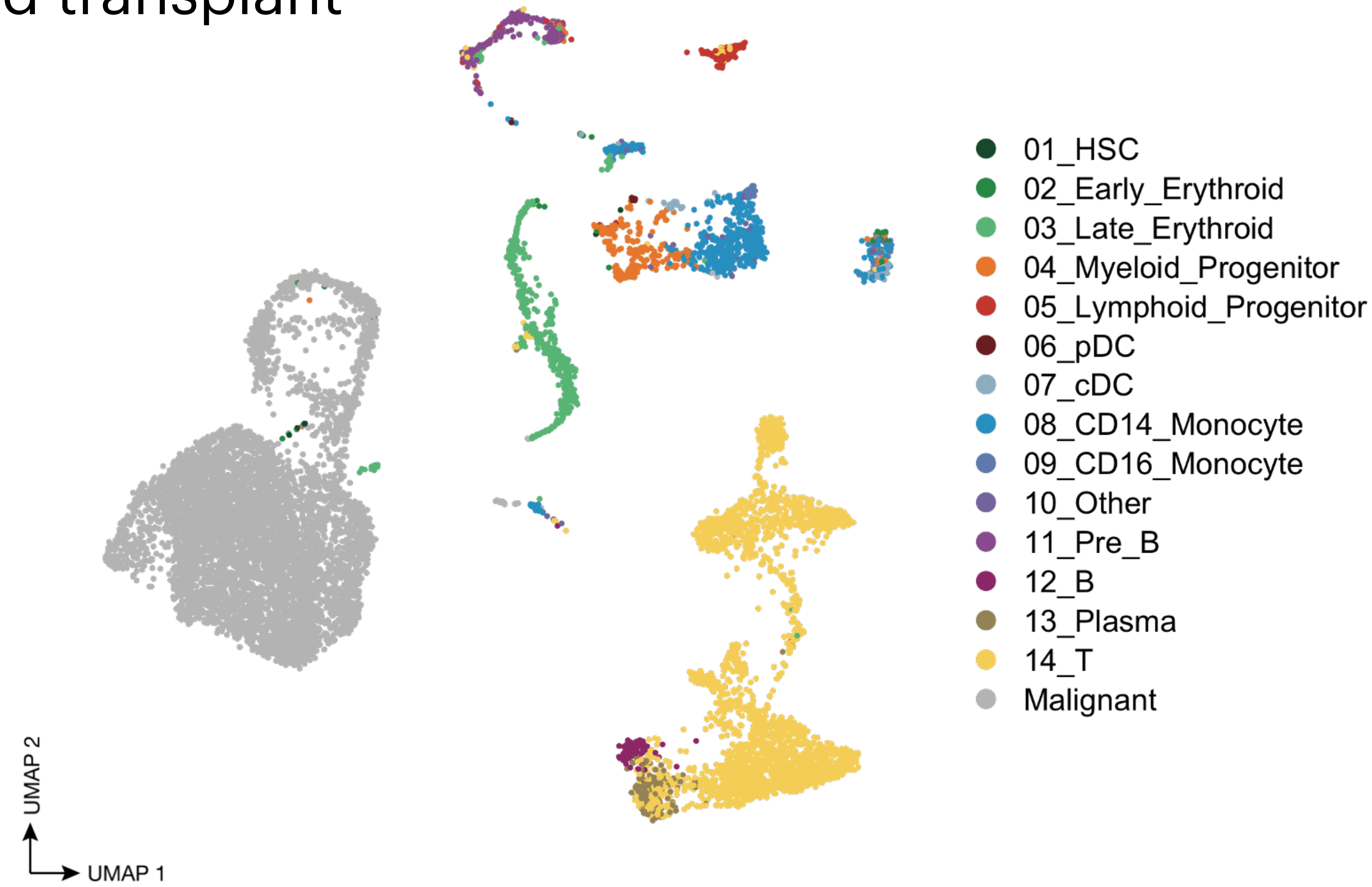


How does this extend to other patients?

- 2 yo with undifferentiated leukemia, with suspected relapse after unrelated cord blood transplant



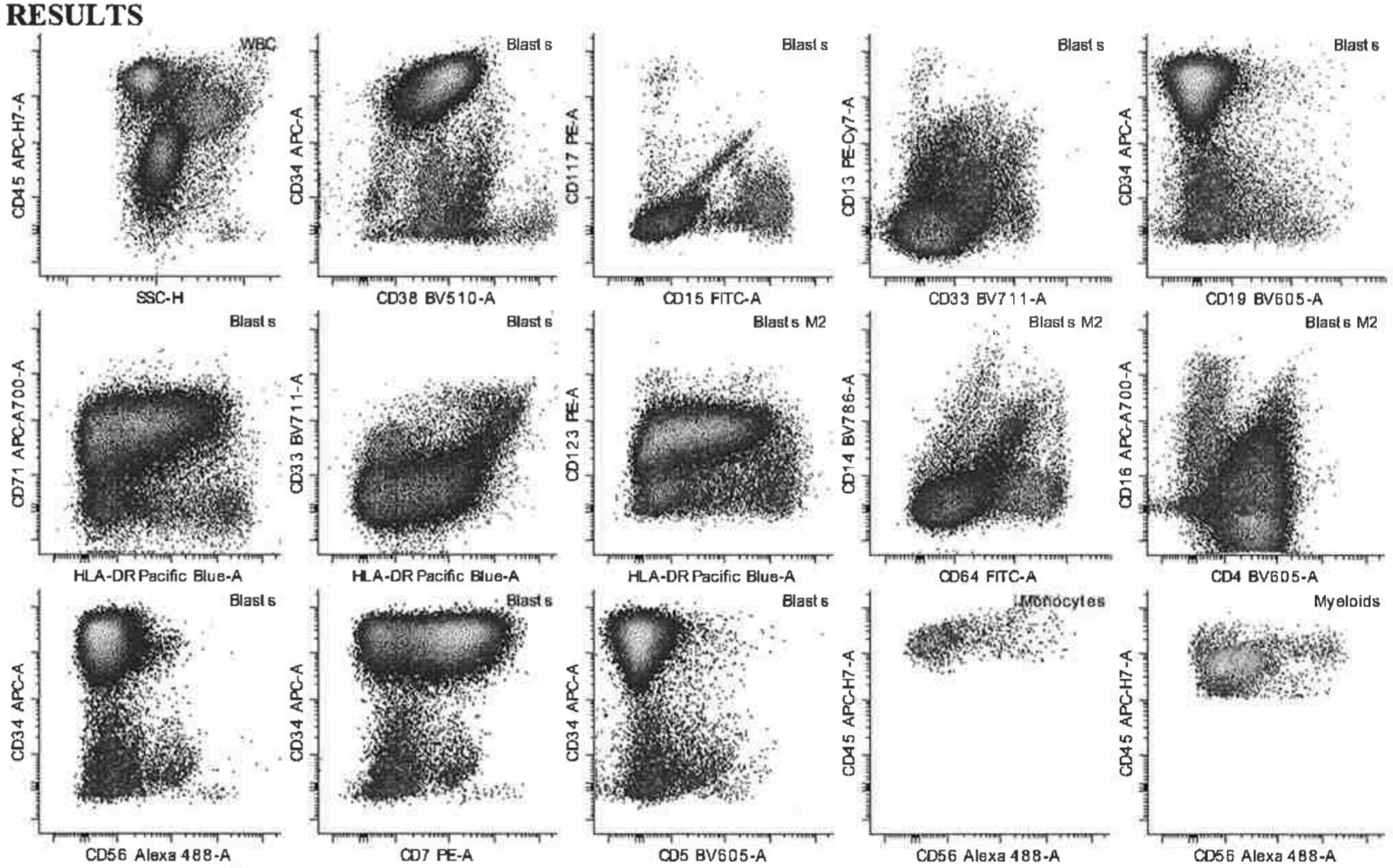
\+
CITEseq

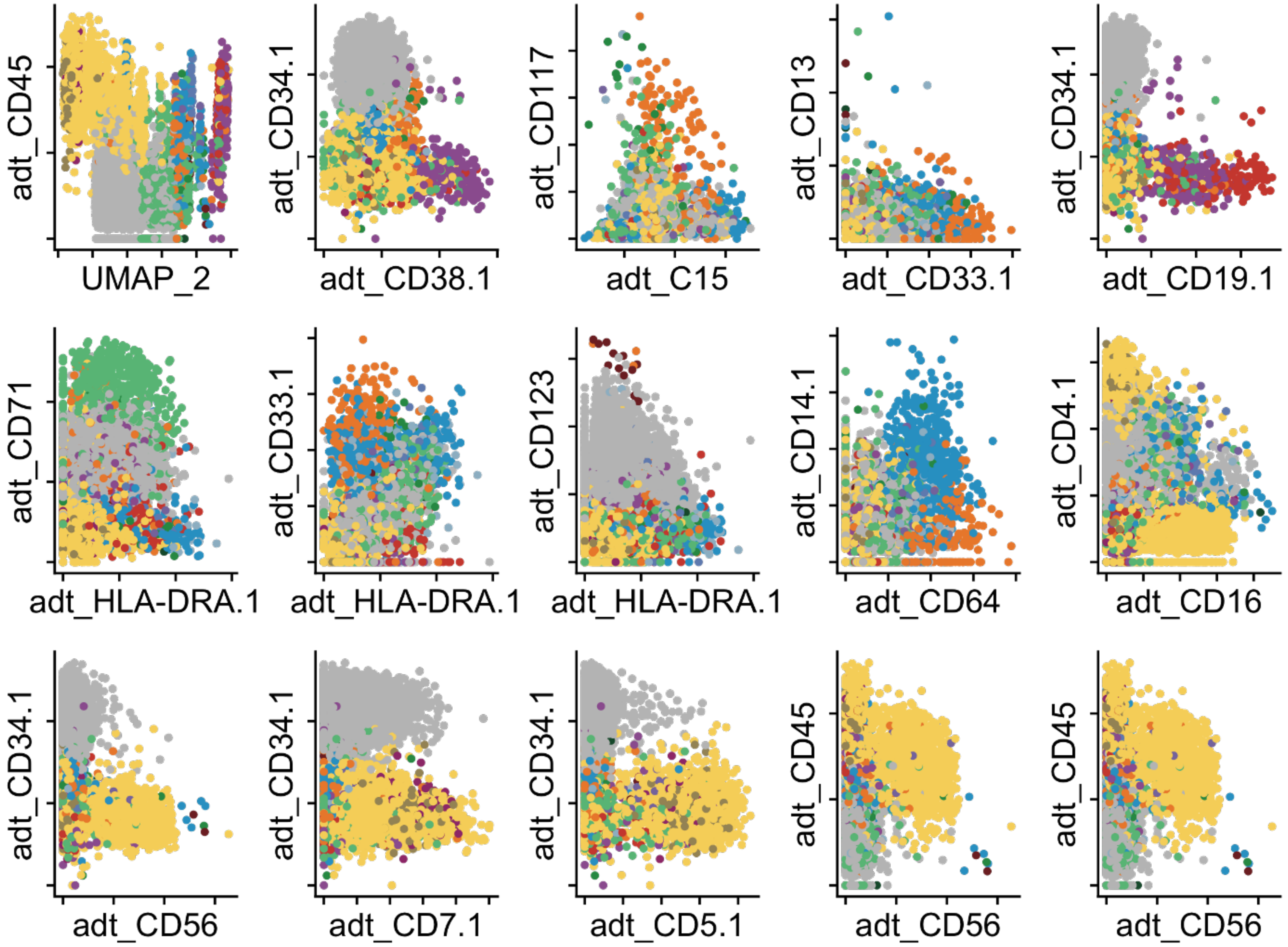


Can we leverage cell surface immunophenotype?

CLINICAL FLOW CYTOMETRY

CITE Seq



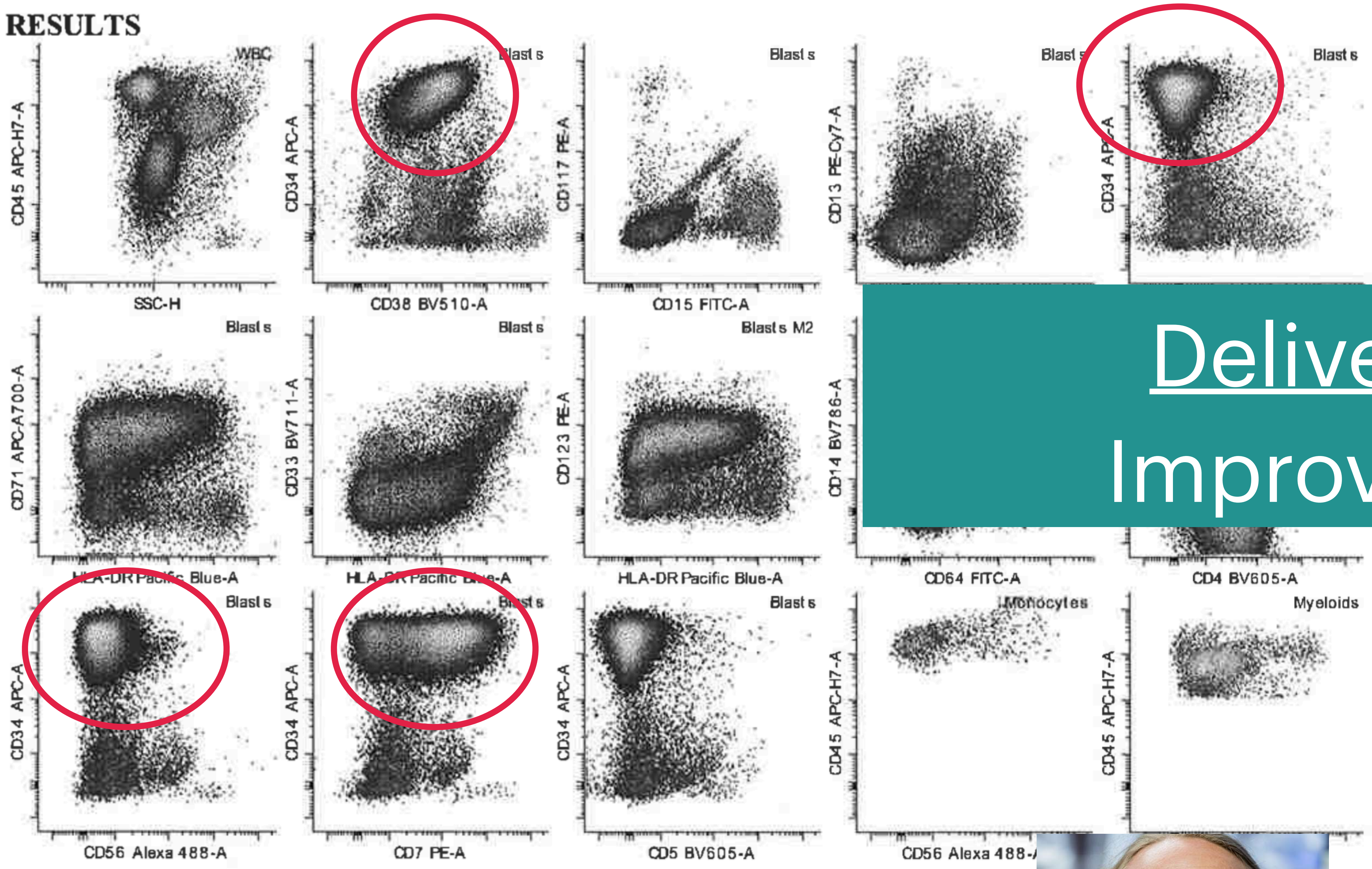


10-20 Cell surface antibodies

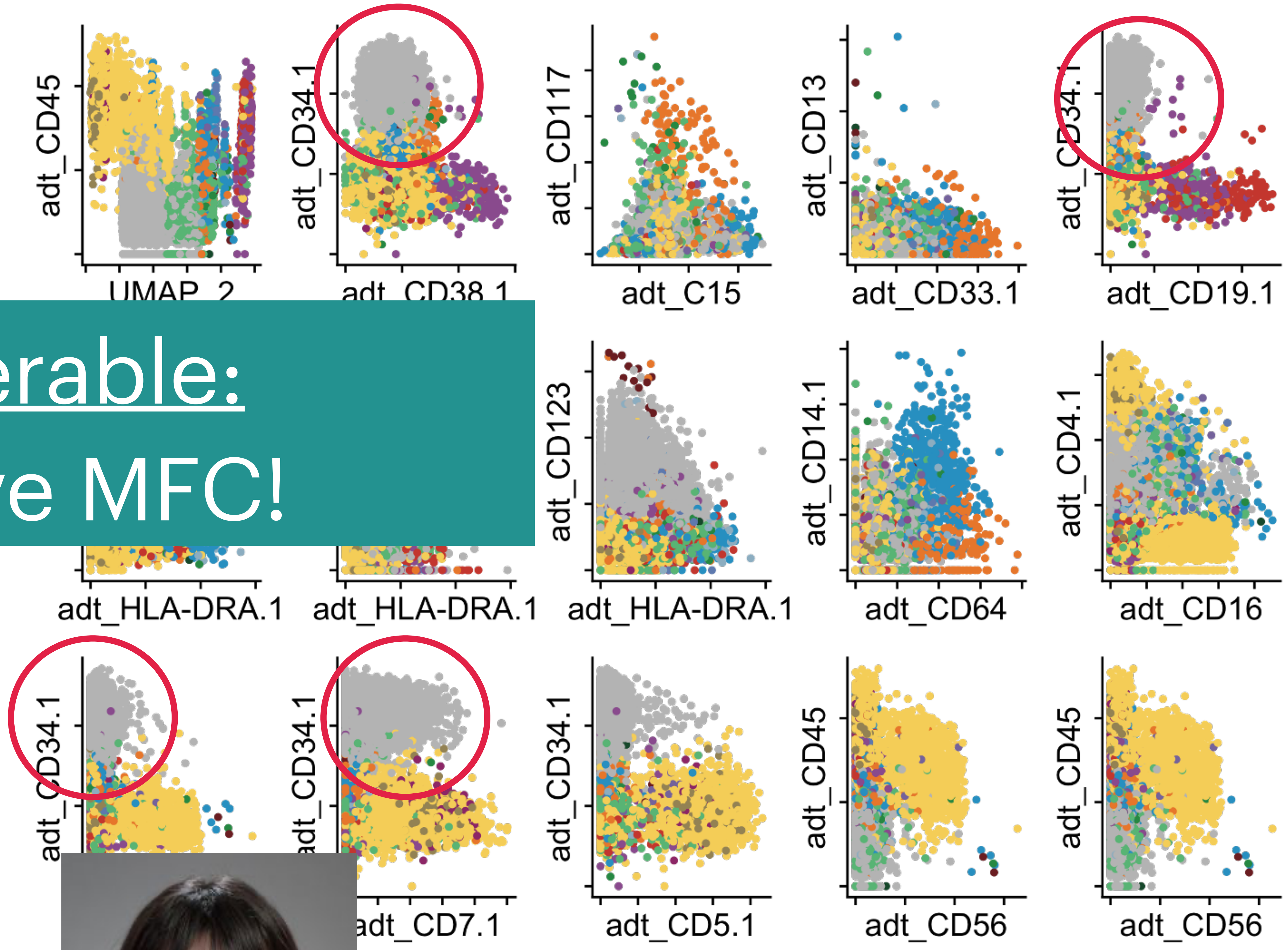
150 Cell surface antibodies

Can we leverage cell surface immunophenotype?

CLINICAL FLOW CYTOMETRY



CITE Seq



Deliverable:

Improve MFC!

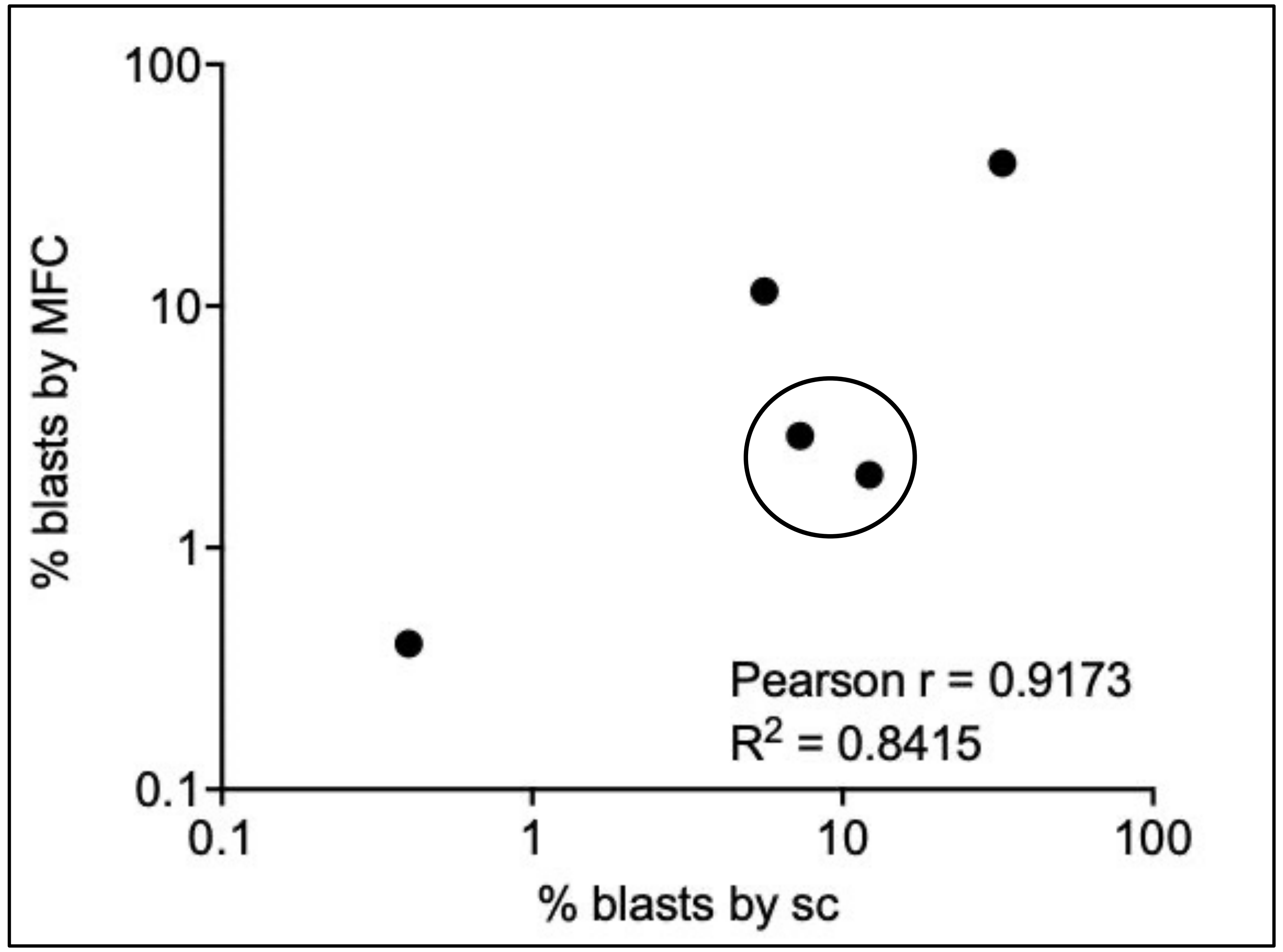
10-20 Cell surface antibodies

150 Cell surface antibodies

Melinda Biernacki MD

Xueyan Chen MD PhD

Where are we now?



- Two patients with PCR+/MFC negative
- One cell of early myeloid lineage and recipient origin

Summary

- Inconsistencies in clinical assays are important motivators to improve diagnostics
- Integration of single expression data with genetic demultiplexing can provide a confident assessment of burden of relapsed leukemia
- Promising preliminary data suggesting that we can augment coverage of specific loci
- Immunoproteomic data show promise in recapitulating clinical flow cytometric data.

Future directions

- Increase sample numbers
- Working to detect fusion transcripts using PacBio sequencing
- Mechanisms of relapse
    - HLA expression / Antigen expression
    - T cell exhaustion
    - Myeloid suppressor cells
- Resources…

Data Scientist Positions at SCRI

- The Ben Towne Center for Childhood Cancer Research (BTCCCR) at SCRI is looking for Computational Biologists/Data Scientists

- Positions are available for Lead Data Scientist and Data Scientist I positions

- Experience with omics or other high dimensional data analysis and pipelines, e.g. next-generation sequencing, proteomics, metabolomics, epigenomics, phenotypic readouts, imaging, and clinical data, including experience developing and using statistical models and algorithms is required

- Work together with pediatric cancer researchers to improve the lives of children with cancer!

https://careers-seattlechildrens.icims.com/

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Email: Sean.Taylor@seattlechildrens.org or jay.sarthy@seattlechildrens.org for more information

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Monica Thakar

Cole Trapnell

Jason Underwood
Vijay Ramani

Soheil Meshinchi
Rhonda Reis
Jenny Smith

OUR PATIENTS AND THEIR FAMILIES

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Questions?