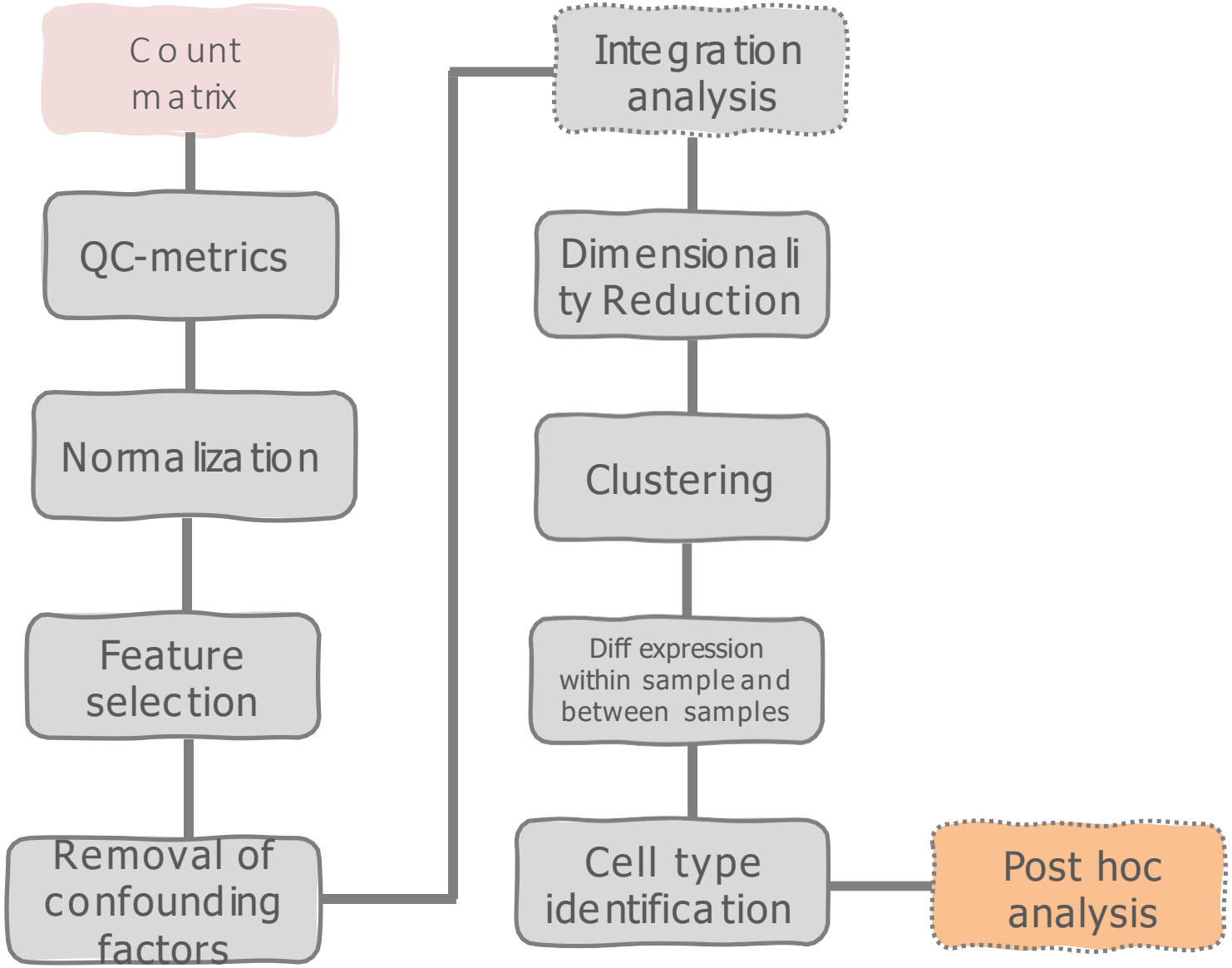
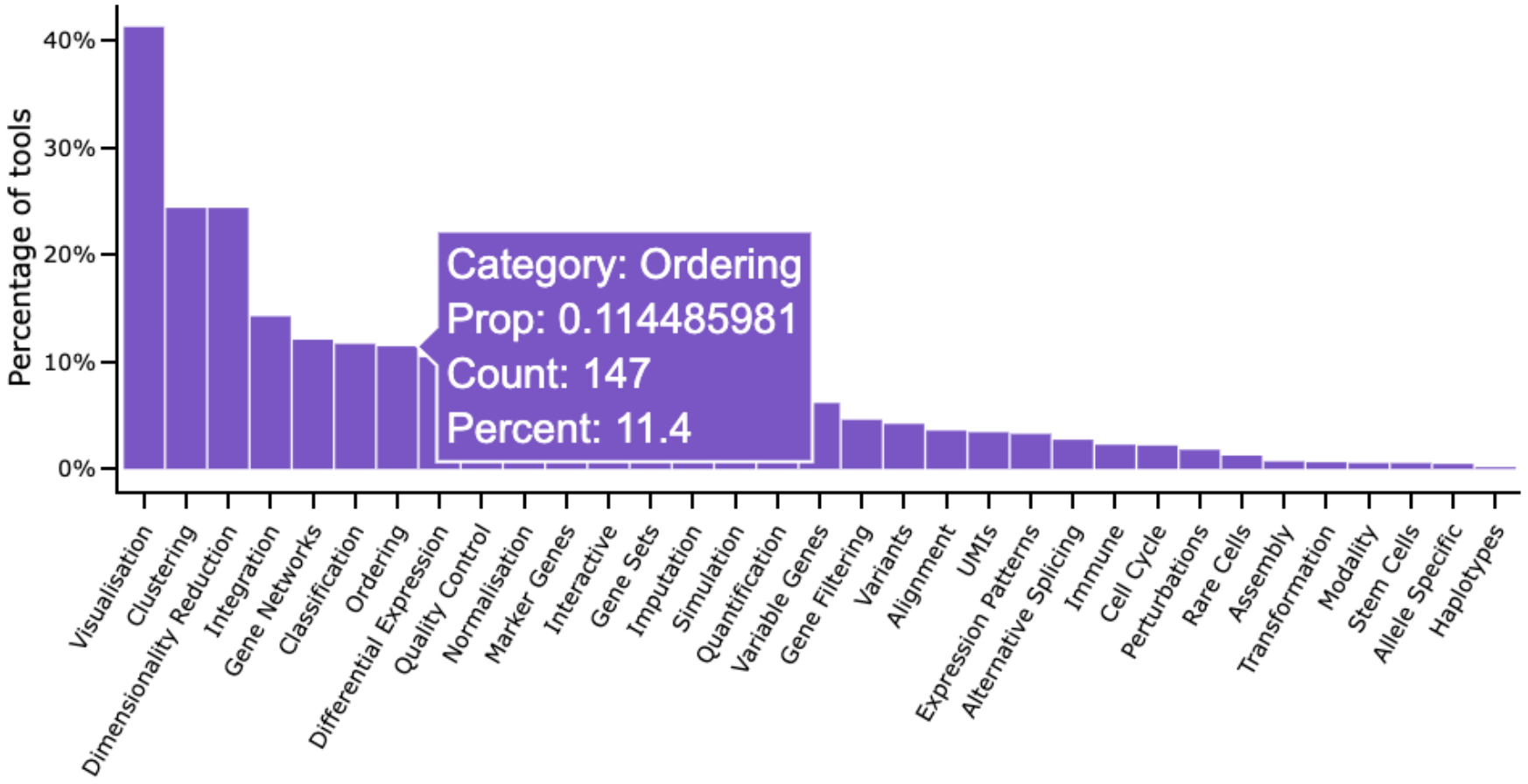

Count matrix
QC-metrics
Normalization
Feature selection
Removal of confounding factors
Integration analysis
Dimensionality Reduction
Clustering
Diff expression within sample and between samples
Cell type identification
Post hoc analysis

Possible interest after cell classification and DGE

- Trajectory Analysis
- Ordering of cells or clusters
- Metacell analysis
- Cell-cell communication
- Pathway analysis
- Deep Neural Network

Again long list of possible tools



https://www.scrna-tools.org/

Example of Trajectories/Order

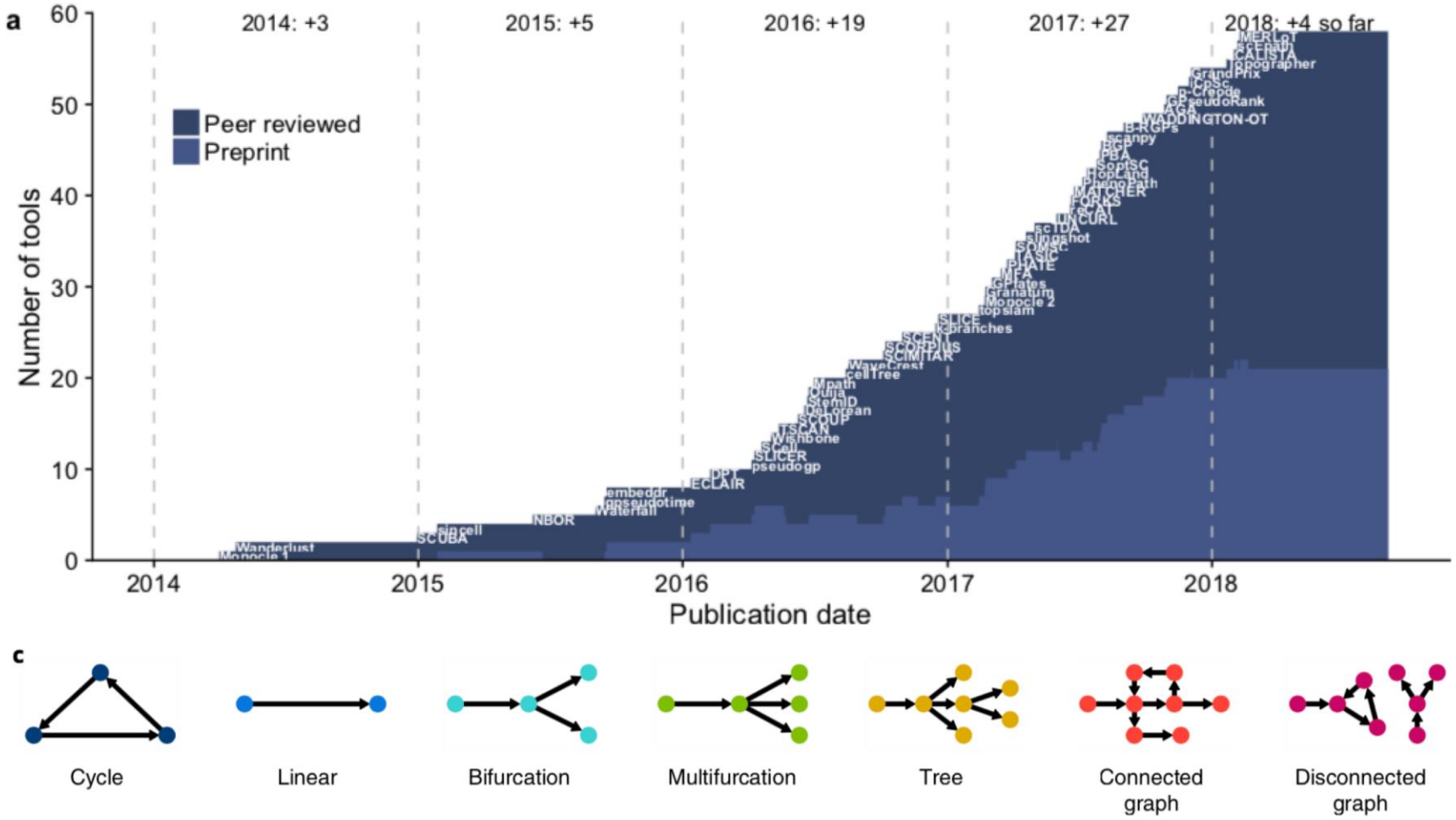
•In the analysed data set one might encounter :
•1. Cells that differentiate and display a **continuous spectrum** of states Transcriptional program for activation and differentiation (from a naïve inactivated stage to activated for instance)
•2. Individual cells will differentiate in an unsynchronized manner Each cell is a snapshot of **differentiation** time
•3. Pseudotime – abstract **unit of progress**
Distance between a cell and the start of the trajectory

Should you run trajectory inference

- Are you sure that you have a developmental trajectory?
- Do you have intermediate states?
- Do you believe that you have branching in your trajectory?
- Do you have a time scale on your cells ?
- Do you have a starting state or an ending state ?

Be aware, any dataset can be forced into a trajectory without any biological meaning!

Different type of trajectory analysis



Saelens et al (2019) Nat Biotechnology

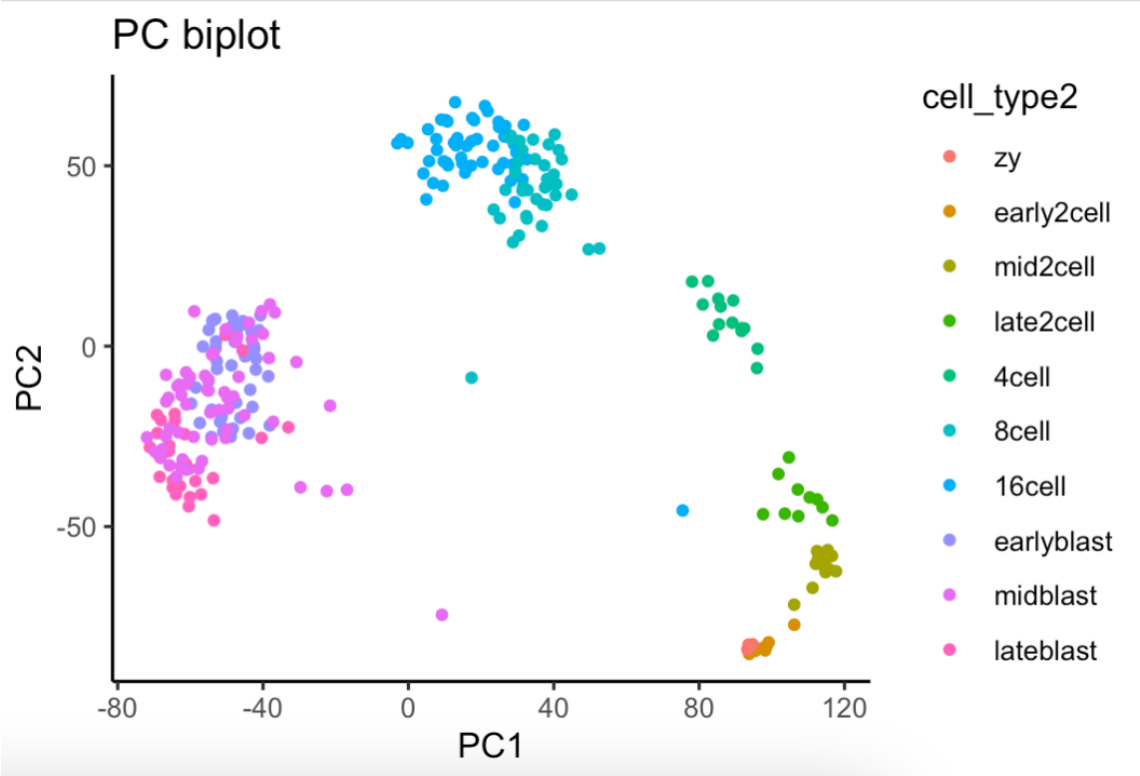
Trajectory analysis

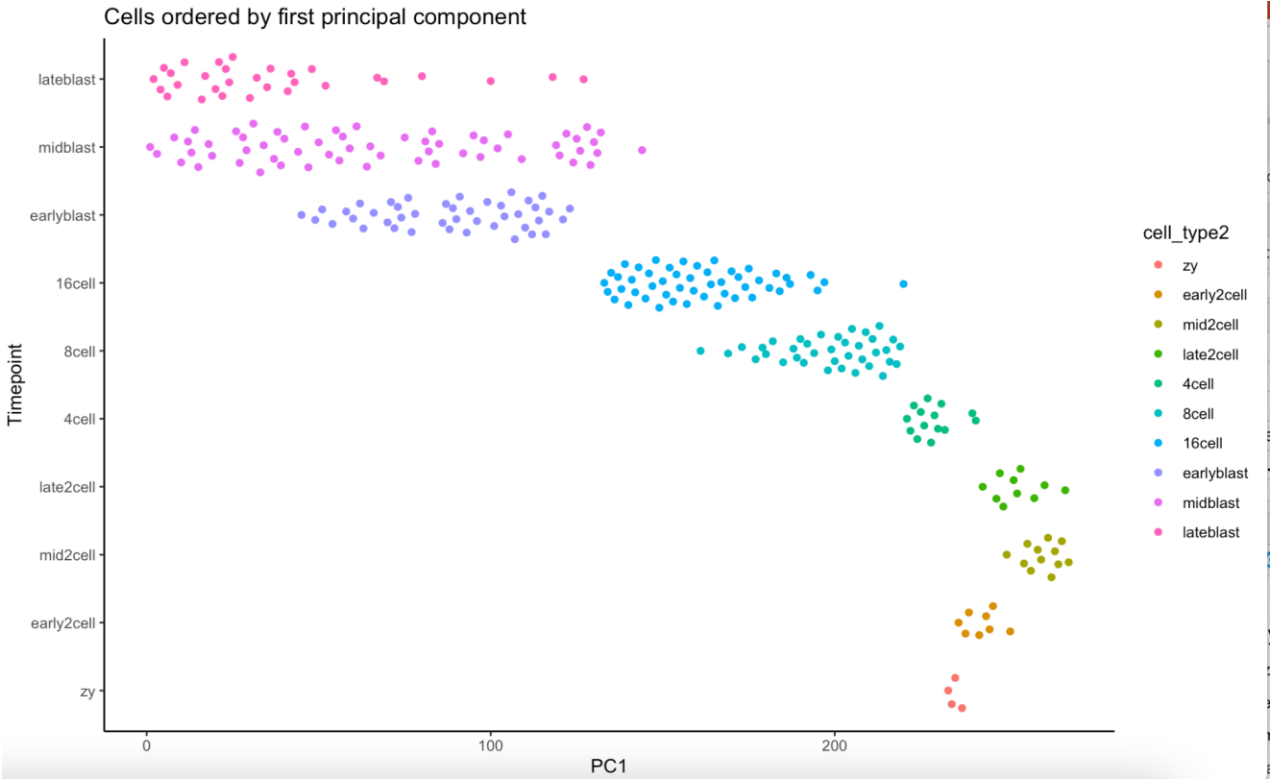
- Differences in gene expression between cells, might be attributed to dynamic processes:
  - Cell cycle
  - Cell differentiation
  - Response to an external stimuli
- Trajectory inference can order a set of individual cells along a path / trajectory / lineage
- Some methods project cells onto a **pseudotime axis** others project each cell along a path.
- This can be a starting point for further analysis to determine gene expression programs driving interesting cell phenotypes or discussions on changes in conditions that drive cells towards more or less differentiated states (Number of cells in the begining of the pseudotime axis vs end).

Example of application

- From the paper Single-Cell RNA-Seq Reveals Dynamic, Random Monoallelic Gene Expression in Mammalian Cells (Deng et al. 2014)
- « To investigate allele-specific gene expression at single-cell resolution, we isolated 269 individual cells dissociated from in vivo F1 embryos (CAST/EiJ × C57BL/6J, hereafter abbreviated as CAST and C57, respectively) from oocyte to blastocyst stages of mouse preimplantation development (PD)»
- Here finding a trajectory between the cells might be of high interest.

A trajectory could be to order the cells according to PC1



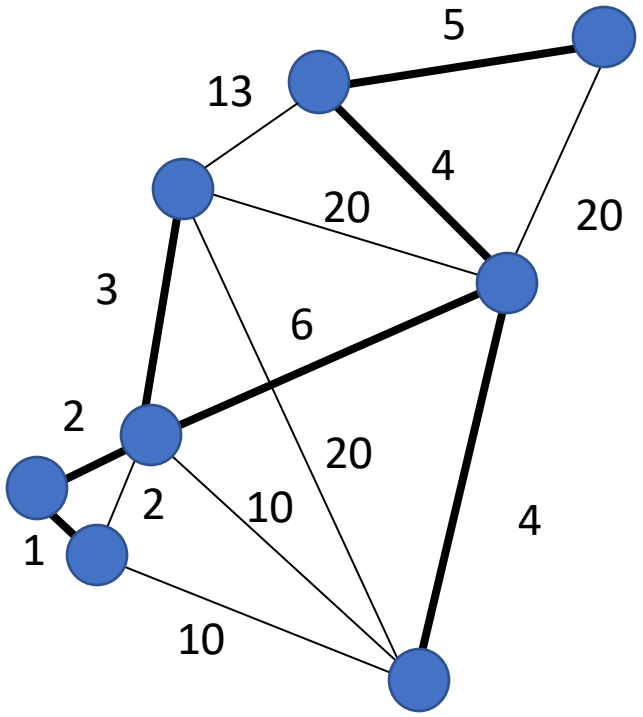


But can we do better ?

Standard process of trajectory analyses

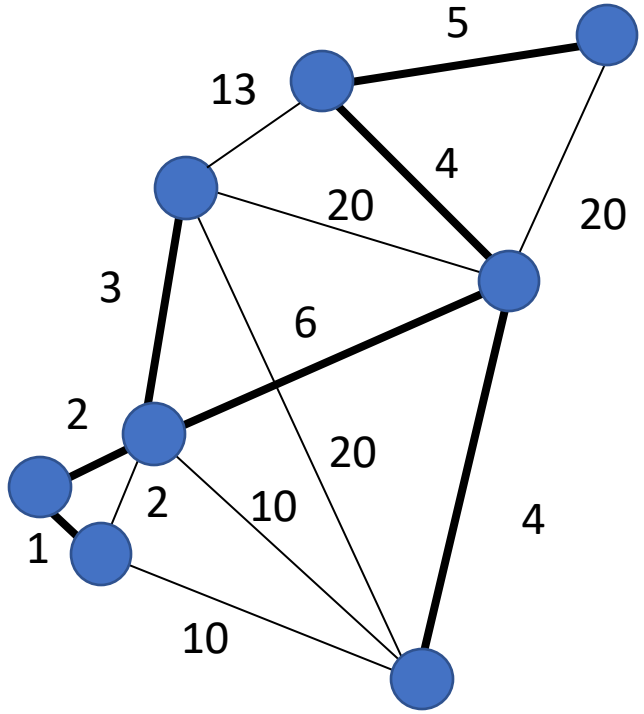
- Take a weighted graph.
- Take a spanning tree
- Take the minimum of all spanning trees.

Example

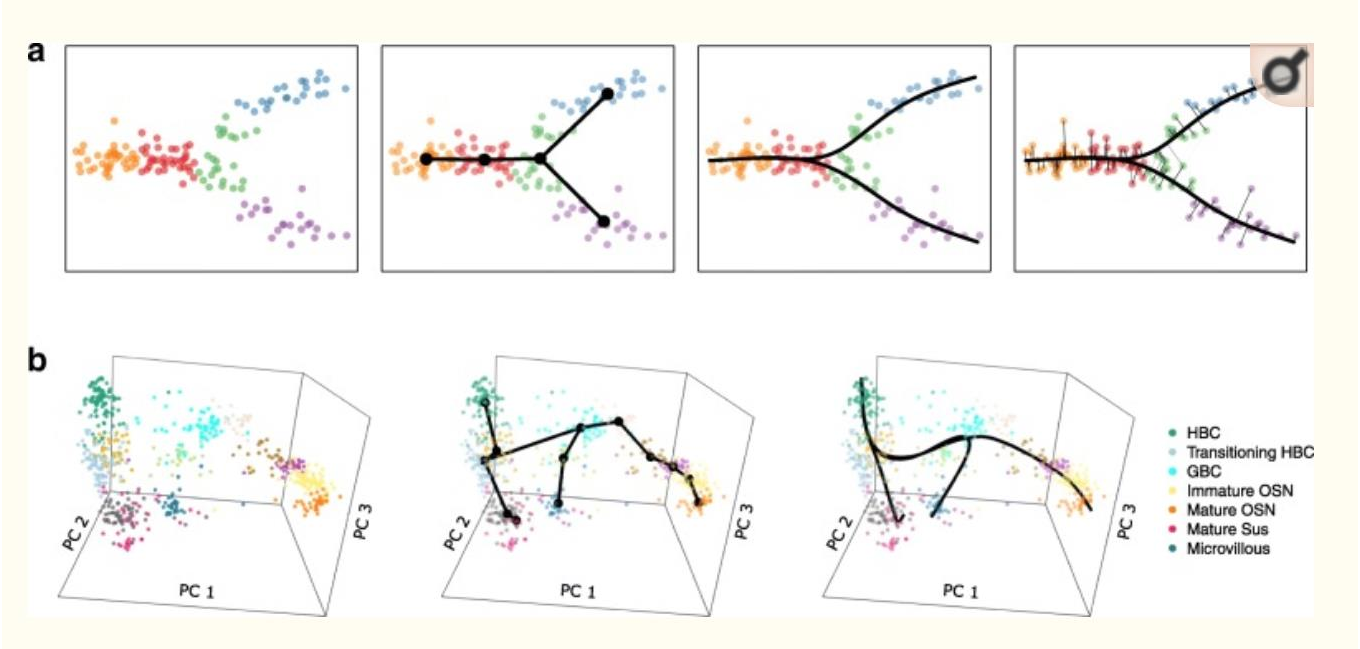


Minimum spanning tree (MST)

- **Sum of all distances in the tree (graph) is at its minimum**
- Having more transitional cells improves the definition of the tree
- The weights can be a distance in the dimensionality reduction space (ICA, T-SNE, UMAP, diffusion maps) or a correlation between cells, etc.
- MST has no cycles, cell cycles will not work in here



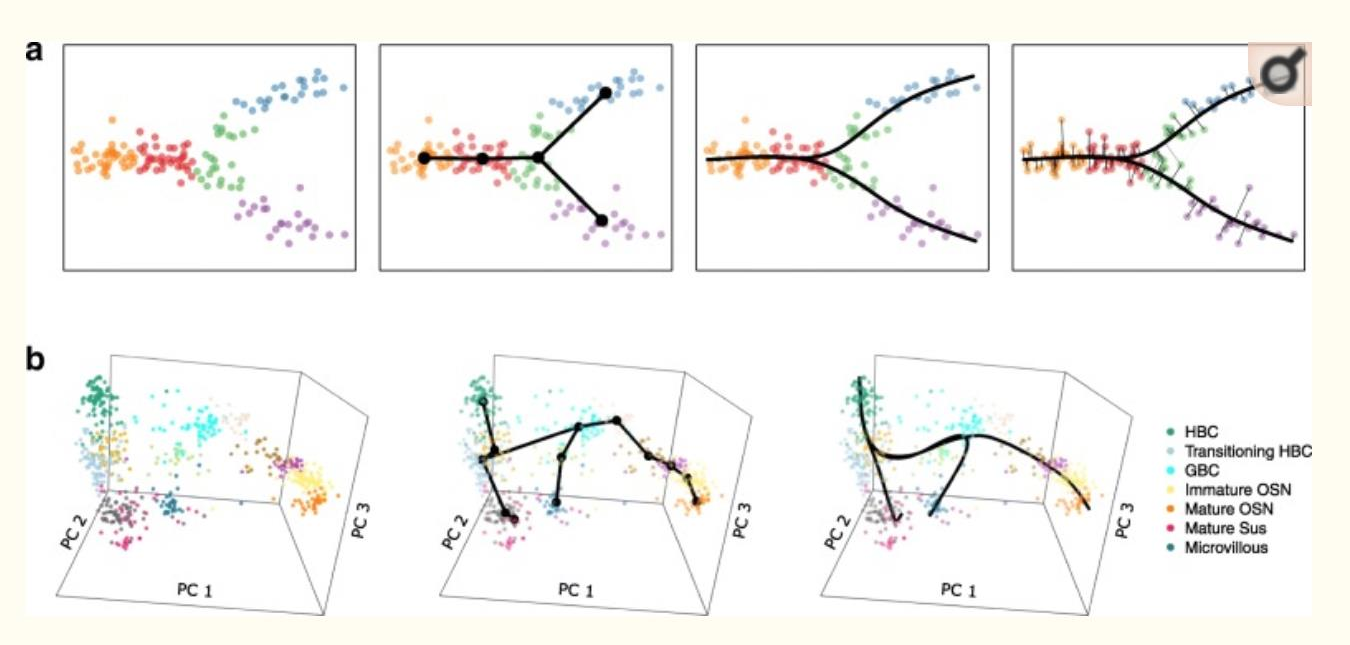
Slingshot (Street et al 2018)



1. Distance between clusters

$$d^2\left(\mathscr{C}_i, \mathscr{C}_j\right) \equiv \left(\overline{X}_i - \overline{X}_j\right)^T \left(S_i + S_j\right)^{-1} \left(\overline{X}_i - \overline{X}_j\right),$$

Slingshot (Street et al 2018)



1. Distance between clusters
2. Infer lineages by ordering cell clusters and construct MST
3. Construct principal curves*

*Principal curves are smooth one-dimensional curves that pass through the middle of a p-dimensional data set, providing a nonlinear summary of the data. They are nonparametric, and their shape is suggested by the data

Slingshot on Deng data

- sce <- slingshot::slingshot(deng_SCE, clusterLabels = 'Seurat_clusters', reducedDim = 'PCA', start.clus = "2")

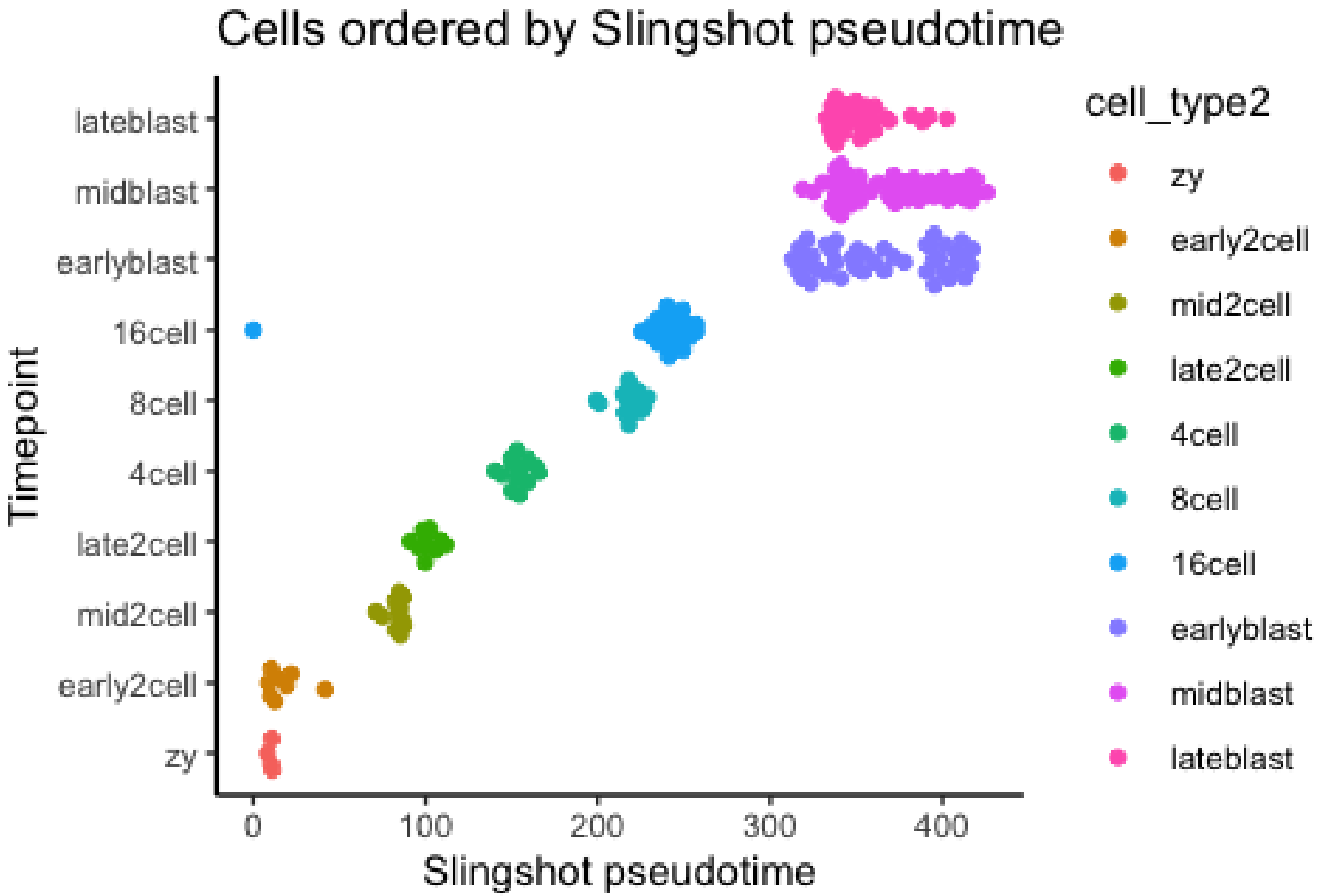
```
> SlingshotDataSet(sce)
class: SlingshotDataSet

 Samples Dimensions
     268          50

lineages: 2
Lineage1: 2  4  0  5  3
Lineage2: 2  4  1

curves: 2
Curve1: Length: 425.93  Samples: 234.68
Curve2: Length: 340.91  Samples: 132.37
```

Slingshot on Deng data



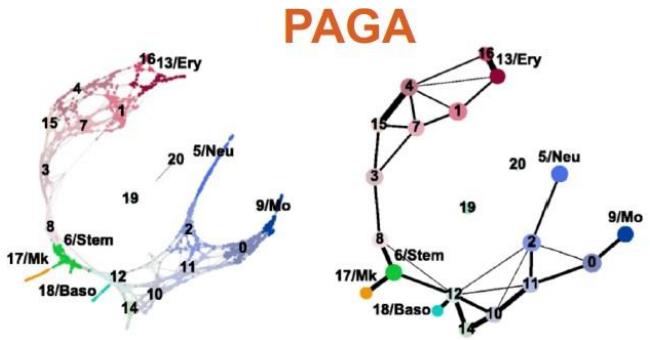
Monocle3 uses an algorithm based on PAGA (python)

- **PAGA** constructs a **k-nearest neighbour** graph on cells and then identifies 'communities' of cells via the Louvain method.
- Two vertices (**Louvain communities**) are linked with an edge, when the cells in the respective communities are neighbours in the *k*-nearest neighbour graph.

- **Monocle 3** constructs a *k*-nearest neighbour graph (*k* = 20) on cells in the UMAP space, then grouping them into Louvain communities, and testing each pair of communities for a **significant number** of links between their respective cells.

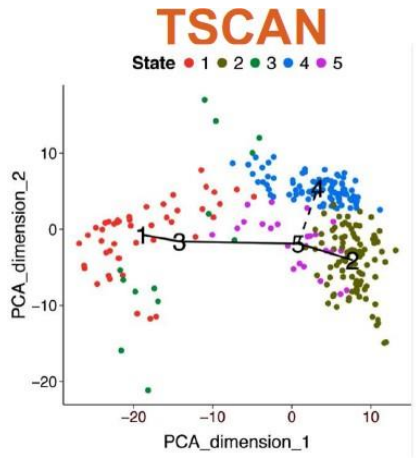
- Those communities that have more links than expected under the null hypothesis of spurious linkage (FDR <1%) remain connected in the PAGA graph, and those links that fail this test are severed. (correction of **spurious linkage**)

Some additional tools

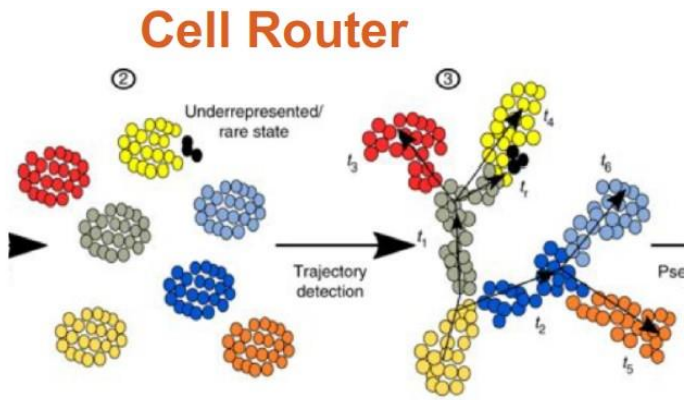


Street et al (2019) Genome Biology

Spade, StemID 2, Eclair, TSCAN and Mpath use different clustering algorithms such as k-means, k-medoids, hierarchical clustering or DBSCAN in a dimensionality-reduced space, Psupertime for time-series data.



Zhicheng et al (2016) Nuc Acid Res



Da Rocha et al (2018) Nat Commun

RNA Velocity a quite different algorithm

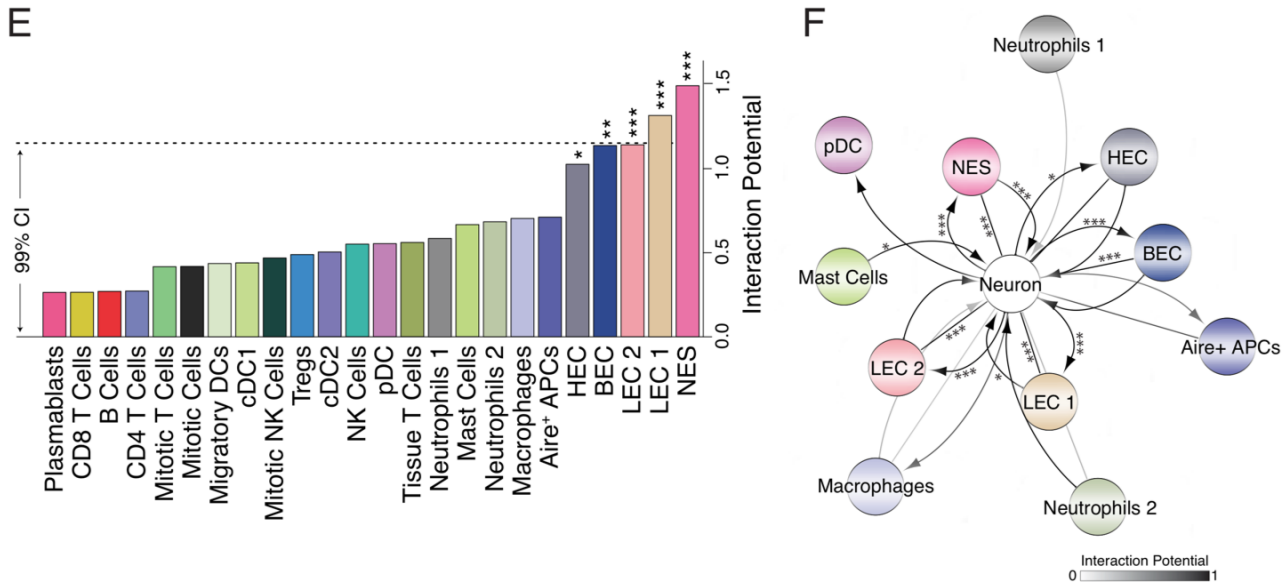
- « RNA velocity is a high-dimensional vector that **predicts** the future state of individual cells on a timescale of hours»
- «aid the analysis of **developmental lineages** and **cellular dynamics**»
- Method : calculate the relative abundance of nascent (unspliced) and mature (spliced) mRNA to estimate the rates of gene splicing and degradation
- During a dynamic process:
  - increase in the transcription rate=> rapid increase in unspliced mRNA=> increase in spliced mRNA until a new steady state is reached.
  - a drop in the rate of transcription => drop in unspliced mRNA => reduction in spliced mRNAs.
- During induction of gene expression: => unspliced mRNAs are present in excess,
- During repression: => unspliced mRNAs are present in lower amounts.
- Hence: The balance of unspliced and spliced mRNA abundance is, therefore, an indicator of the future state of mature mRNA abundance, and thus the future state of the cell.

Gioele La Manno (2018) Nature

Cell-cell communication

Cell-cell communications

- LRIP – Ramilowski et al, Nat comm, 2015- bioarxiv, Huang et al,xmol, 2020
- CellphoneDB - https://www.cellphonedb.org/ - online « clickable » Mirjana Efremova, Nat protocols, 2020.
- NicheNet – needs apriori knowledge, Robin Browaeys, Nat met, 2020.
- CellChat- http://www.cellchat.org/



LRIP – Bioarxiv, https://doi.org/10.1101/833509

NicheNet- Ligand receptor analysis

- Question : In your analysis you have a certain list of differentially expressed genes. Can one associate a pair of Ligand and Receptor responsible for the change in expression observed?

- This is extremely useful as it will point biologist to possible pathways to target to block the changes observed and which cell types communicate.

NicheNet- Ligand receptor analysis-How it works

Prior model of ligand-target regulatory potential

	Target_1	Target_2	Target_3	…	Target_n
Ligand_1	P11	P12	P13	…	P1n
Ligand_2	P21				
…	…				
Ligand_m	Pm1	Pm2	Pm3	…	Pmn

NicheNet- Ligand receptor analysis-How it works

Prior model of ligand-target regulatory potential

Calculate correlation scores

Select highest scores (scores are not meant to be so high due to high number of 0s)

	Target_1	Target_2	Target_3	…	Target_n
Ligand_1	P11	P12	P13	…	P1n
Ligand_2	P21				
…	…				
Ligand_m	Pm1	Pm2	Pm3	…	Pmn

Select only expressed ligands

	Target_1	Target_2	Target_3	…	Target_n
DGE	1	1	0		1

Assumptions and calculation

- How likely ligands in sender cells affected the gene expression in interacting receiver cells.
- Under the assumption that true downstream target genes of a ligand will be **differentially expressed after treatment** of cells with this ligand

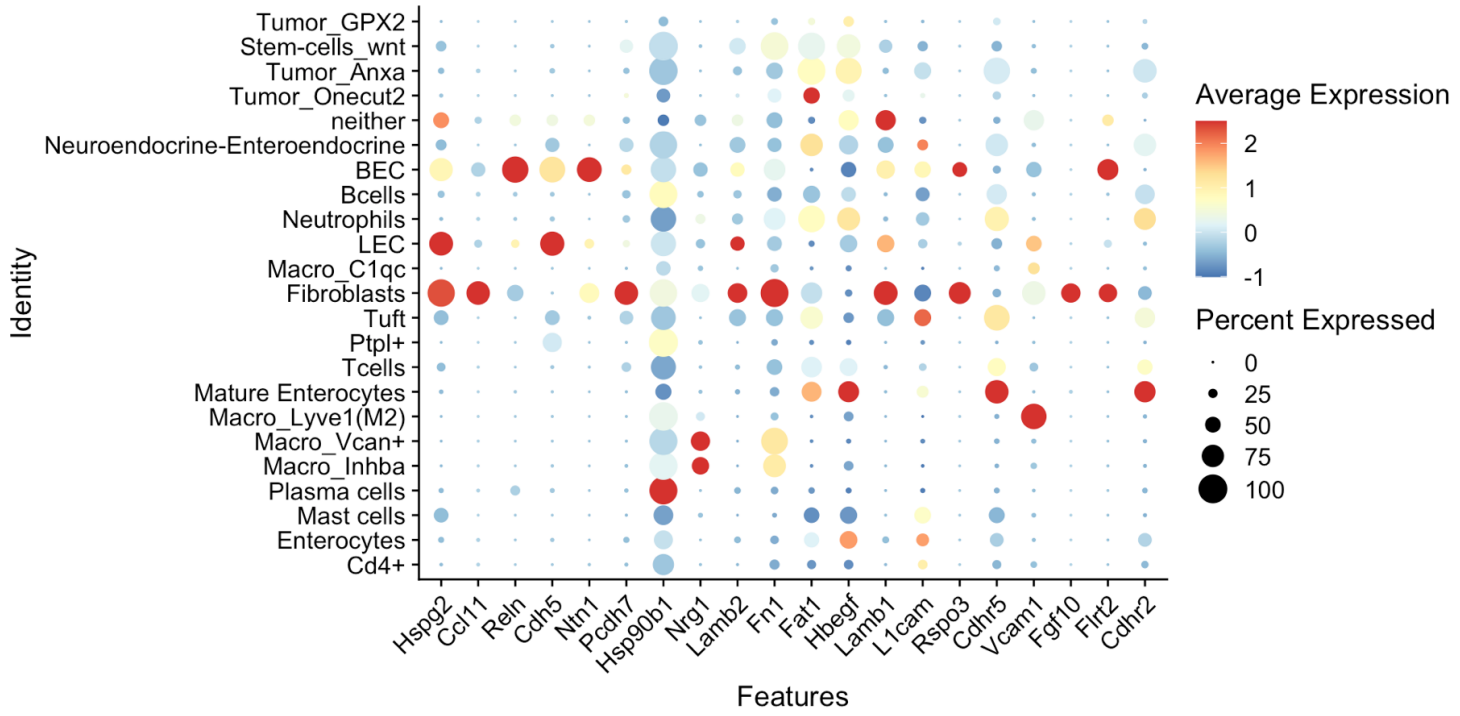
- **Ligand activity scores** :
- AUROC (area under receiver operating characteristic curve)
- AUPR (area under the precision–recall curve)
- **Pearson correlation**
- Spearman’s rank correlation
- …

NicheNet- Ligand receptor analysis-How it works

List of Ligands

L1
L4
L8
L23
L50

Where are they expressed + Are they actually Differentially expressed as well in that cell type

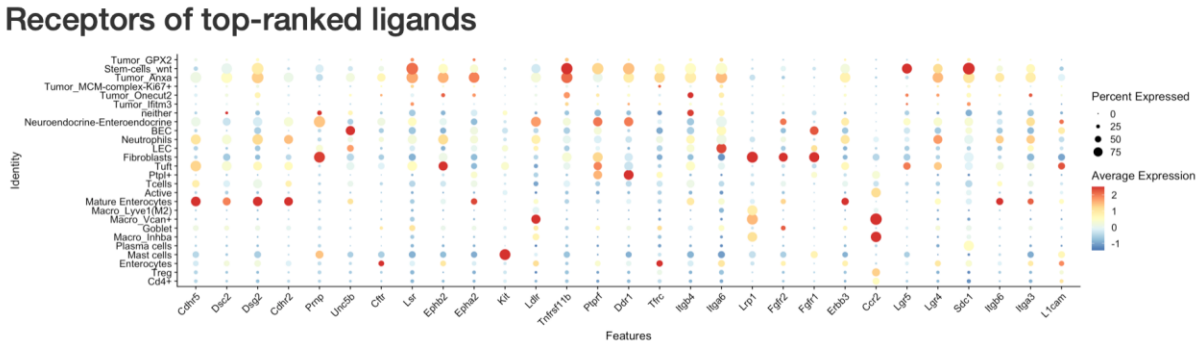


*Courtesy Amber Bowler

NicheNet- Ligand receptor analysis-How it works

List of Receptors associated to the potential ligands

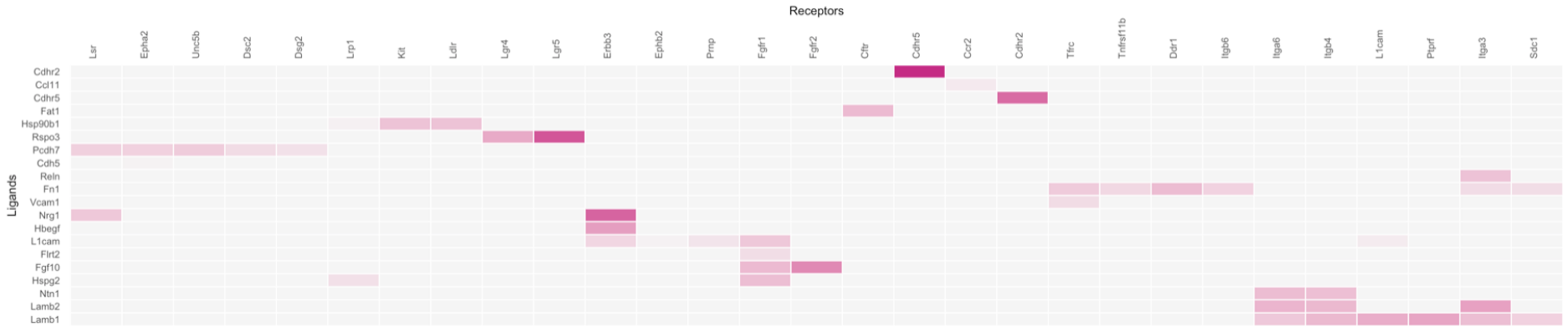
Where are they expressed + Are they actually Differentially expressed as well in that cell type



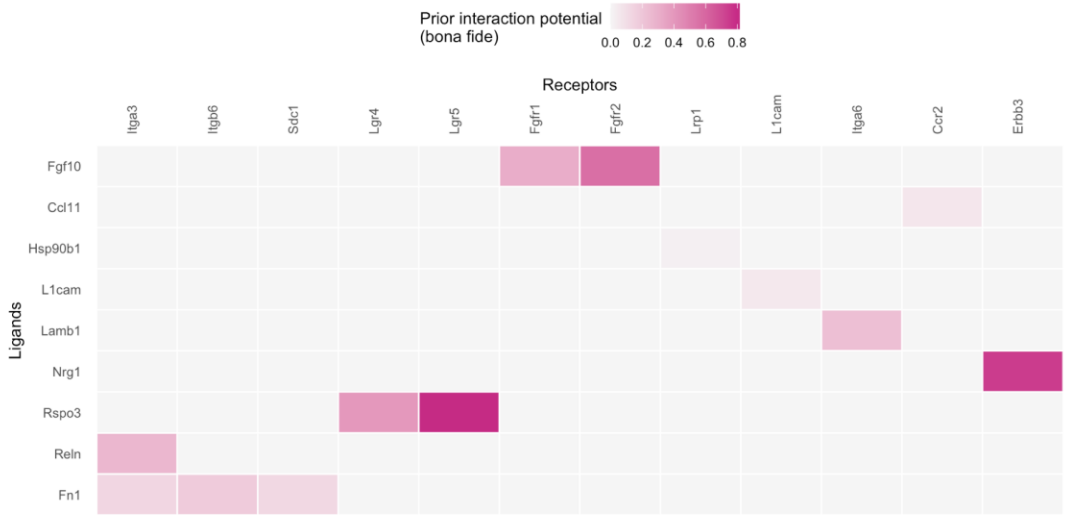
*Courtesy Amber Bowler

NicheNet- Ligand receptor analysis-How it works

List of Ligand Receptors with interaction potential



Bona fide



*Courtesy Amber Bowler