An atlas of the tumor immune ecosystem

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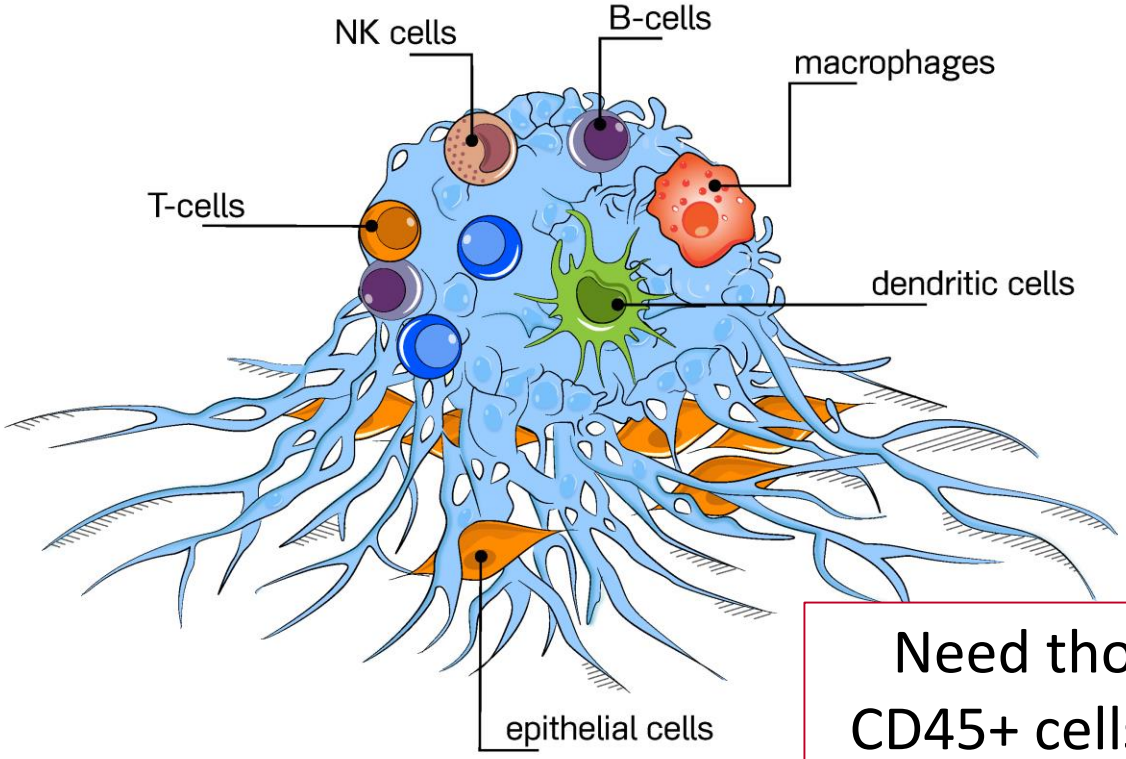
Disclosure slide

- The speaker discloses that she is a strongly opinionated person.
- However, these strong opinions are not driven by any financial interests, but rather her scientific passion.

A Human Cell Atlas

- Our genes are well mapped, but most of cell types remain unknown
- Cells are basic biological units
- Diseases are caused by malfunction of specific cell types.
- Goal: Construct a comprehensive map of all cell types in our body

Tumor Eco-system



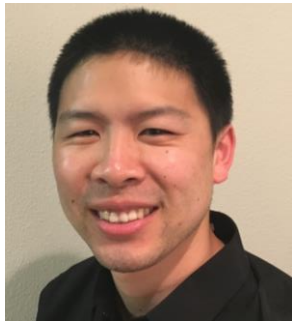
Need thousands of CD45+ cells per tumor

- Goal: Characterize sub-populations in tumor immune ecosystem.
- Challenge: Substantial unknown diversity.
- A better understanding of tumor immune eco-system will aid the development strategies to activate it against the tumor.

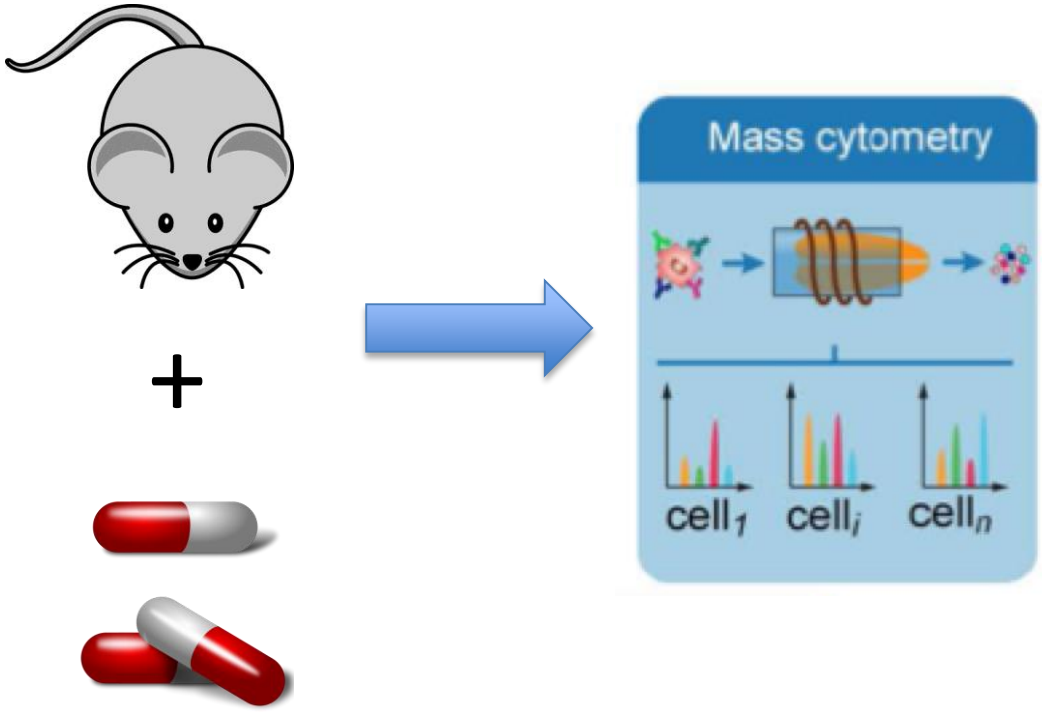
With Allison lab, MD anderson

Understanding response to immunotherapy

Spencer Wei
Jacob Levine
Jim Allison

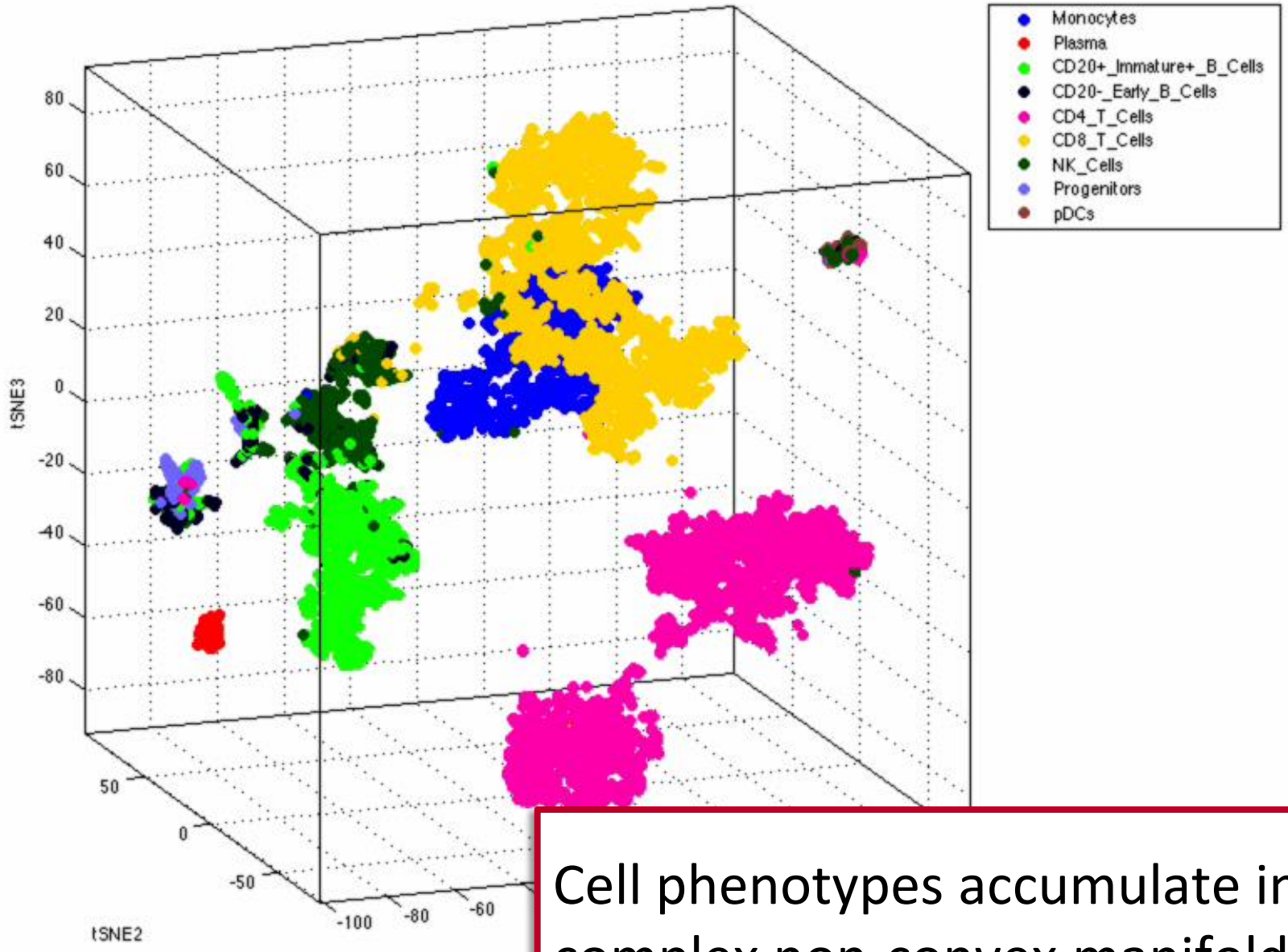


Data Collection



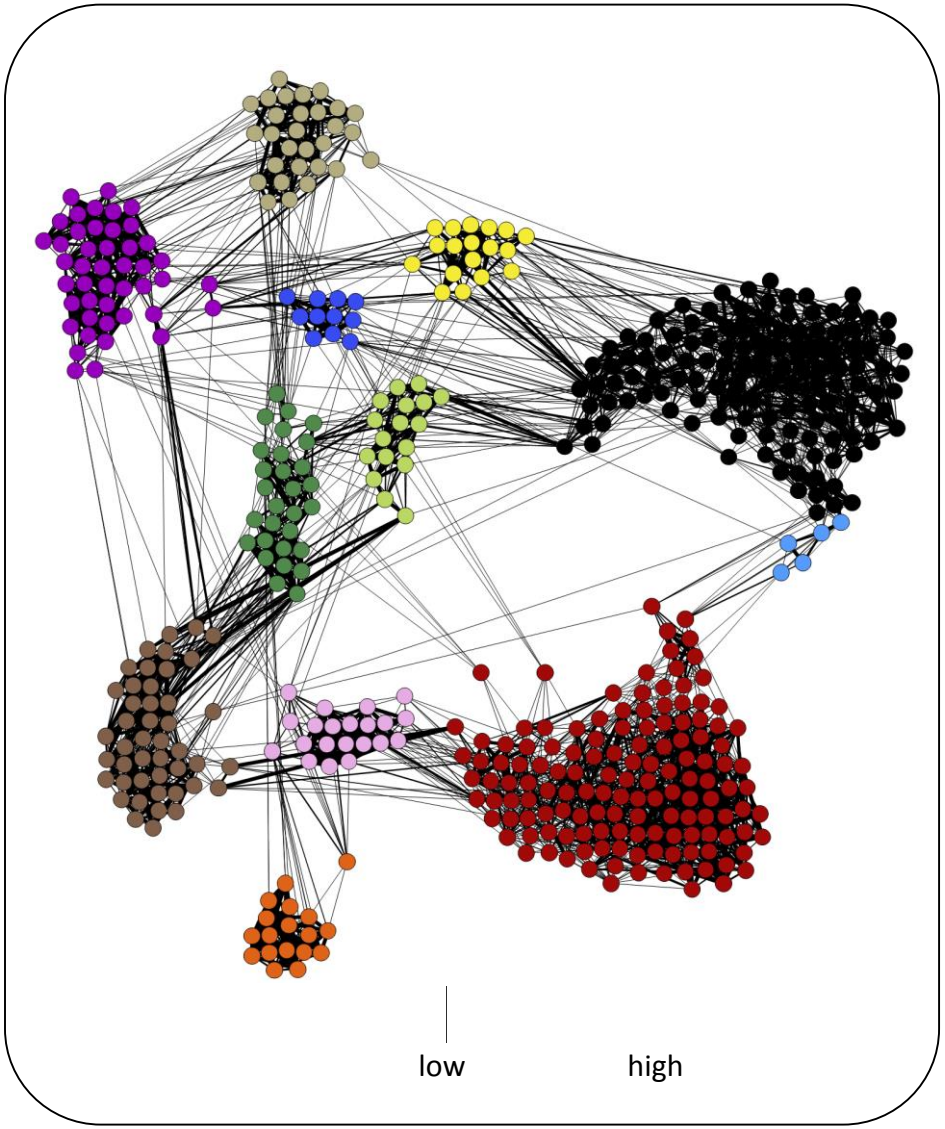
- Measured response to anti-PD1 and anti-CTLA4
- Measured immunogenic MC38 colorectal tumors and the poorly immunogenic B16BL6 melanoma mode
- 33 surface and 10 intracellular markers: Cell type(e.g. CD8, CD4, CD11b, CD19), T cell differentiation and activation markers (e.g. PD-1, ICOS, TIM3, KLRG1, CD127), T cell lineage transcription factors (e.g. T-BET, EOMES, GATA3, BCL6).

viSNE map of healthy bone-marrow



Cell phenotypes accumulate in complex non-convex manifolds

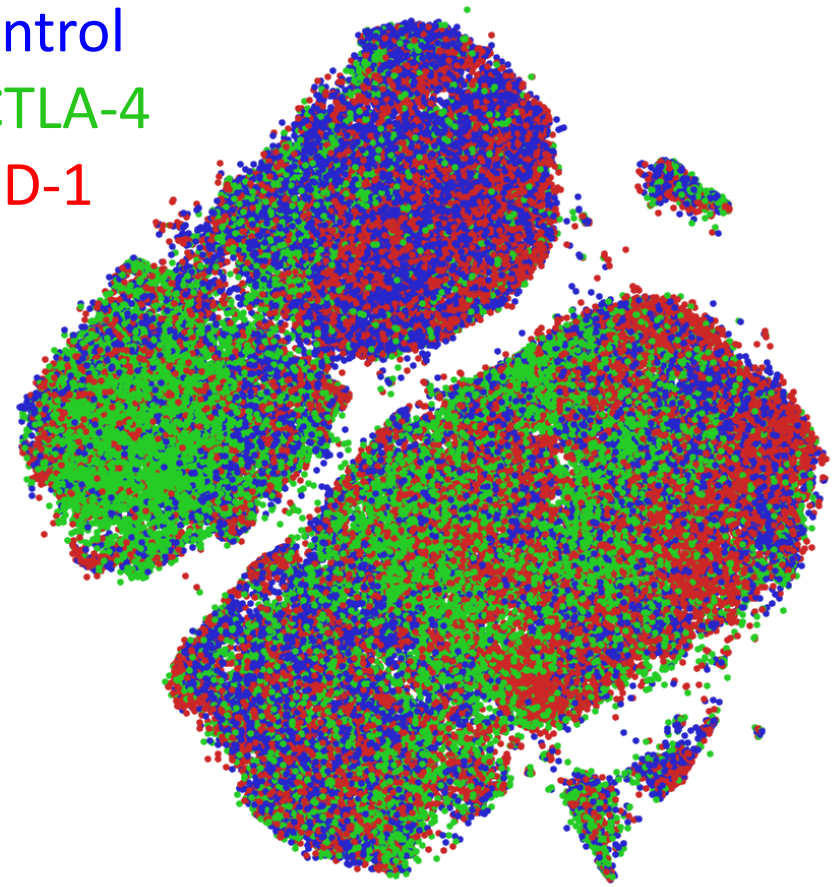
PhenoGraph: graph for single-cell phenotypes



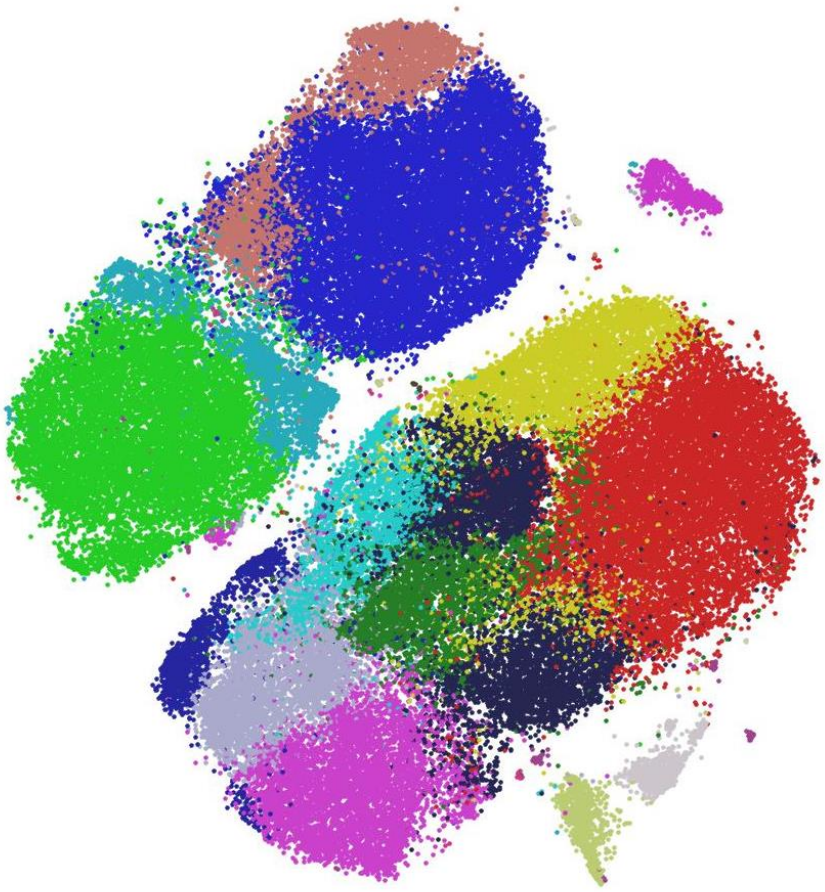
- Each node is a cell
- Each cell is connected to a neighborhood of “similar” cells
- Key to graph construction is a good distance measure:
- We use methods derived from social networks

MC38 tumor infiltrating T cell subsets

Control
αCTLA-4
αPD-1

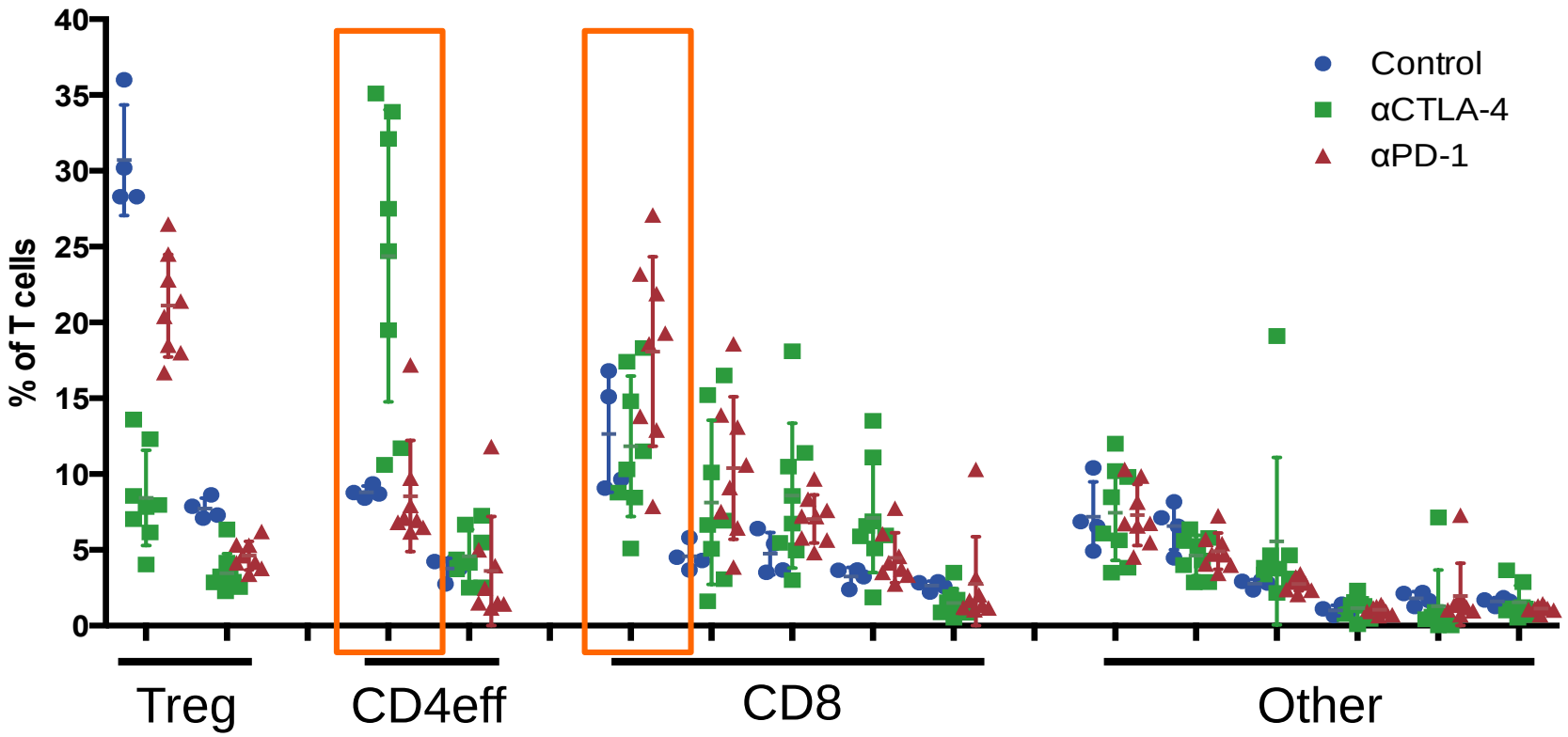


Clusters



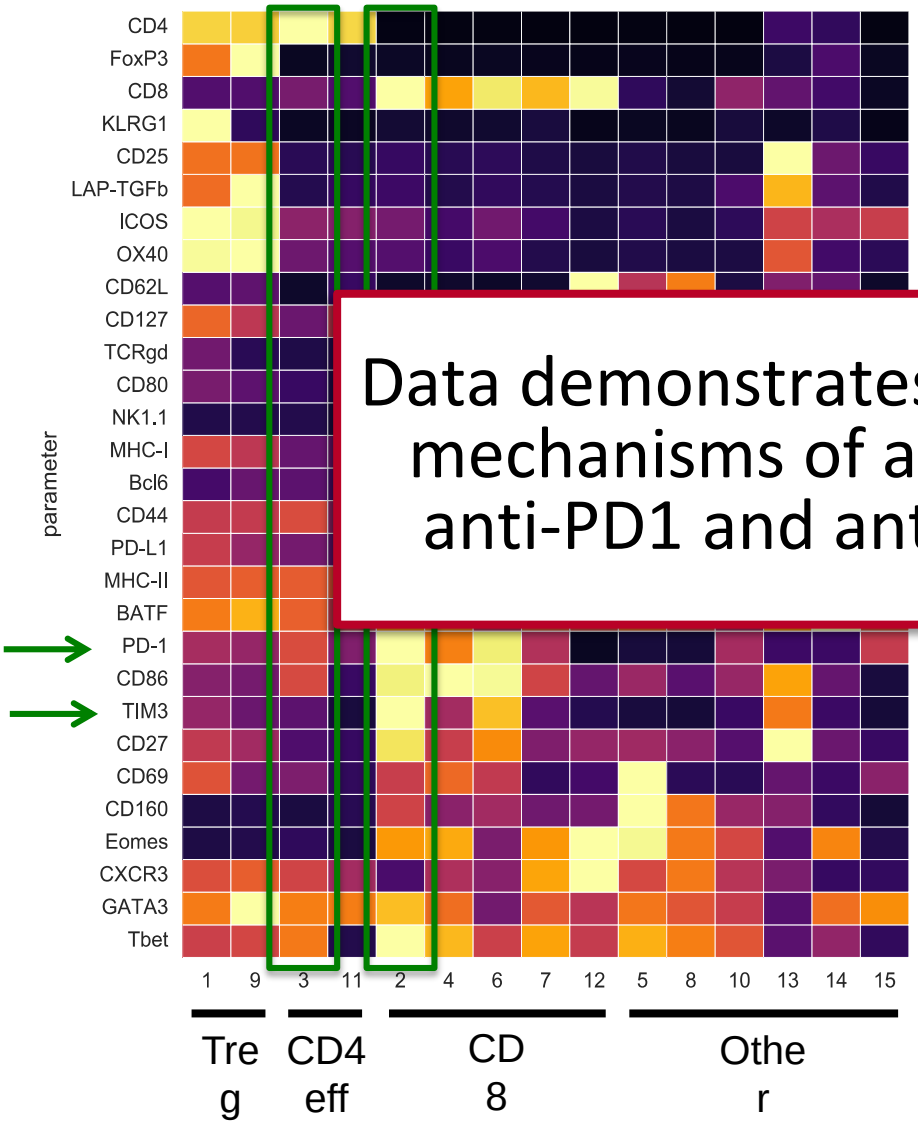
- 15 distinct tumor infiltrating T cell clusters found

Population dynamics in response to therapy



- CD8 expanded following both therapies, but not all CD8 clusters expanded equally
- Expansion of CD4 phenotype only in response to CTLA-4, but does not change after PD-1

MC38 infiltrating T cell populations

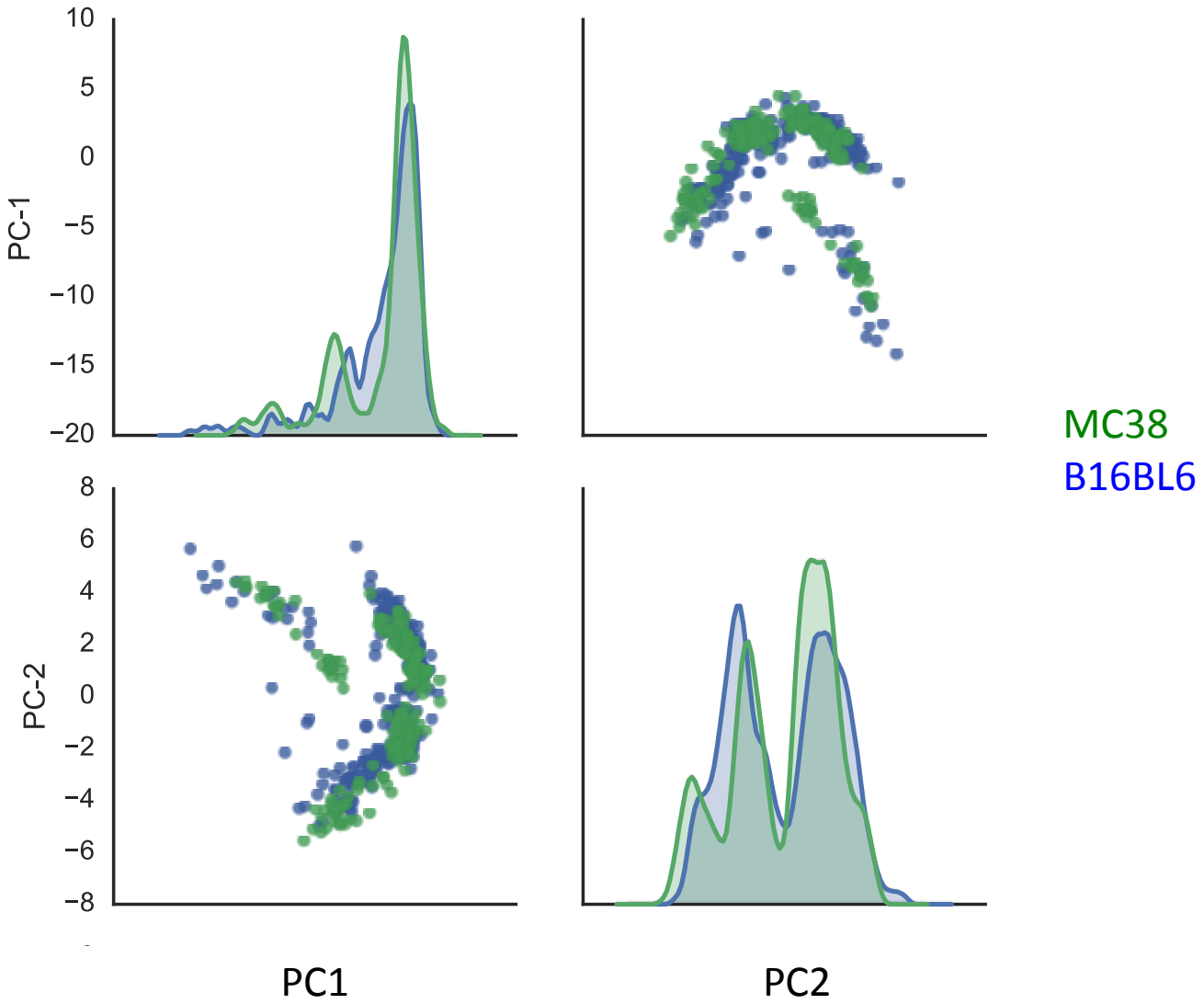


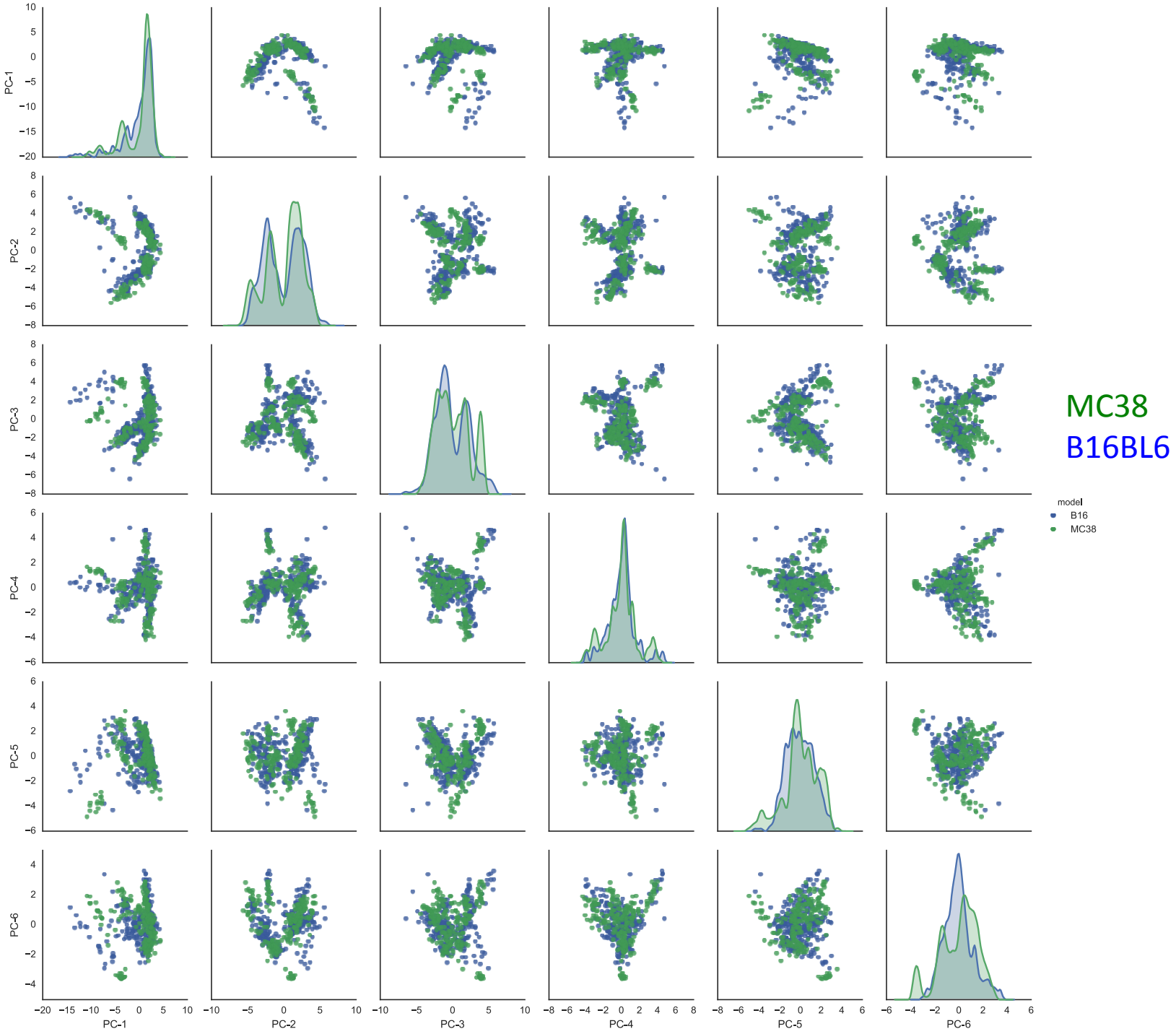
Data demonstrates different mechanisms of action for anti-PD1 and anti-CTLA4

- TIM3+ PD-1^{high}: Expansion of exhausted population (IdU staining) -> reinvigoration of exhausted phenotype

1-like phenotype (PD-
COSint CD86+ TBET+)
s only in response to
CTLA4, low IdU staining
suggests increased
differentiation or infiltration.

MC38 and B16BL6 infiltrating T cell populations are strikingly similar



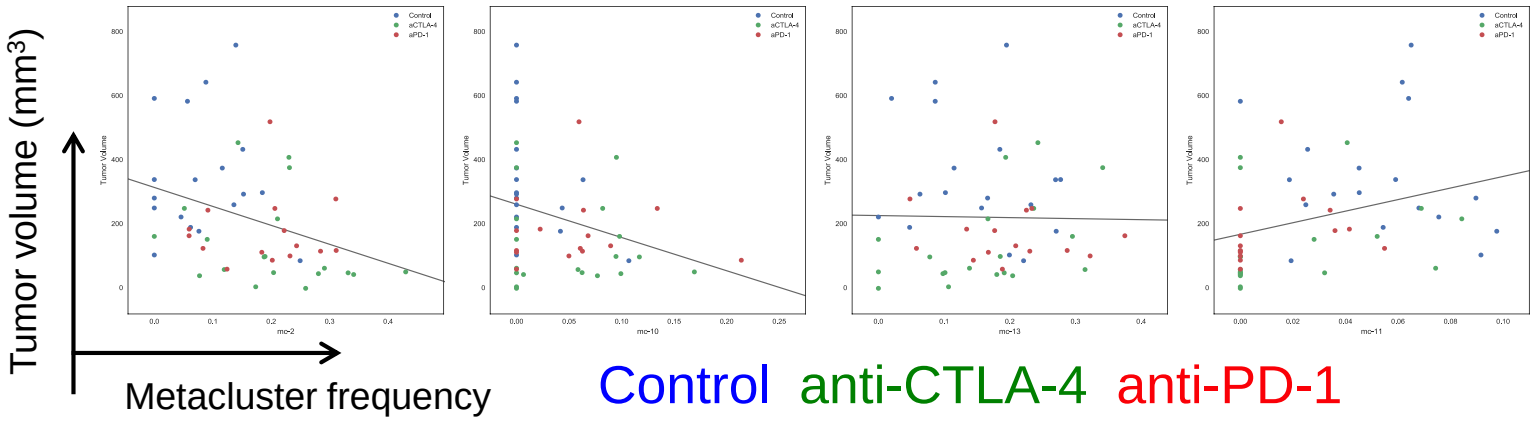

PC-1
PC-2
PC-3
PC-4
PC-5
PC-6
model
B16
MC38
MC38
B16BL6

T cell subsets that correlate with tumor growth

Metacluster 2	Metacluster 10	Metacluster 13	Metacluster 11
PD-1+ Tim3^{low} Tbet+ Eomes-	PD-1++ Tim3+ Tbet+ Eomes+	PD-1++ Tim3+ Tbet+ Eomes-	PD-1- CD62L+ CD127+

Multi-dimensional view is important,

only PD1 is not enough



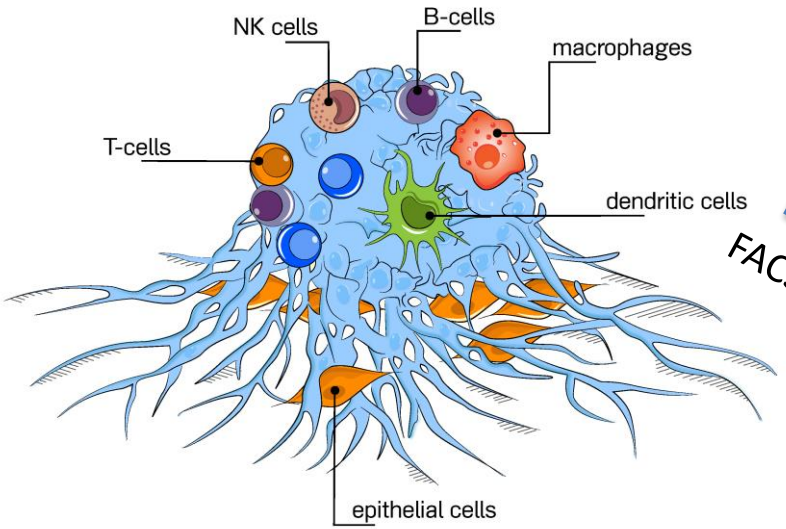
- Only 2 CD8+ populations correlated negatively with tumor growth.
- Subtle multivariate phenotypic differences, distinguish T cell populations with dramatic functional differences

Breast Cancer Immune Cell Atlas

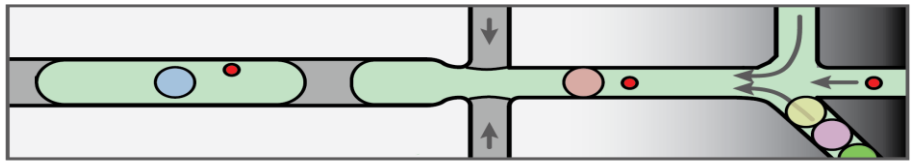
Ambrose Carr
Elham Azizi
George Piltas
Linas Mazutis
Andrew Cornish
Kasia Konopacki
Sasha Rudensky



In-drop characterization of tumor immune cells in breast cancer



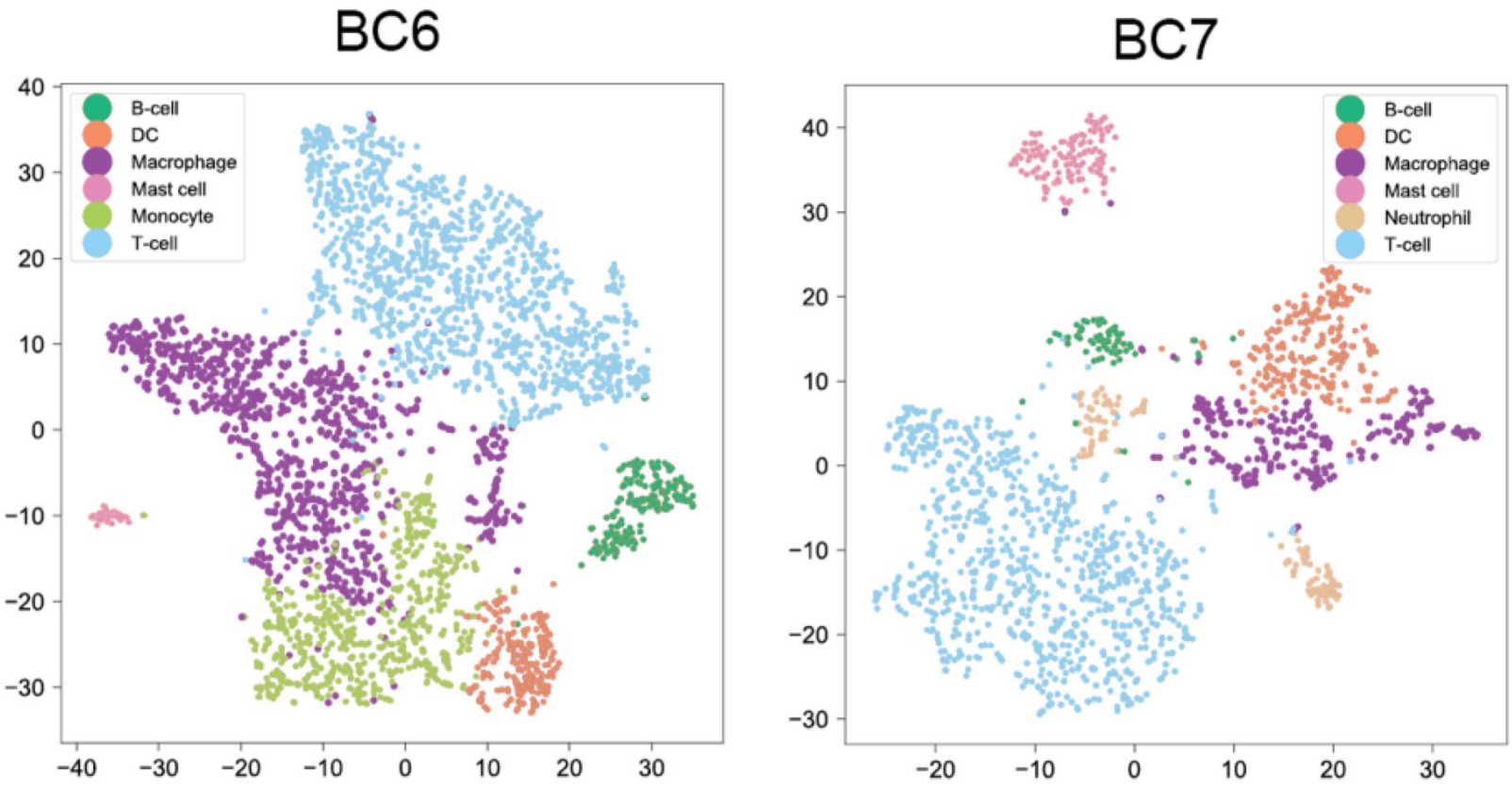
FACS for CD45+ cells



Data-Driven approach: > 3000-10,000 CD45+ collected per tumor

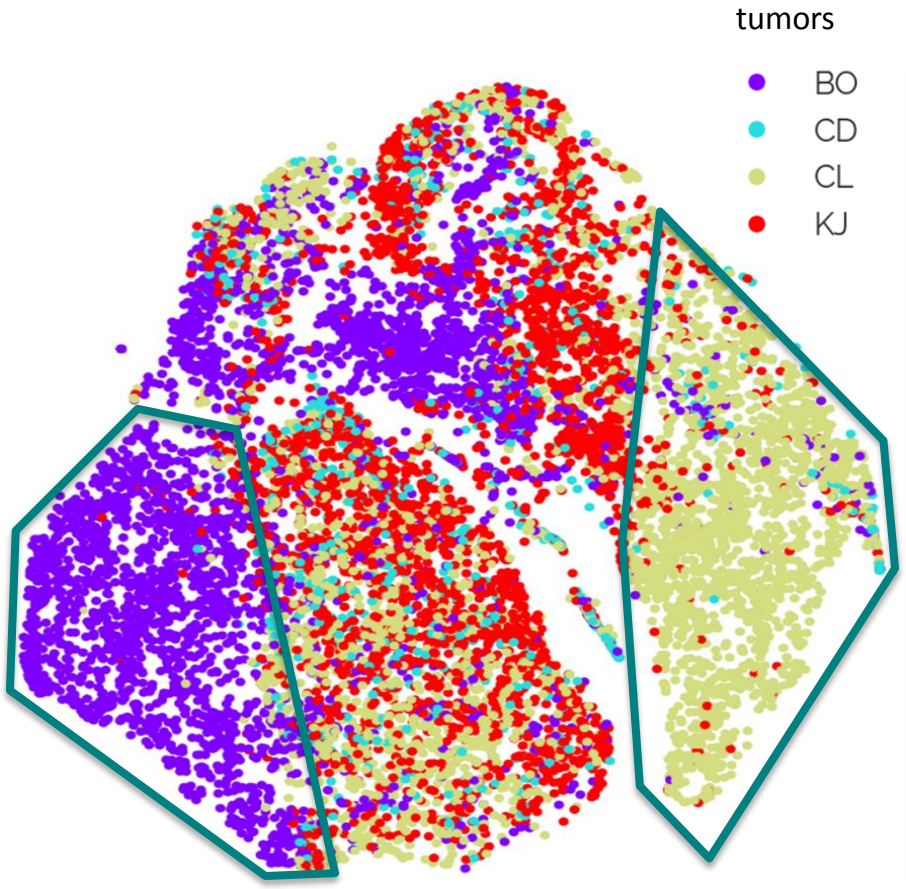
What is the immune states and the structure of the tumor immune ecosystem?

PhenoGraph Clustering



- PhenoGraph found all major immune subtypes in each tumor
- Each tumor is very different

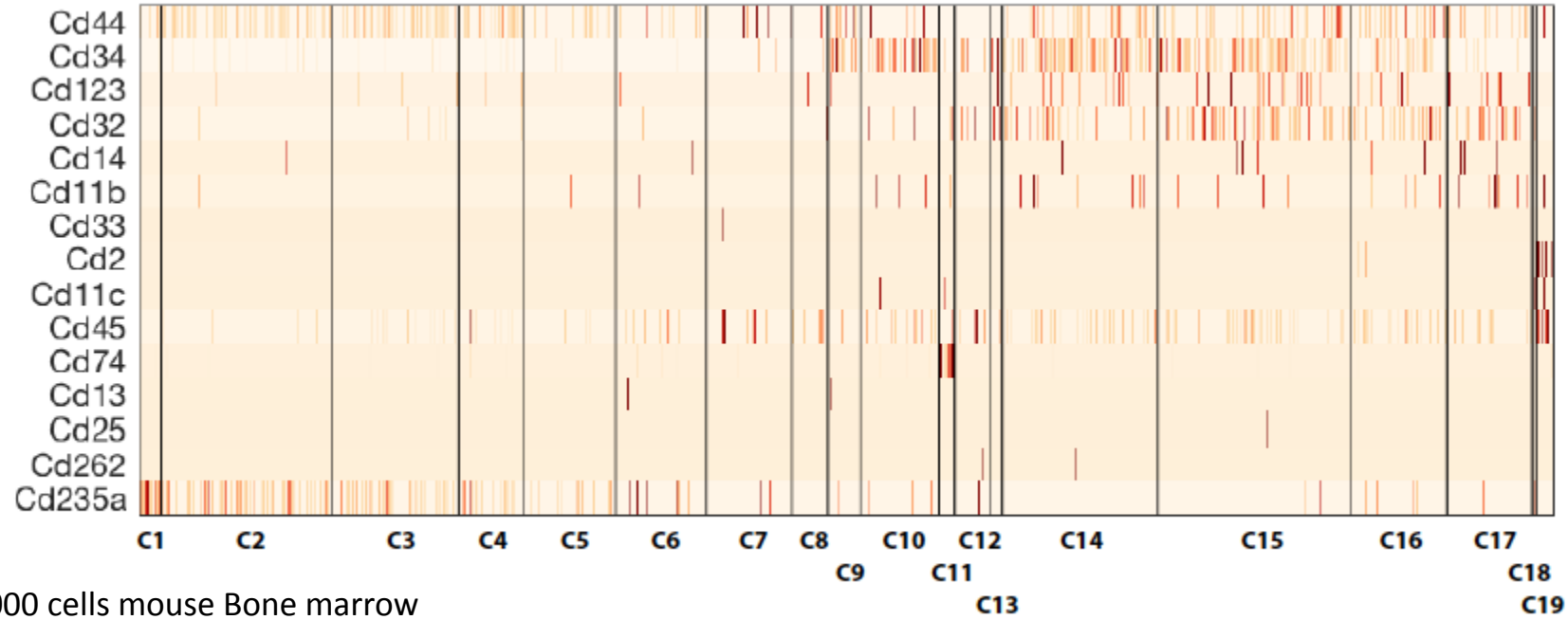
What happens when we compare immune ecosystems across patients?



tSNE 2D projection

- CD45+ TILs from 4 breast tumors
- Entire regions on the map are tumor specific
- Are these differences real biology or technical effects?

Single-Cell RNAseq data is Sparse

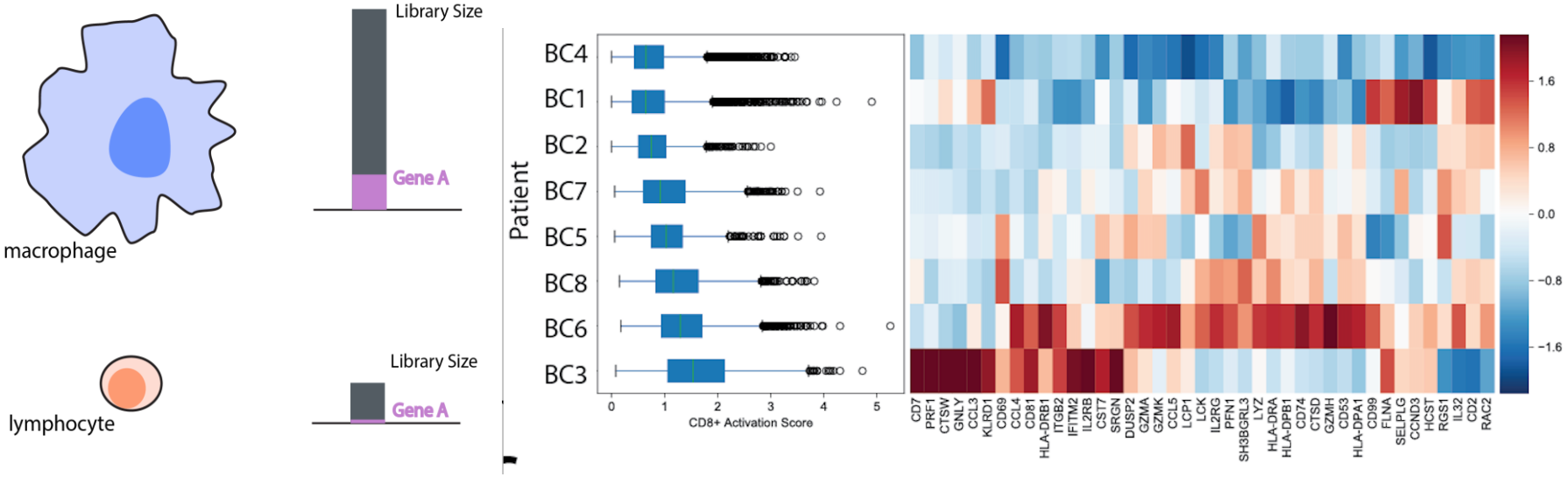


3000 cells mouse Bone marrow
Paul, *et.al. Cell* 2016

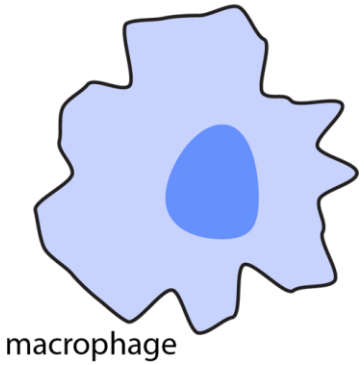
- Surface markers used for gating typically have very low RNA-levels and are poorly captured in most immune cells.
- For example: Monocyte clusters have
  - 1.6% cells expressing CD14
  - 5.8% cells expressing CD11b

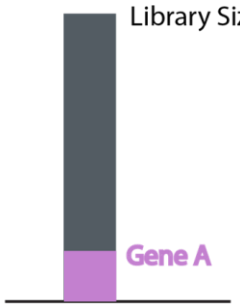
There are strong batch effects with and between samples

- There is a huge variation of transcripts captured per cell, a wide distribution within a sample, and distinctly different distributions between samples.
- The differences convolute both biological and technical reasons



Problem with Global Normalization

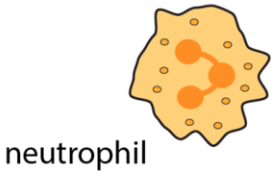


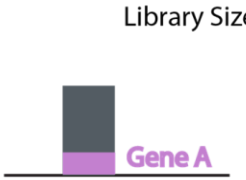


Cells with different sizes have very different total number of transcripts

Example Housekeeping Gene

High chance of Dropouts in smaller cells





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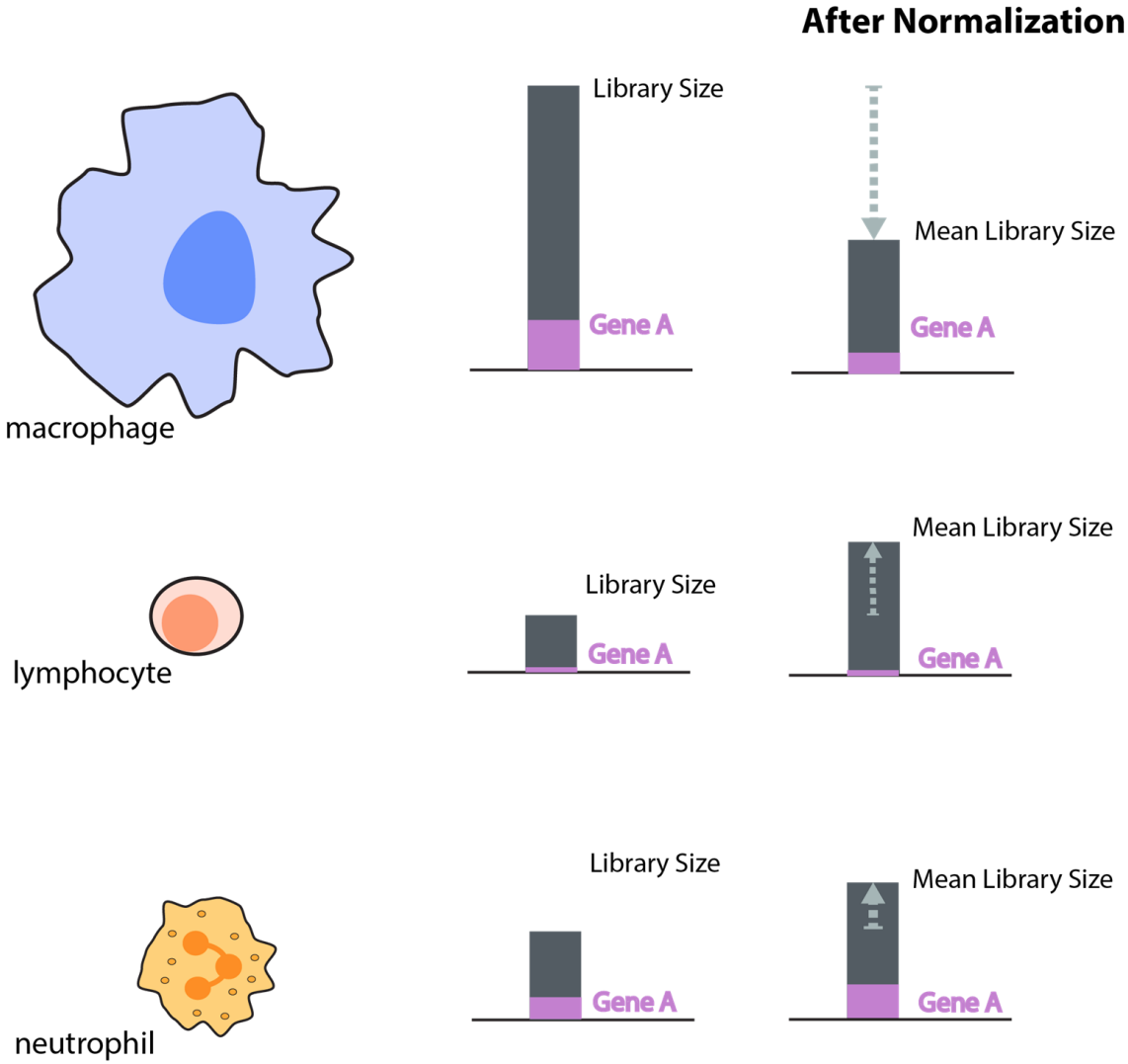
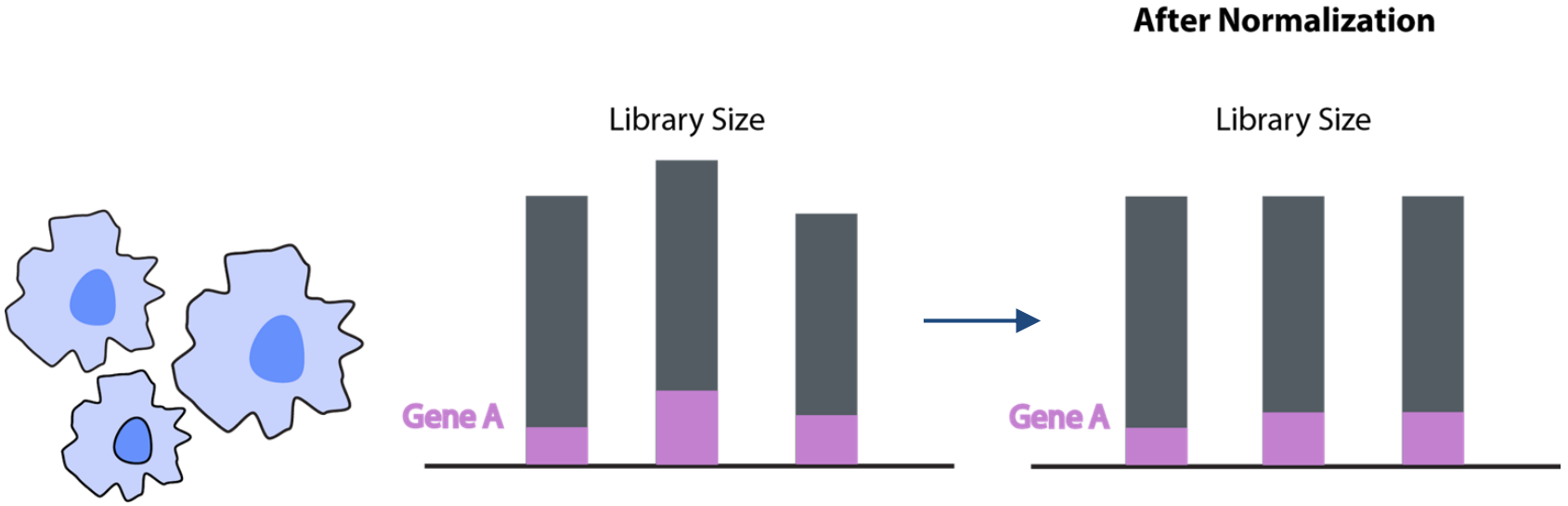


Dropout not resolved

Spurious Differential Expression

Idea: Different normalization for each cell type

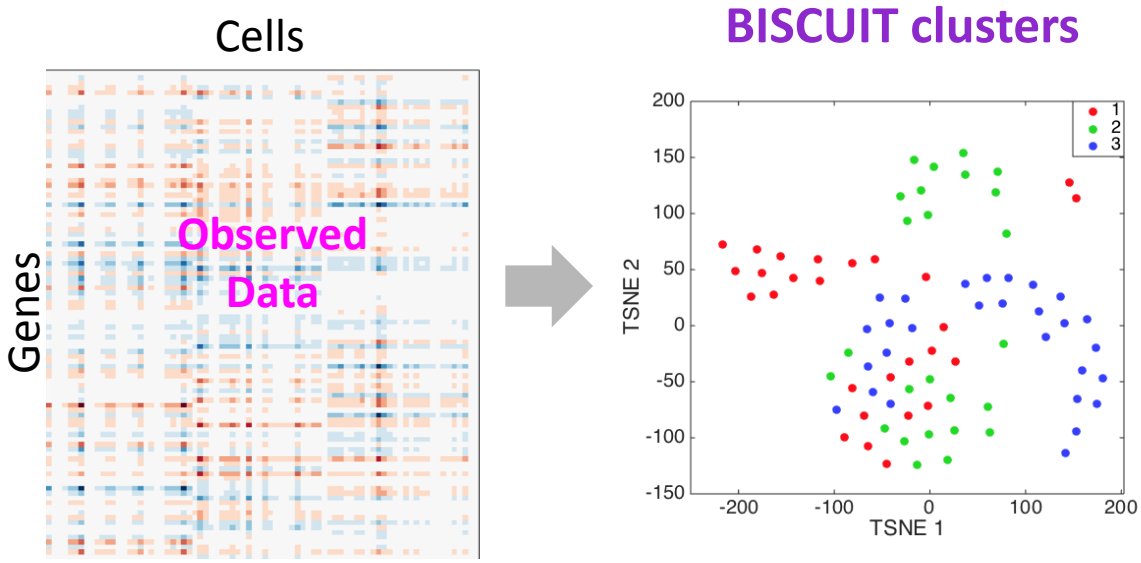


Problem:

We don’t know cell types

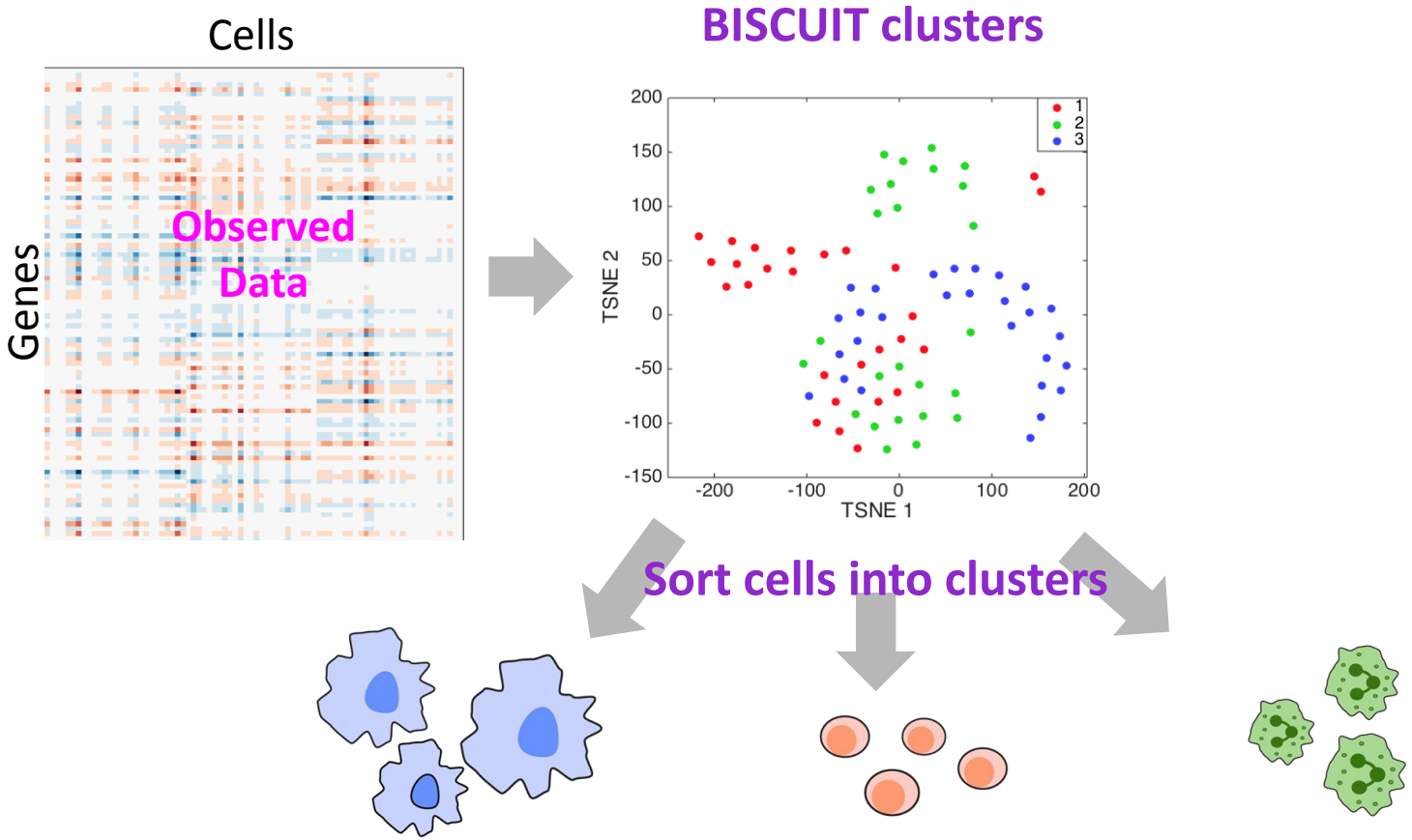
Need to infer **cell clusters**

Cluster-dependent **Imputing & Normalizing**



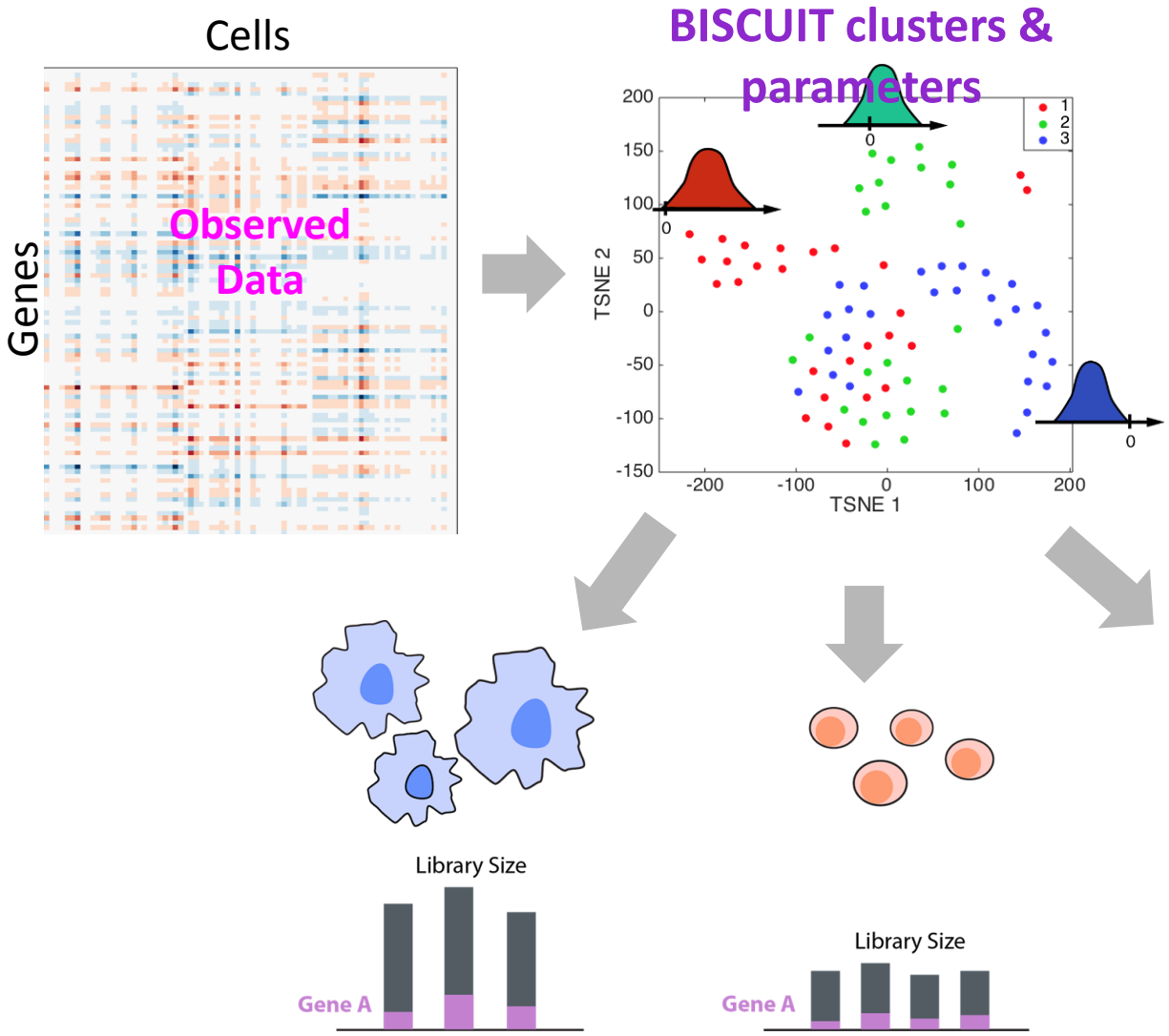
Prabhakaran, Azizi, ICML 2016

Cluster-dependent **Imputing & Normalizing**

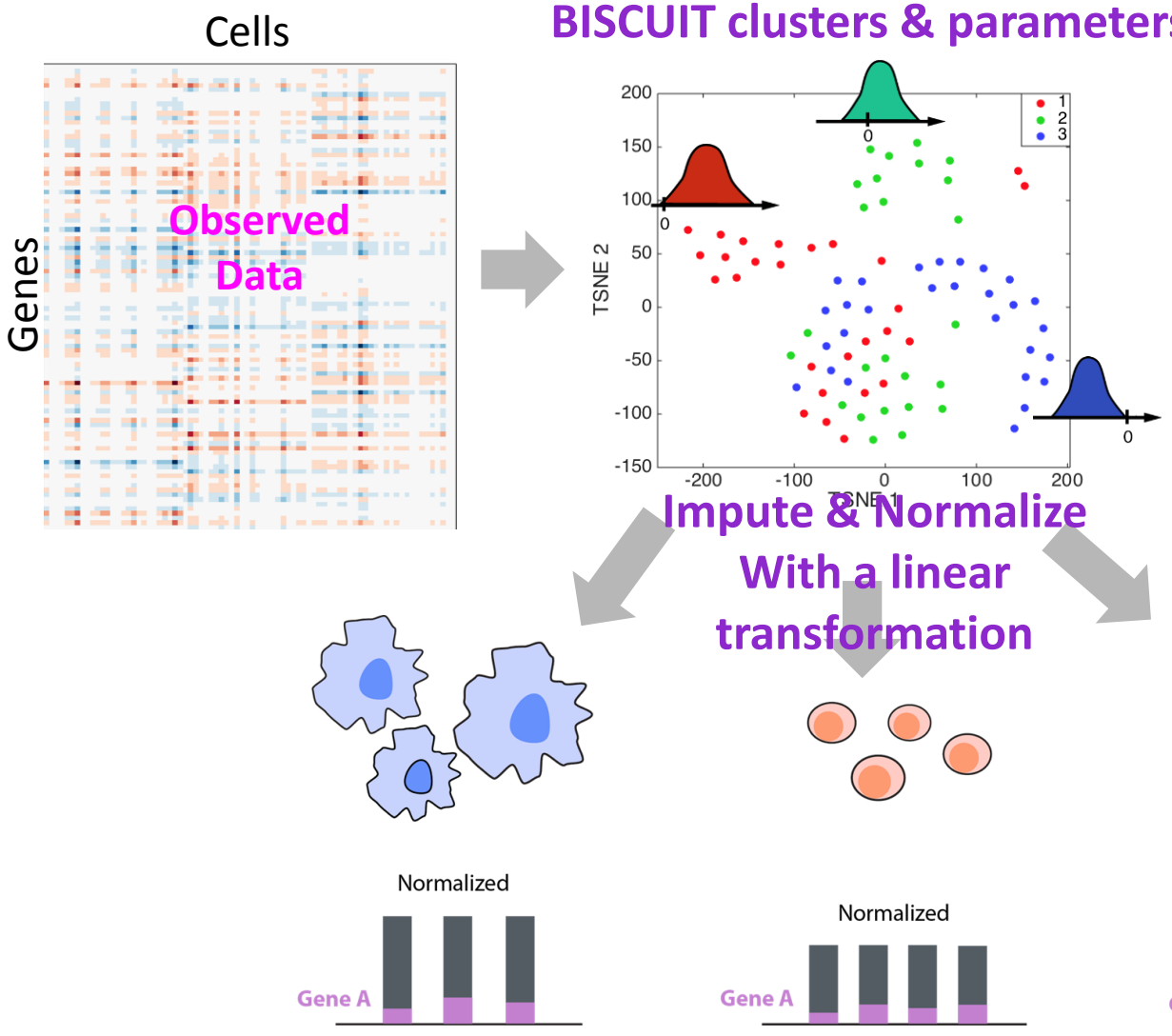


Prabhakaran, Azizi, ICML 2016

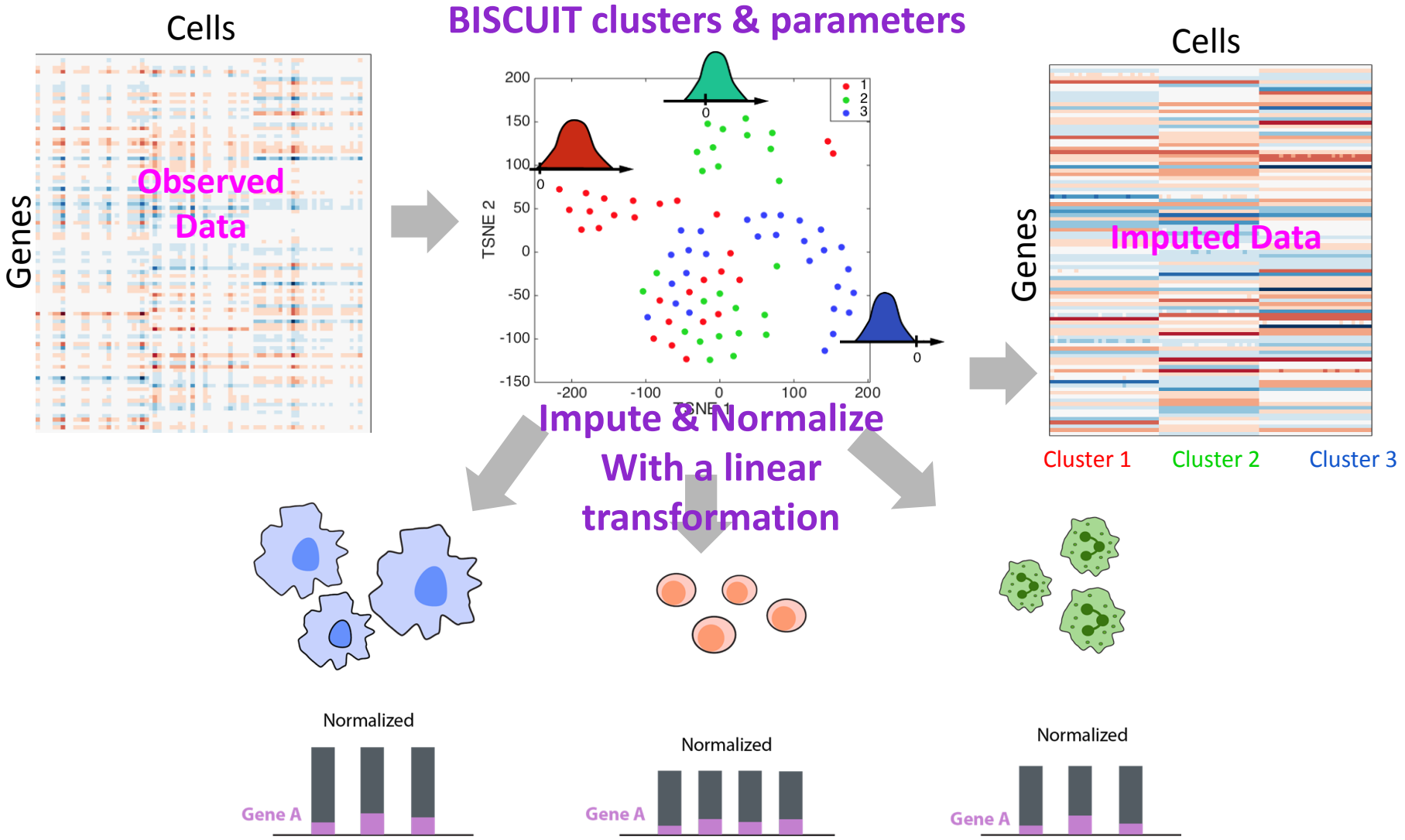
Cluster-dependent **Imputing & Normalizing**



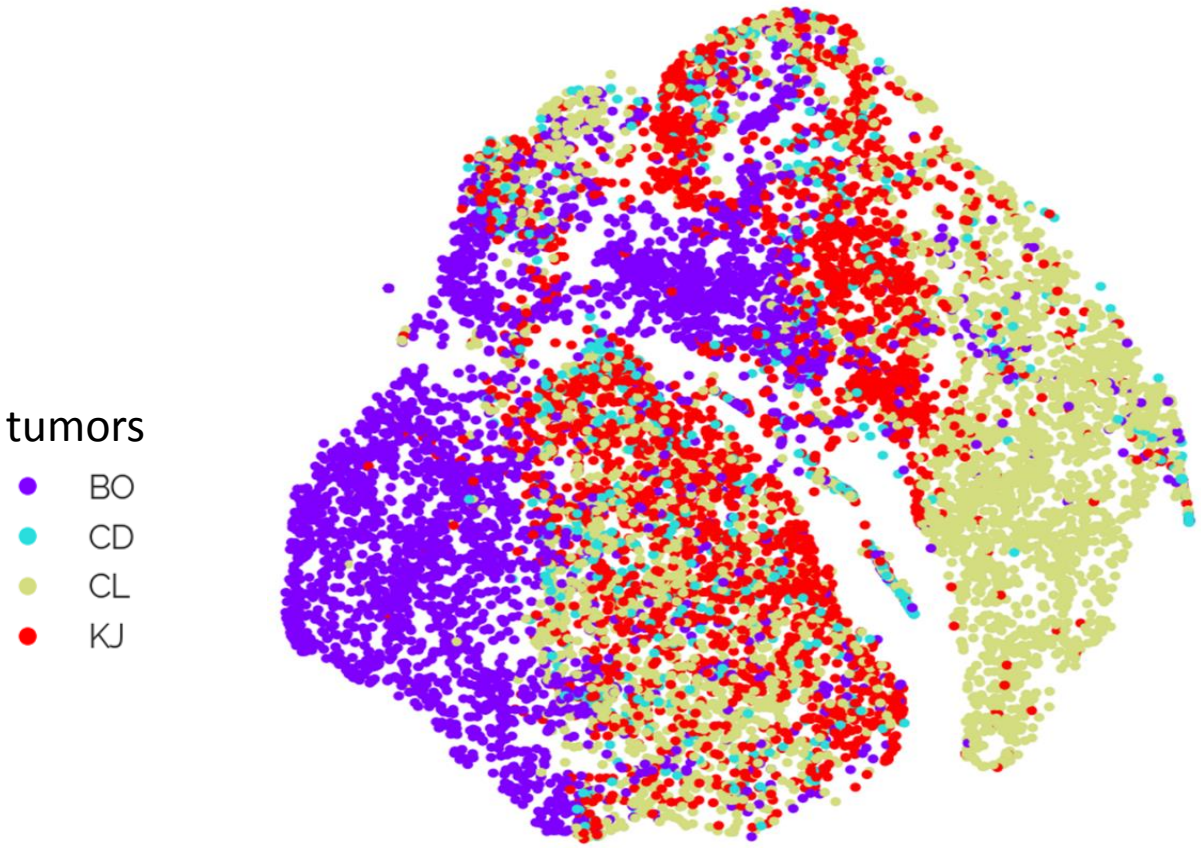
Cluster-dependent Imputing & Normalizing



Cluster-dependent Imputing & Normalizing



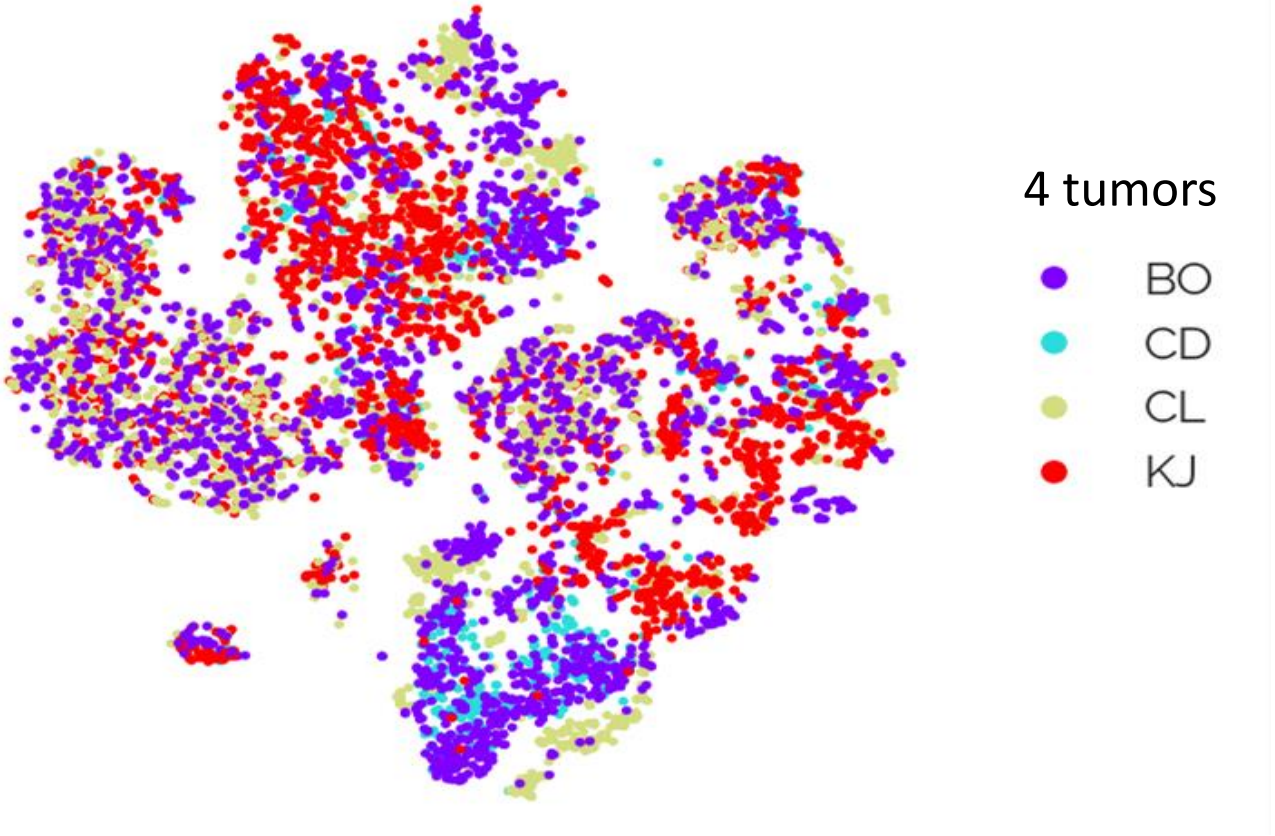
Reminder: Breast TIL data before Biscuit



- Skews data, non-overlapping cells across tumors
- Unclear structure of cell types, mostly distinguishes myeloid from lymphoid cells

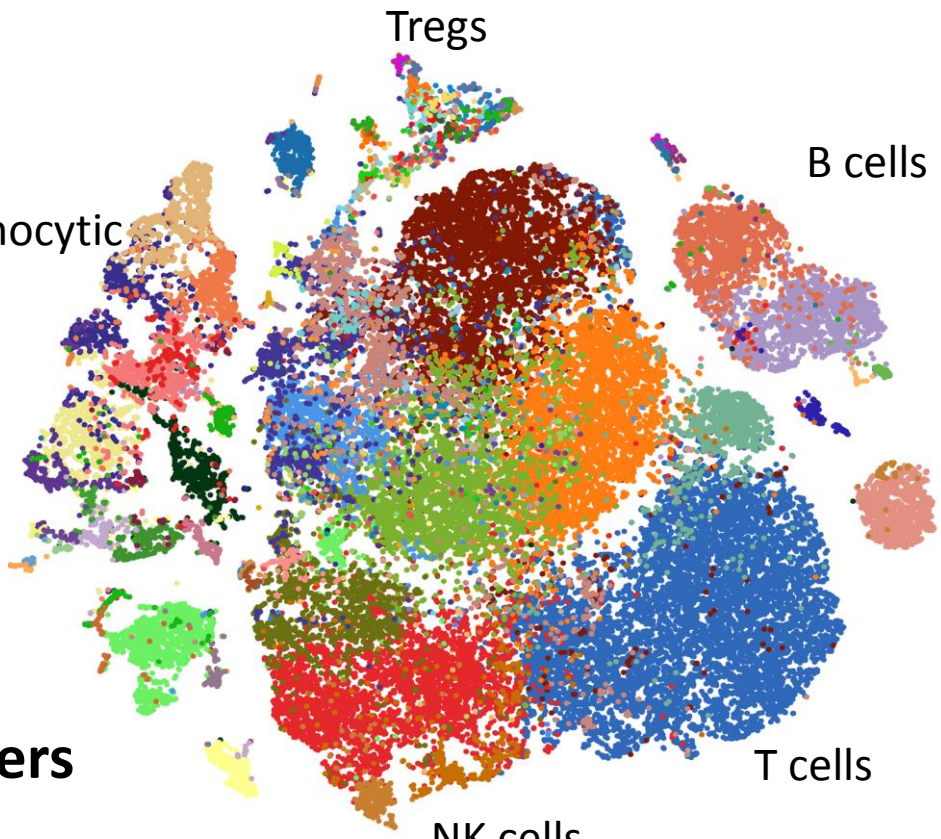
Breast cancer TIL data **after Biscuit**

12,000 Cells, ~2500 molecules per cell

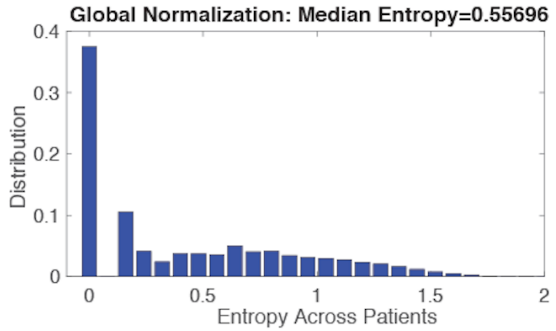


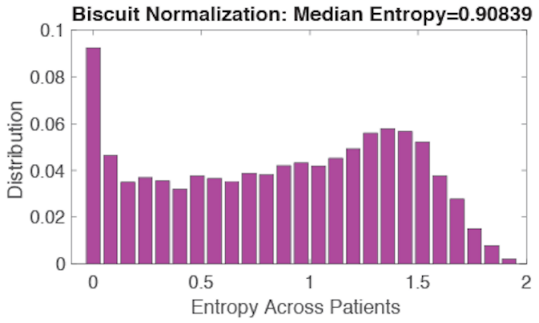
Breast cancer Immune Atlas

60,000 cells, All Tisues



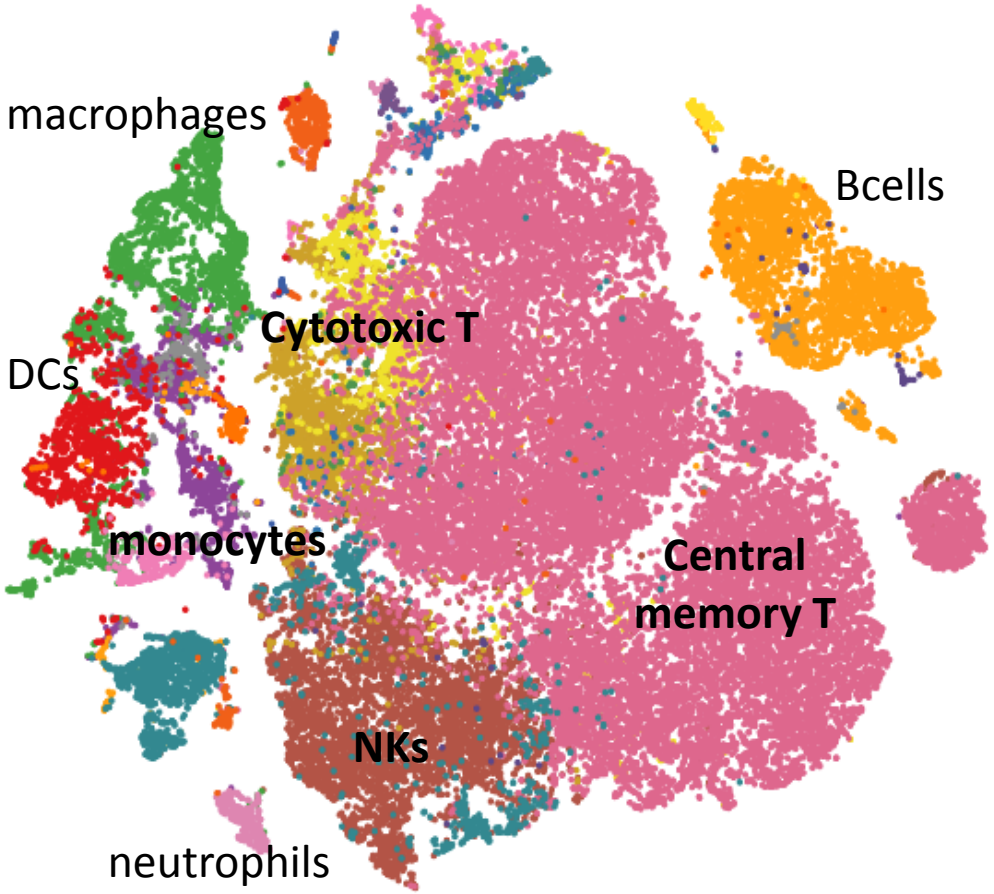
82 clusters

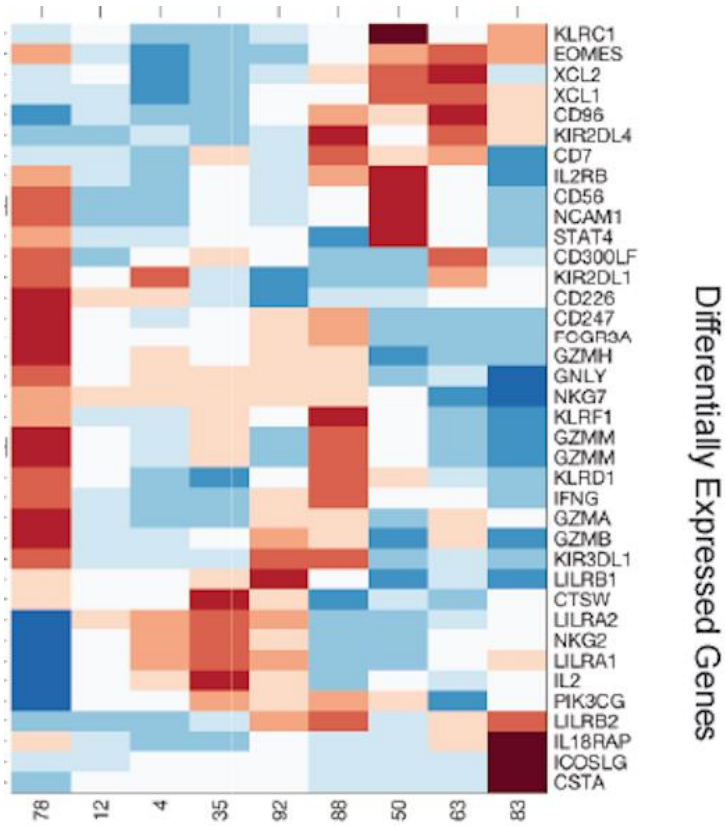




- 8 patients collected from tumor and normal (prophylactic mastectomies) tissue, peripheral blood and lymph-node.
- Entropy confirms mixing of samples
- Clusters are robust

Annotation of the Atlas



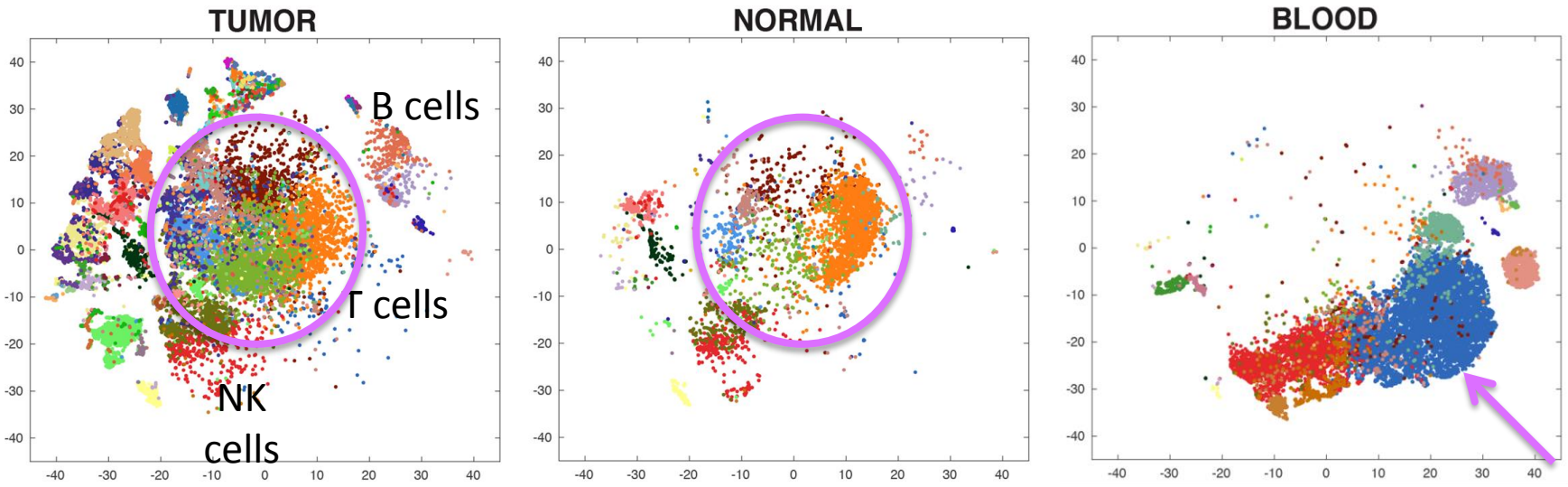


- 36 T-cell clusters
- 9 NK clusters

- Bulk gene signatures can help explain clusters.
- Each cluster has a distinct set of differentially expressed genes

How does the tissue environment influence the immune ecosystem?

Tissue Contribution



- Tumor and tissue have similar T-cells
- Blood T-cells are completely different
  - Blood has more naïve characteristics)
- B-cells largely overlap (across tissues and individuals)

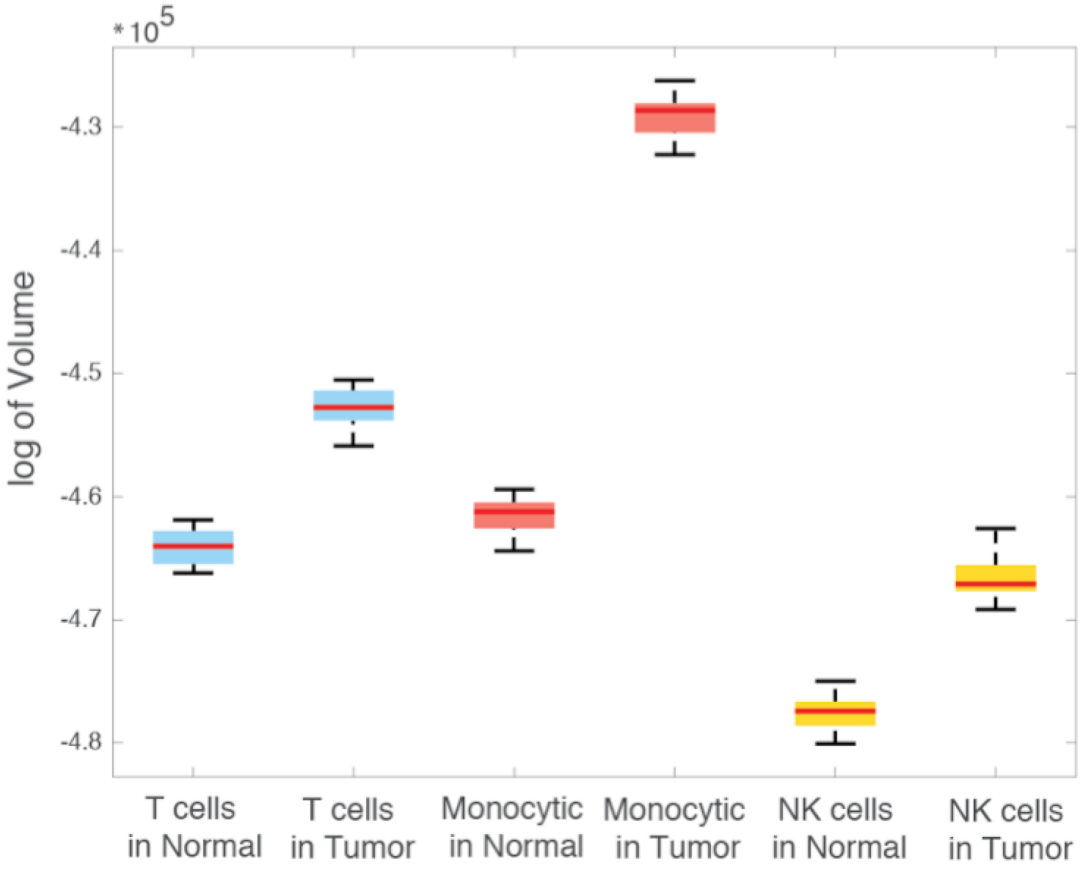
Tissue Contribution

- 19 T-cell clusters shared between tumor and breast-tissue
- 17 T-cell clusters unique to tumor are more activated and more cytotoxic
- 5 T-regs subsets unique to tumor (differ between patients)

Tissue Contribution

- 13 myeloid clusters shared between tumor and breast-tissue
- 14 myeloid clusters unique to tumor are more activated
- 3 TAM subsets unique to tumor (differ between patients)

Tumor infiltrating immune cells explore a more diverse phenotypic space



Oxidative Phosphorylation

Interferon Gamma Response

Apoptosis

Interferon Alpha Response

Tgf Beta Signaling

Hypoxia

Il2 Stat5 Signaling

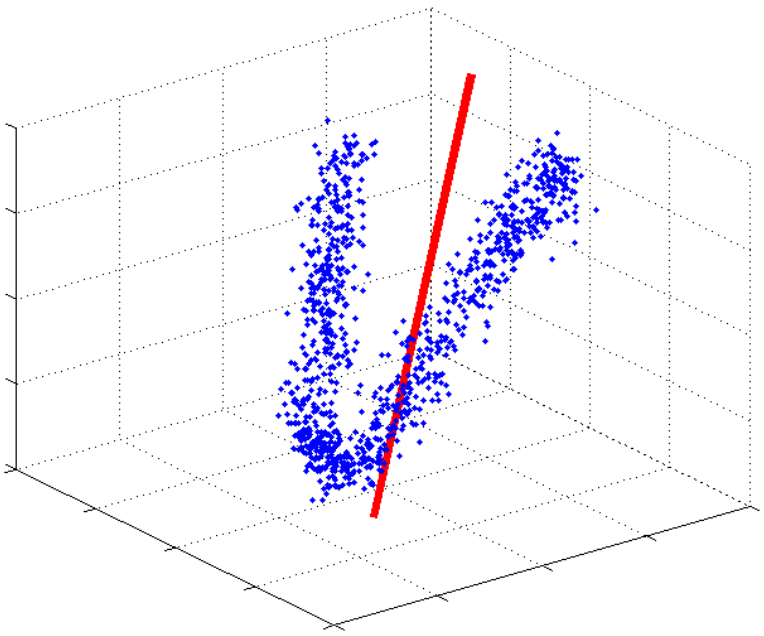
Il6 Jak Stat3 Signaling

- The tumor environment substantially expands the diversity of observed cell states.

What happens to T-cells when they enter the tissue environment?

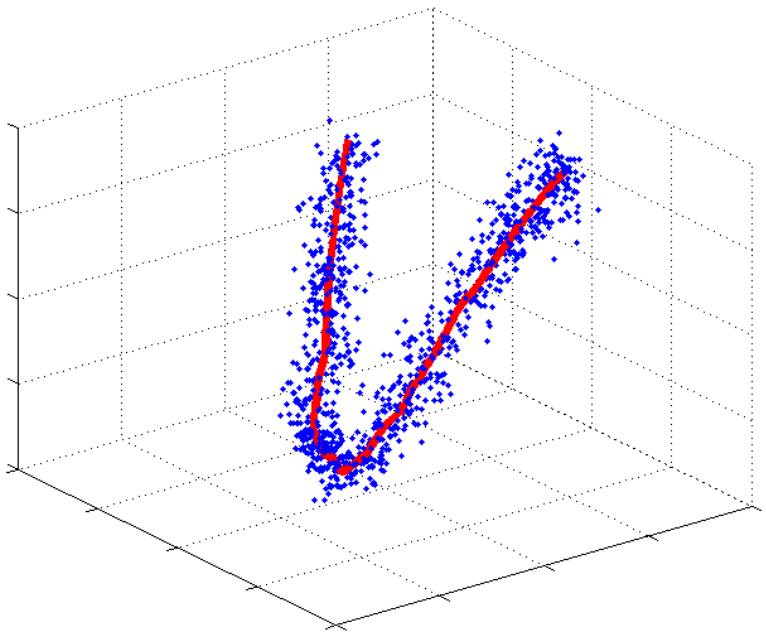
Markov Eigen vectors follow the manifold

PCA



Linear direction of variation

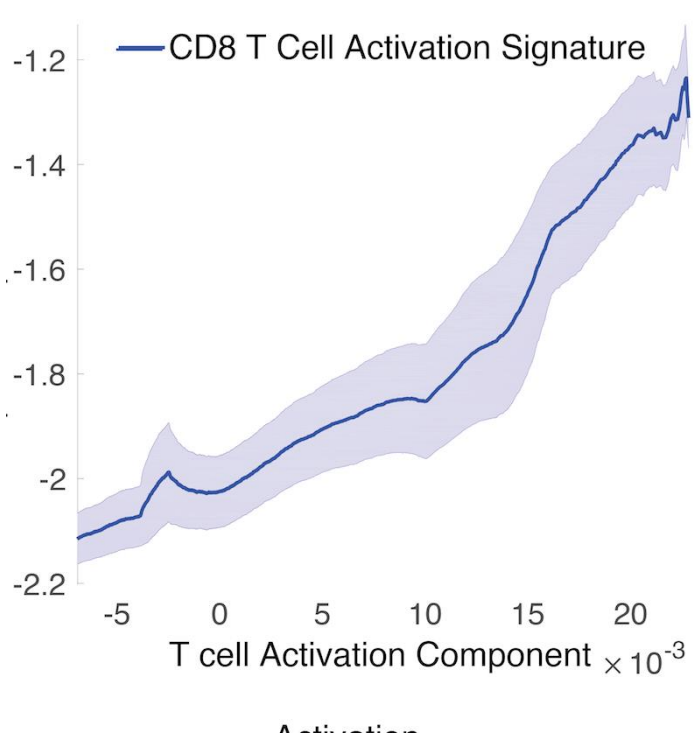
Diffusion maps

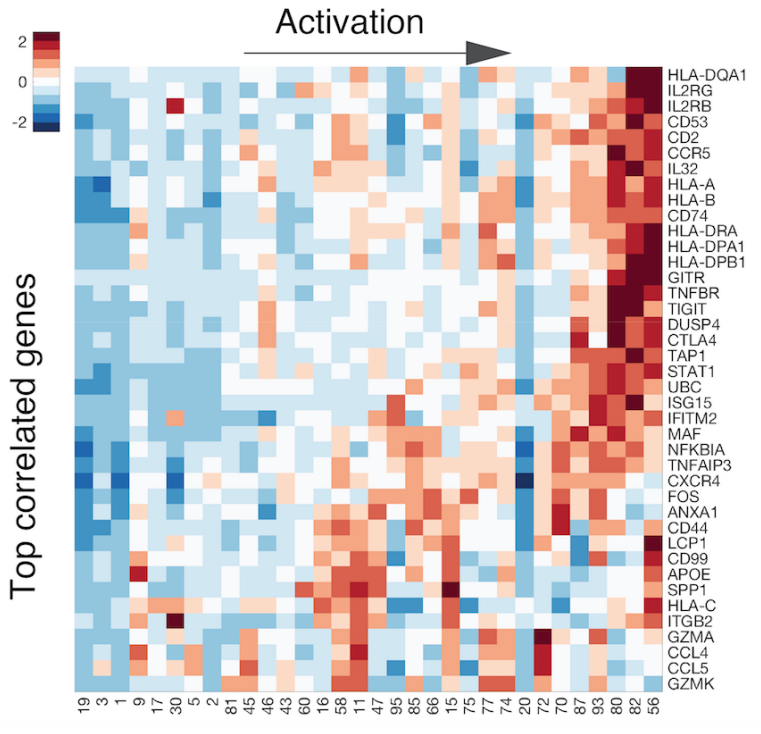


Trajectory through the manifold

Diffusion maps are a “non-linear” version of PCA that follows the data density

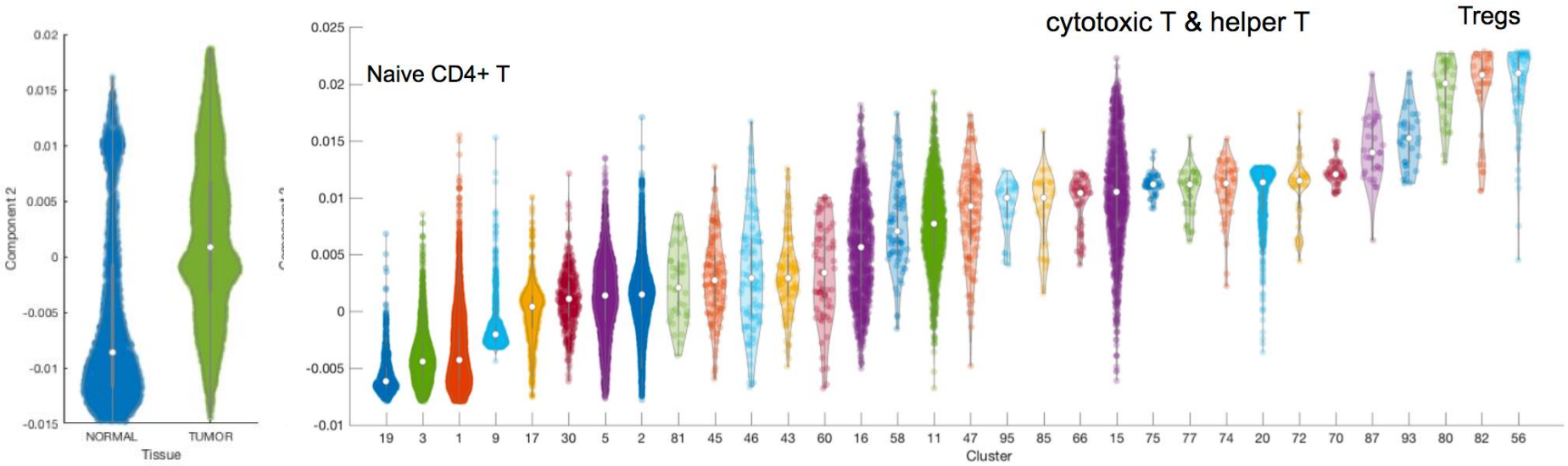
T-cell activation
first diffusion component in lymphocytes





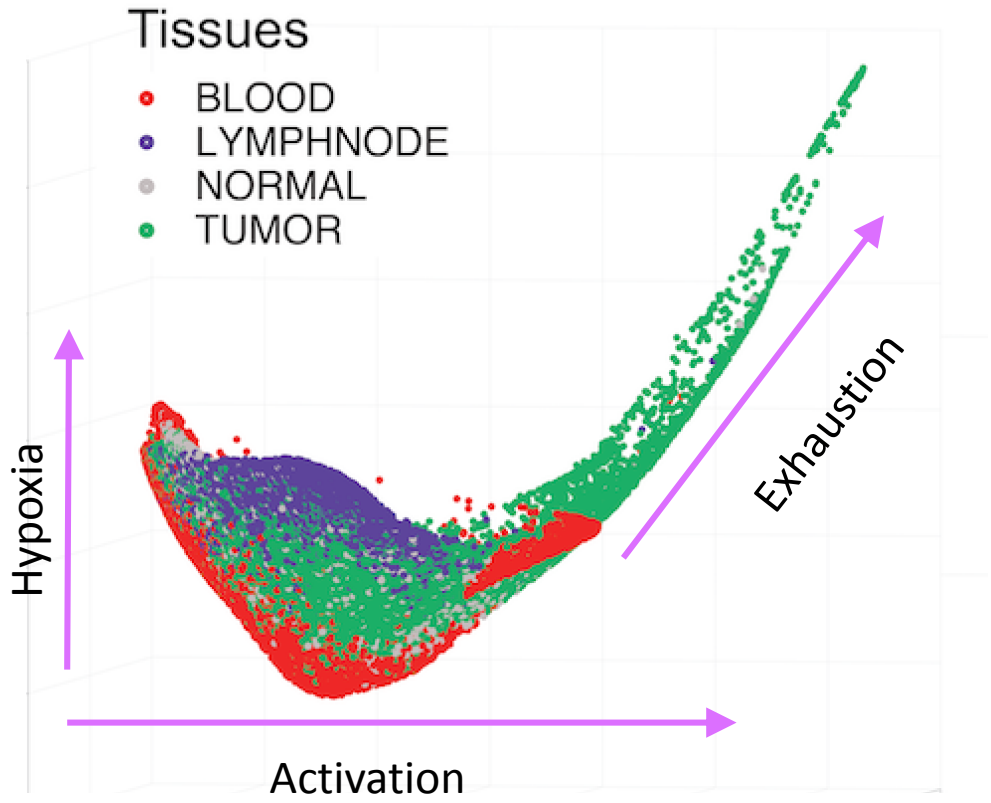
- First diffusion component that explains most of variation is T cell activation
- Enriched for cytokine production & signaling, lymphocyte activation, leukocyte differentiation, ligand receptor interaction

Ordering of clusters based on activation state



- Comparing distribution of cells along the component between tumor and normal shows tumor is more activated.
- Plotting clusters along diffusion component provides an ordering of “activation status” for these clusters.
- Phenotypic space has a gradual rather than a discrete nature

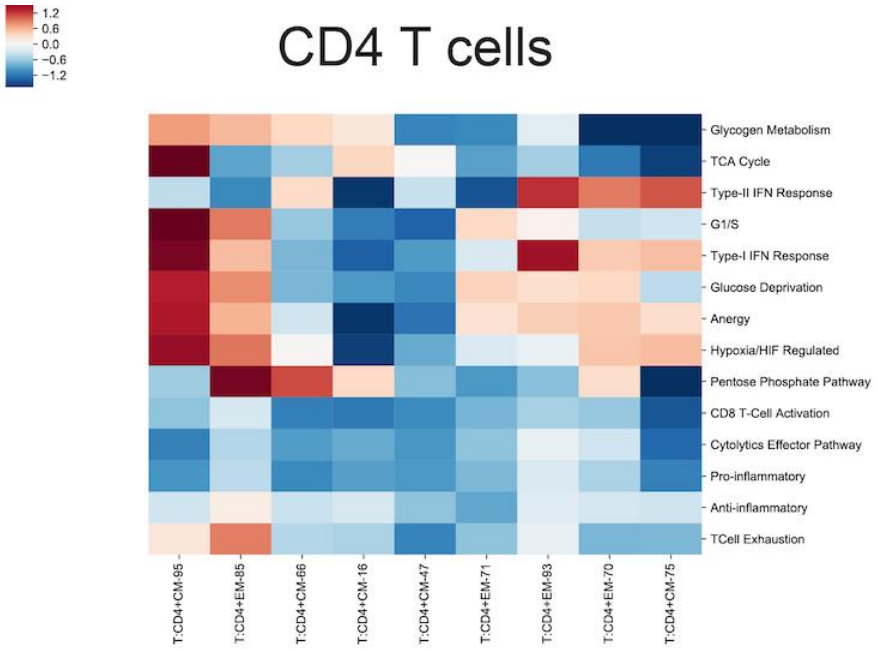
T-cell phenotypic space is continuous

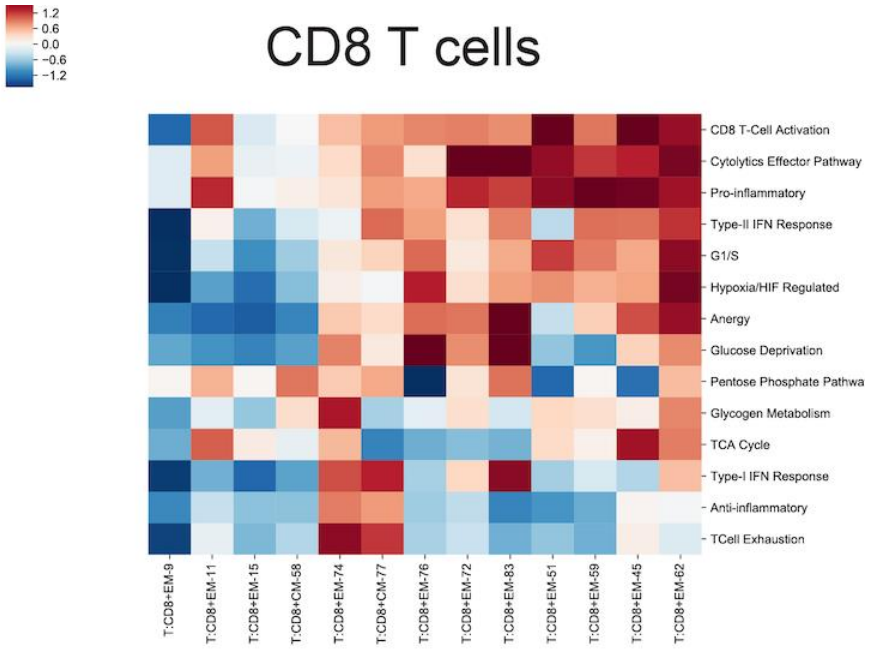


- T-cells define a continuum of states
- Most of the variation can be captured by a few axes of variation
- Tissue context plays a big role!

So what defines the clusters?

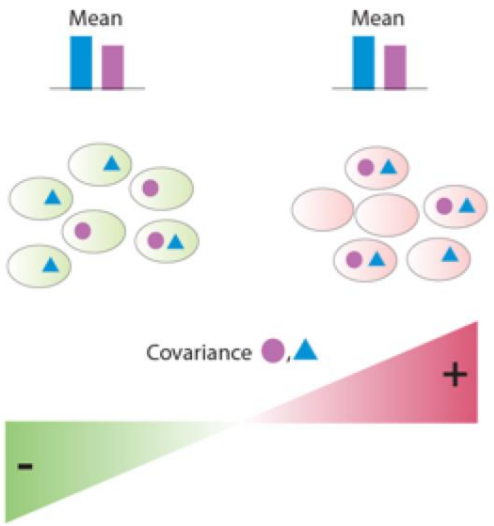
Tissue resident cells experience combinations of environmental conditions

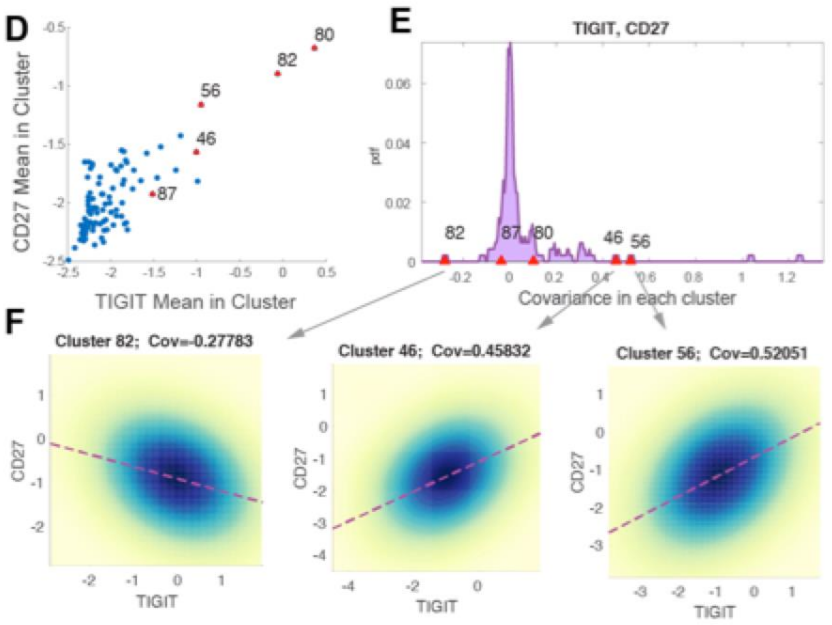




- While the strongest components of variation are a few gradual axes of activation, differentiation, hypoxia, glucose starvation
- Each cell experiences a different combination of these, creating discrete clustering

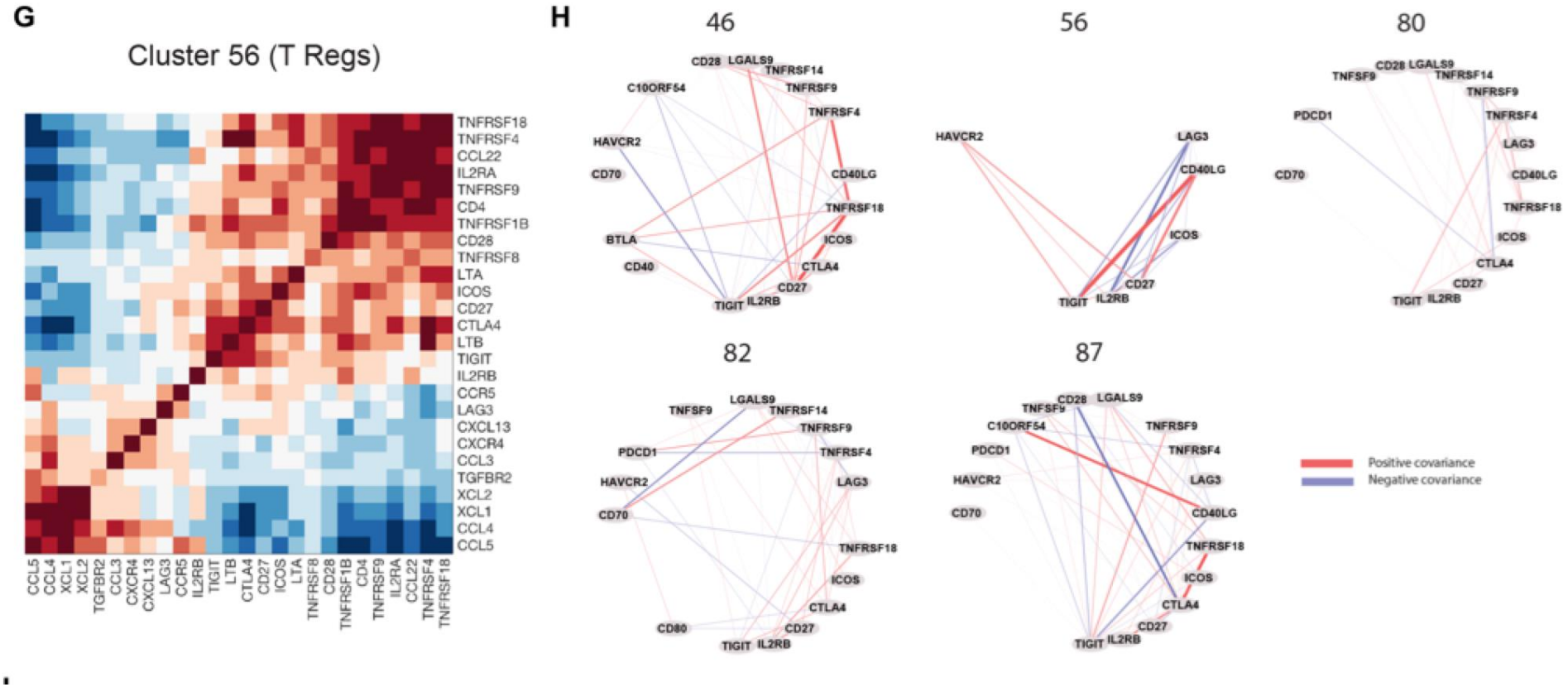
Co-variation drives the clustering





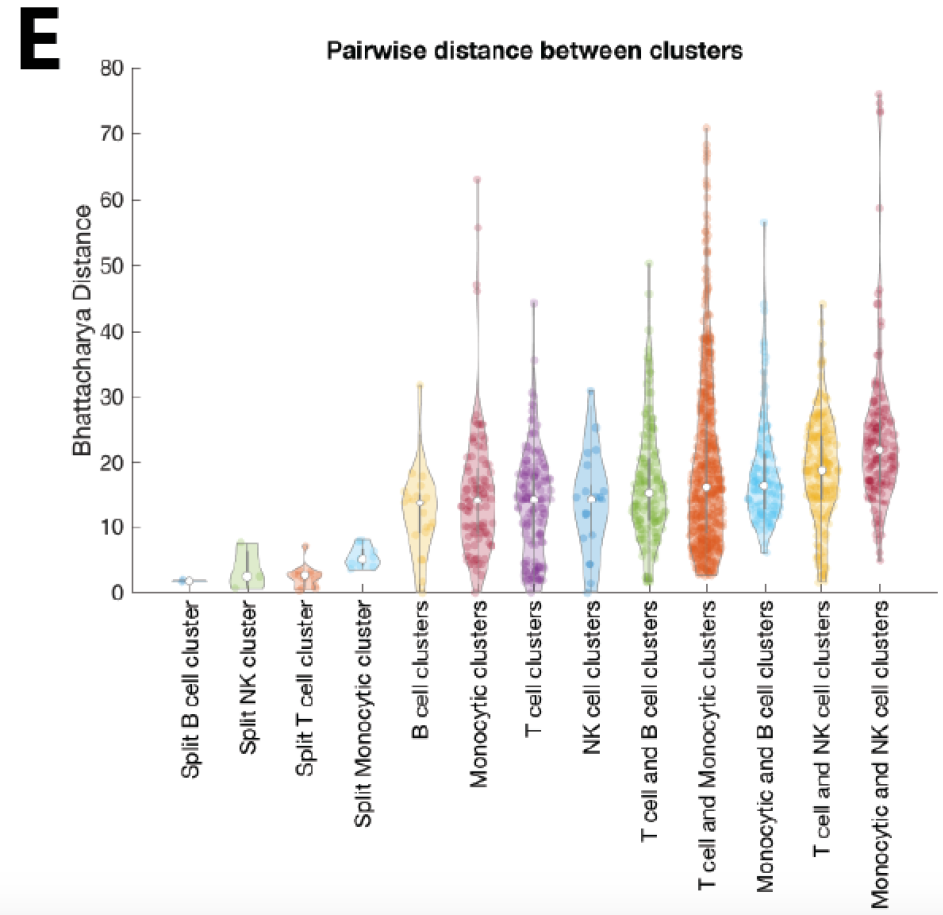
- TIGIT and CD27 are highest in the 5 T-reg clusters
- However they have substantial variation in the co-variation between them

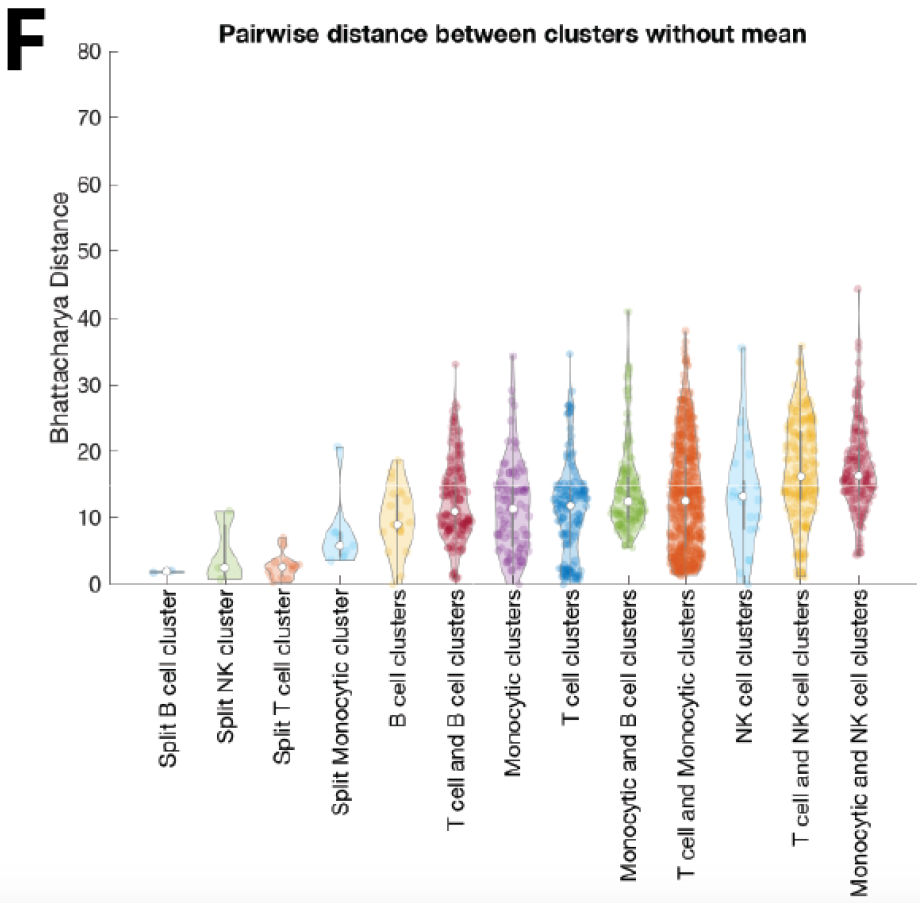
Co-variation drives the clustering



- T-reg clusters have similar “mean” expression profiles
- T-reg clusters are very diverse in their co-variation patterns involving key immune receptors and drug targets

Co-variation drives the clustering

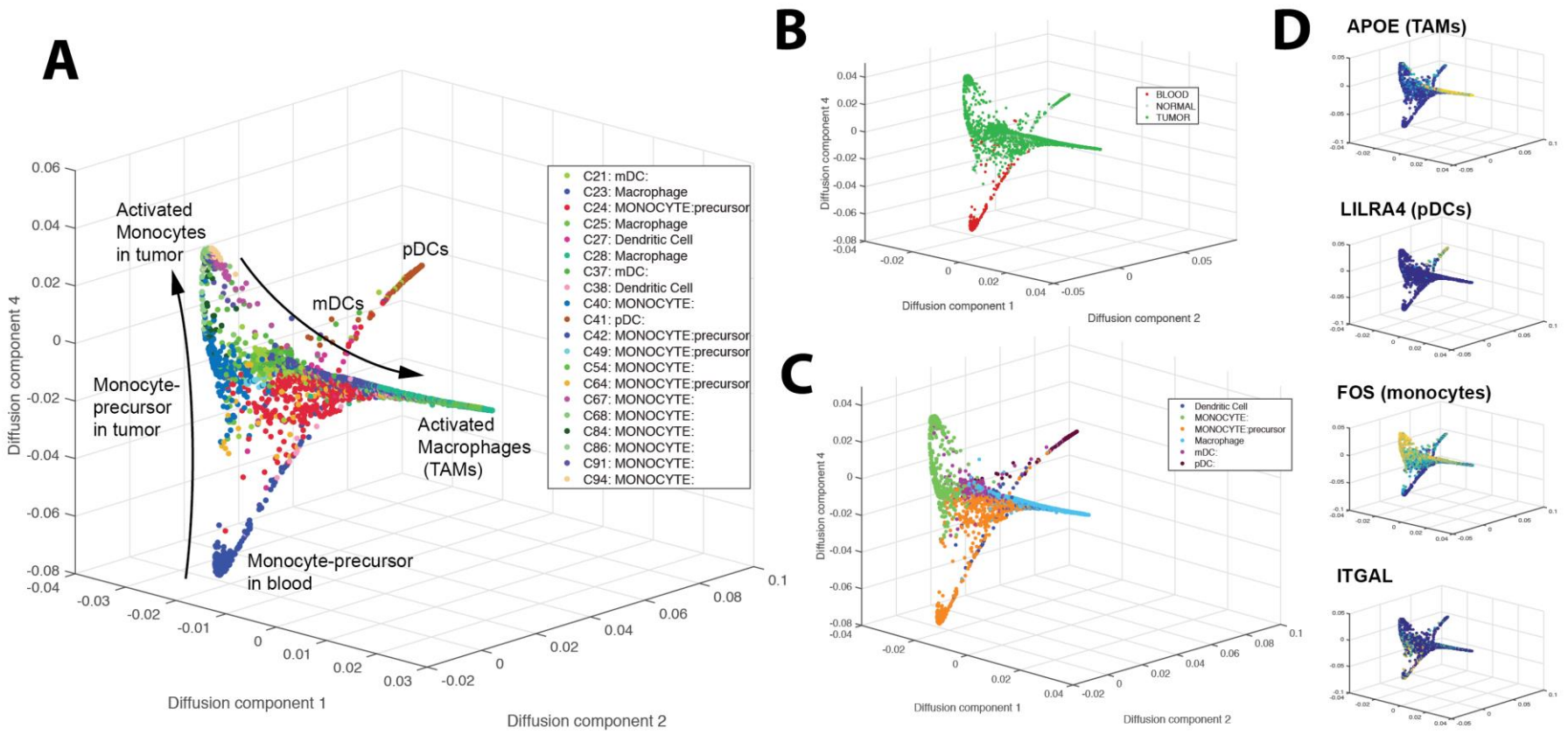




- Even after means are normalized, distances largely remain

What happens to monocytic cells when they enter the tissue environment?

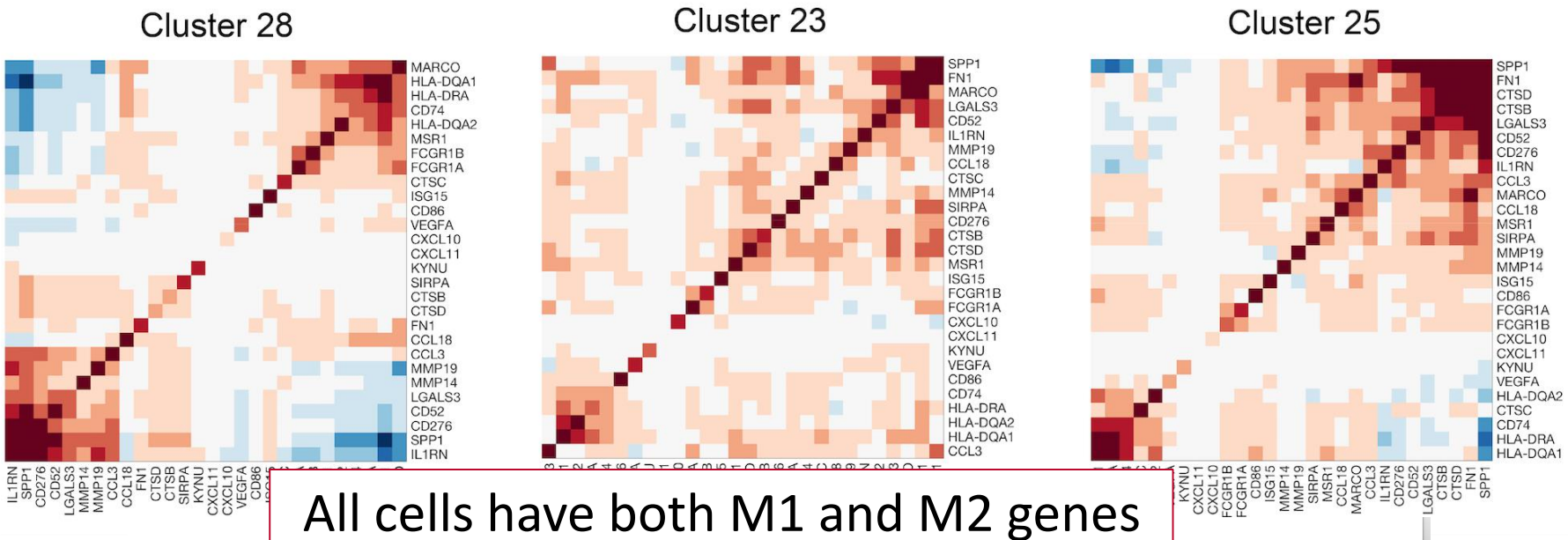
Activation and Differentiation Axes in Tumor Immune Atlas



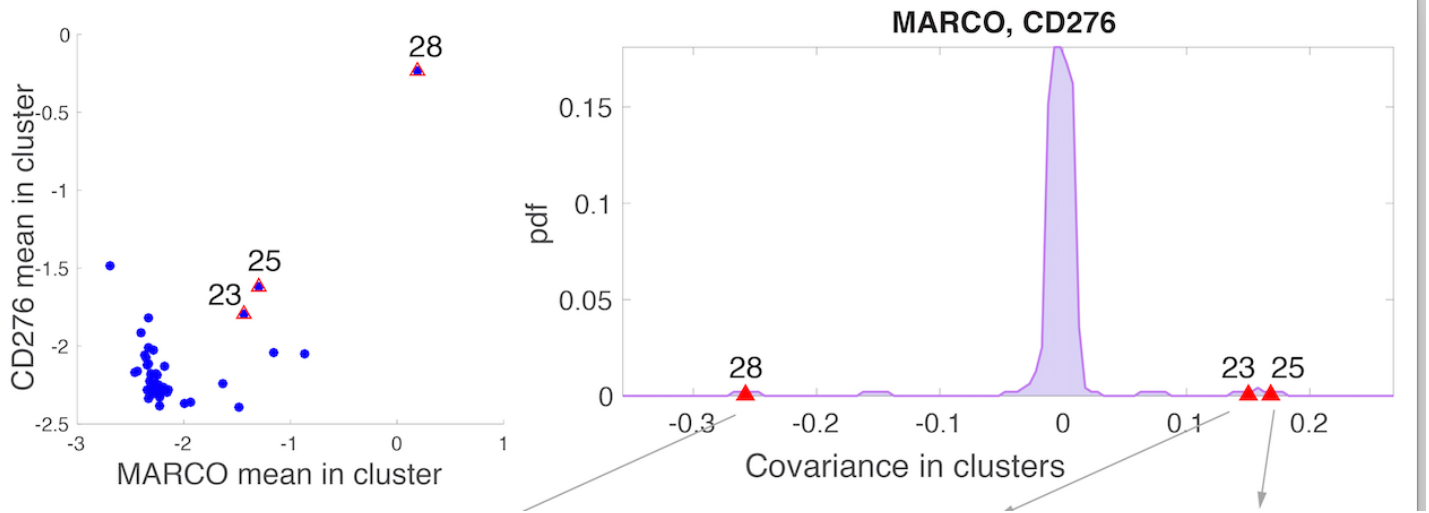
- A large diversity of monocytic cells organize onto distinct axes of differentiation as they change environment / tissue context

Co-variation drives the Myeloid clusters

3 TAM clusters



All cells have both M1 and M2 genes



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