Again long list of possible tools

Developmental time

0h   1h   2h   3h   4h   5h   6h
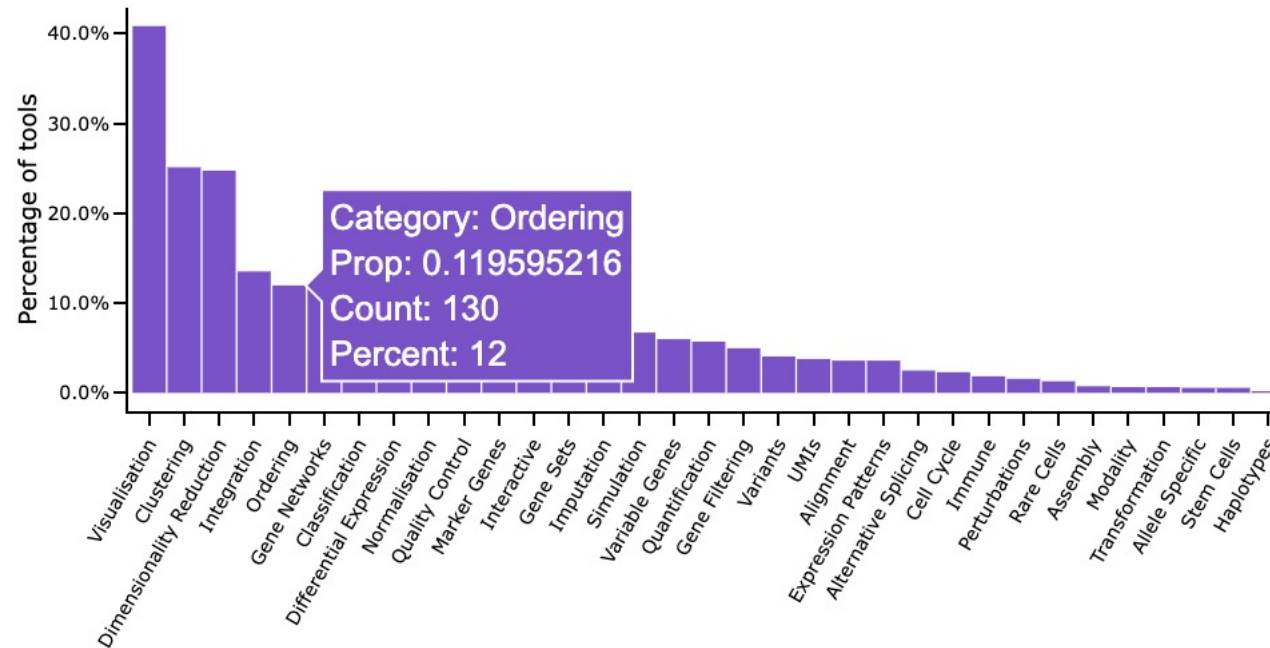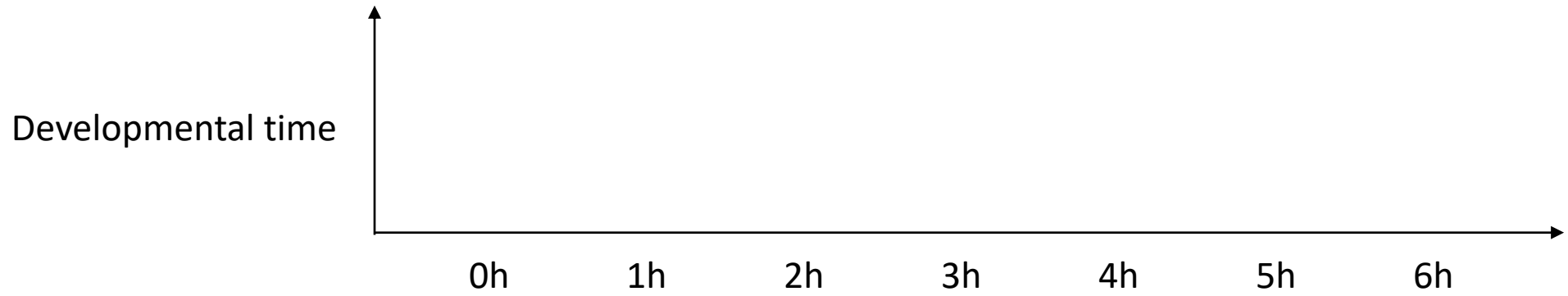
• In the analysed data set one might encounter :
• 1. Cells that differentiate display a continuous spectrum of states Transcriptional program for activation and differentiation
• 2. Individual cells will differentiate in an unsynchronized manner   Each cell is a snapshot of differentiation time
• OR 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?

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

First make sure that gene set and dimensionality reduction captures what you expect.

Saelens et al (2019) Nat Biotechnology
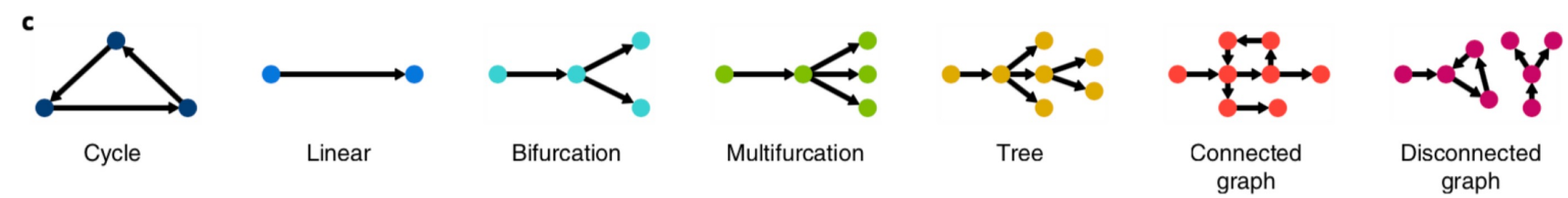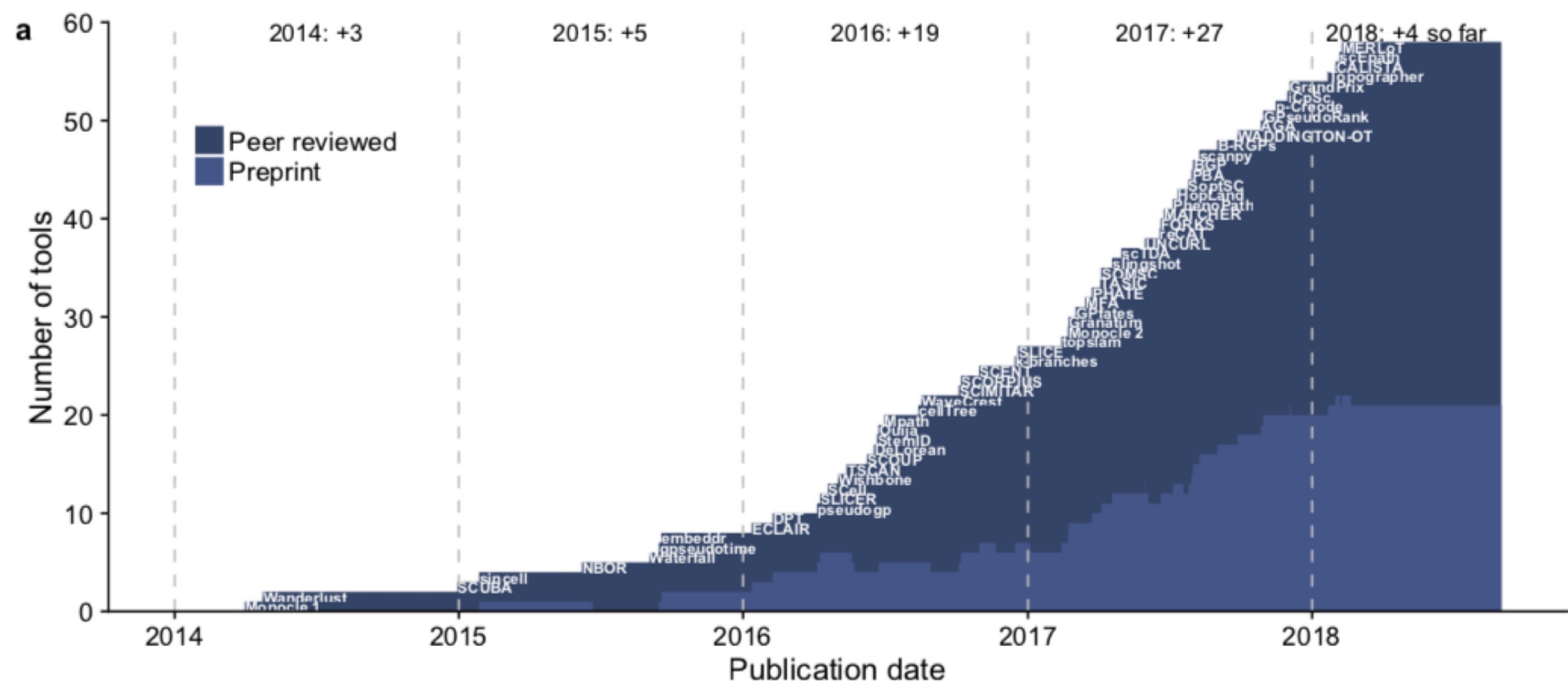
Trajectory analysis

- 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.

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.

Minimum spanning tree

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

Example
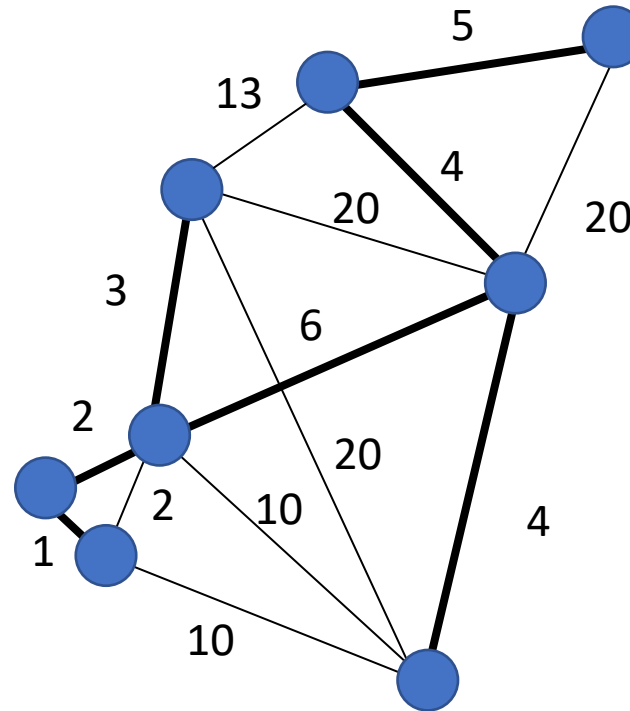
Minimum spanning tree (MST)

- **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.
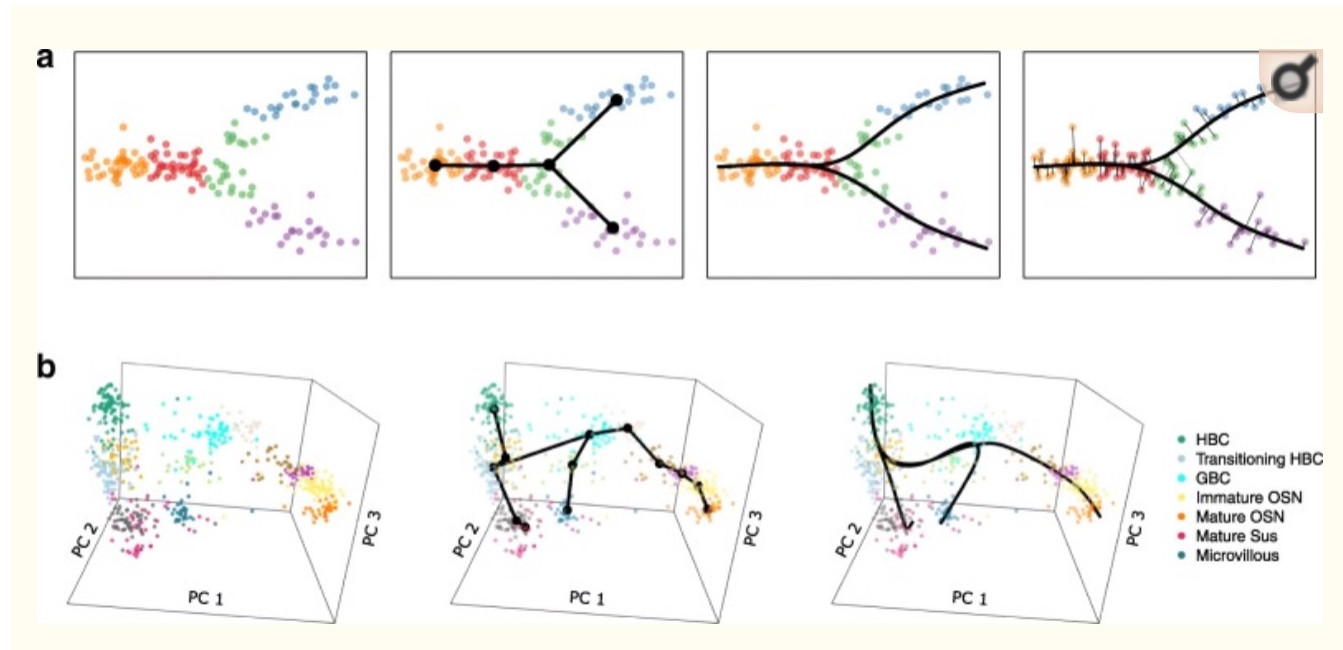- MST has no cycles, cell cycles will not work in here
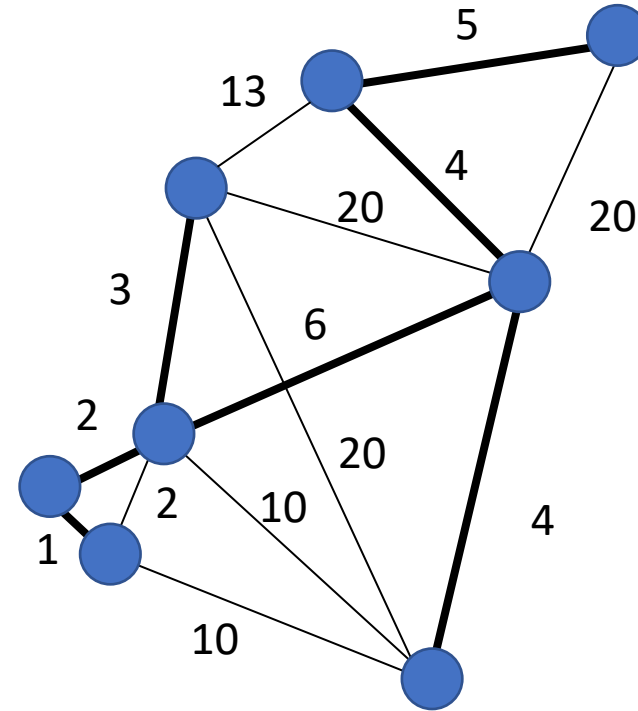
Slingshot (Street et al 2018)
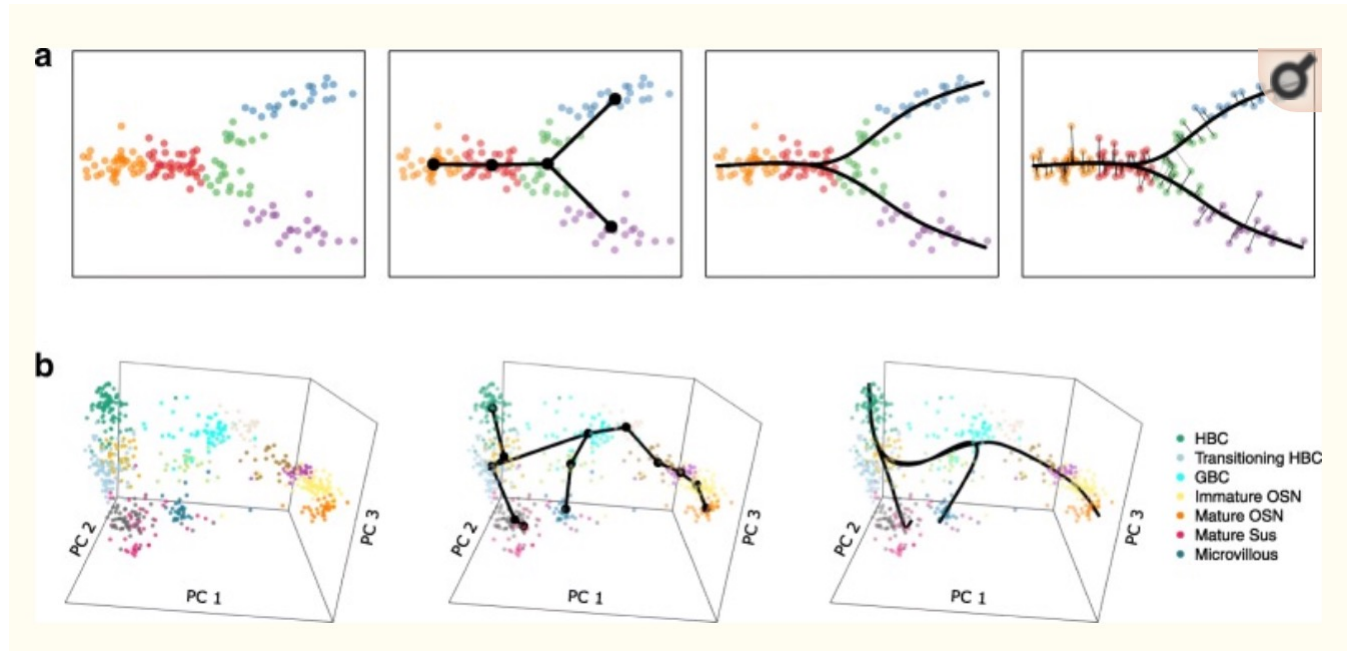
1. Distance between clusters
2. Infer lineages by ordering cell clusters

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

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

# 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
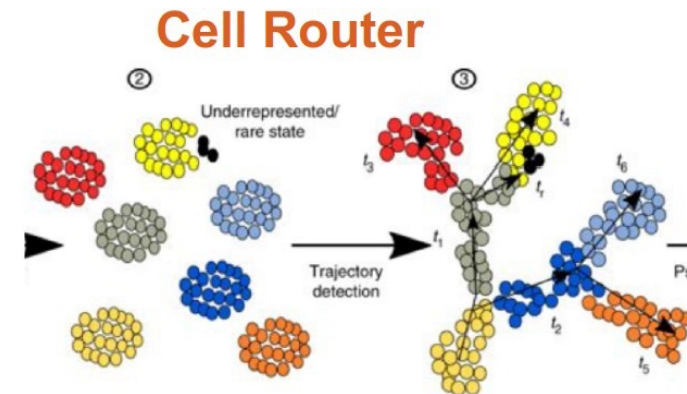
PAGA

Street et al (2019) Genome Biology
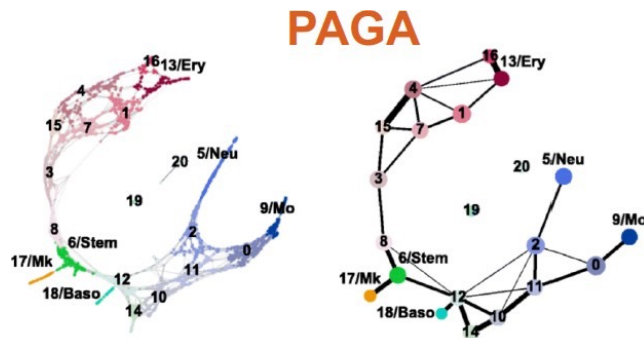
Spade, StemID 2, Eclair, TSCAN and Mpath use different clustering algorithms such as k-means, k-medoids, hierarchical clustering or DBSCAN in a dimensionality-reduced space.

TSCAN
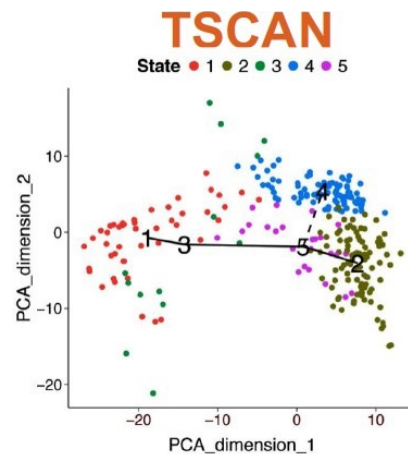
Zhicheng et al (2016) Nuc Acid Res

Cell Router

Da Rocha et al (2018) Nat Commun

Other Post-hoc analysis

- LRIP – Ramilowski et al, Nat comm, 2015- bioarxiv, https://doi.org/10.1101/833509, 2019
- 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
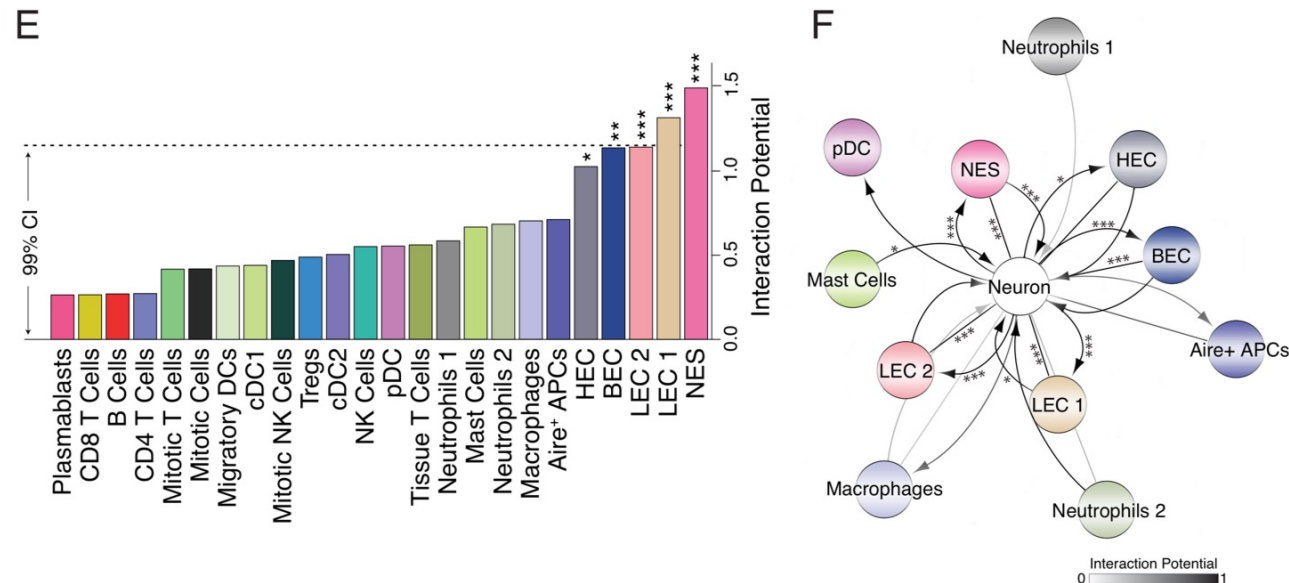
NicheNet- Ligand receptor analysis

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

- This is extremely useful as it will point biologist to possible pathways to target.

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)

Select only expressed ligands

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

	Target_1	Target_2	Target_3	...	Target_n
DGE	0	1	1		0

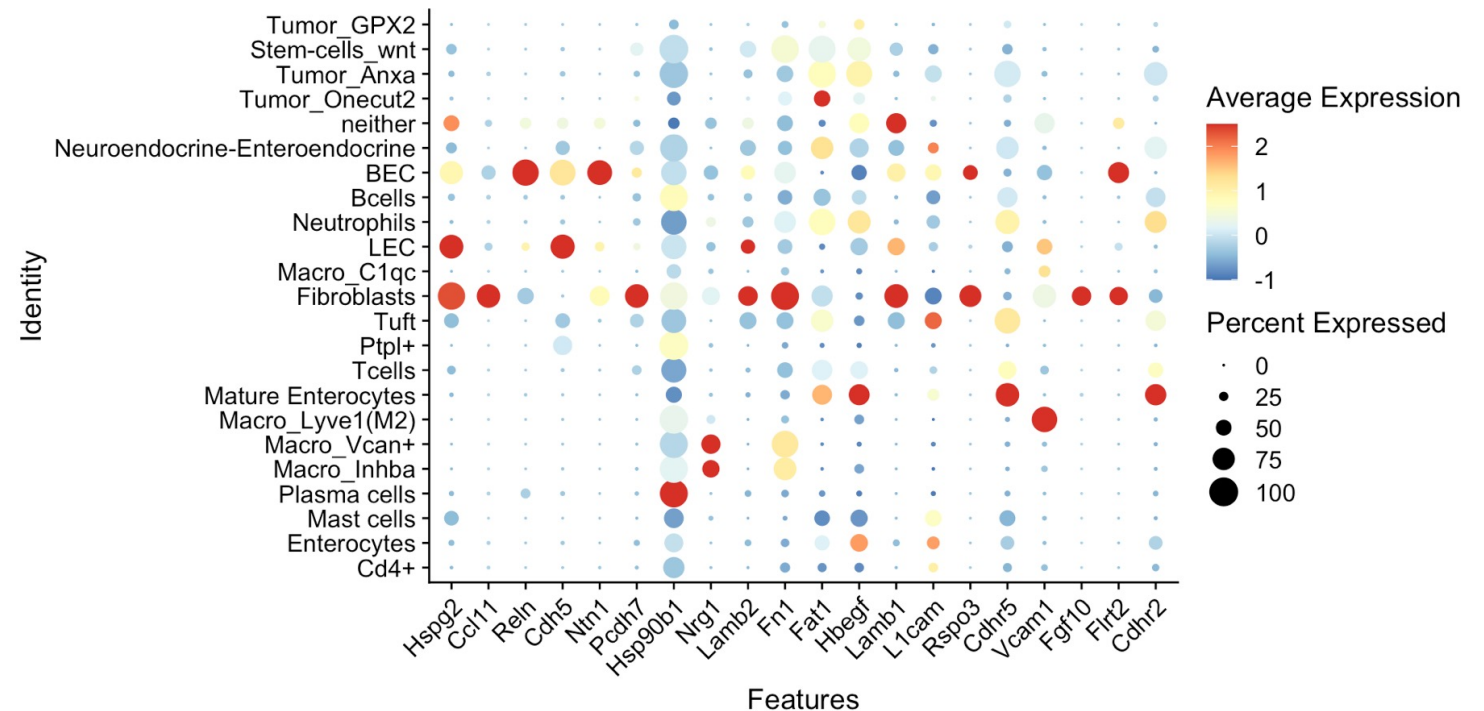# NicheNet- Ligand receptor analysis-How it works

List of Ligands

Where are they expressed + Are they actually Differentially expressed as well in that cell type
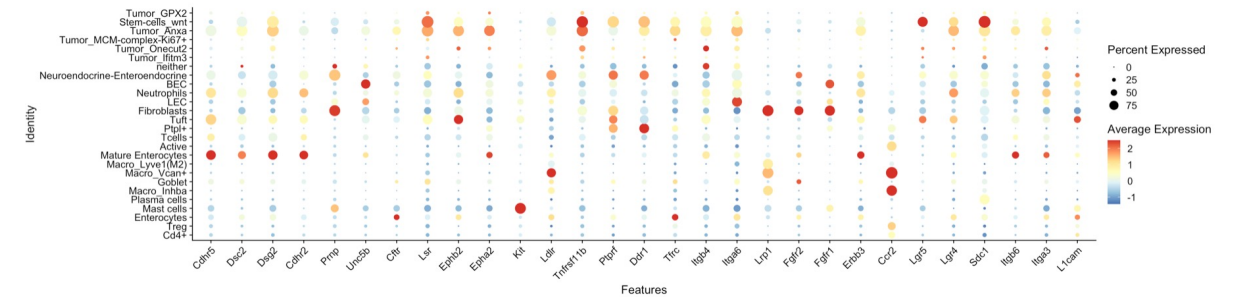
| L1 |
| L4 |
| L8 |
| L23 |
| L50 |

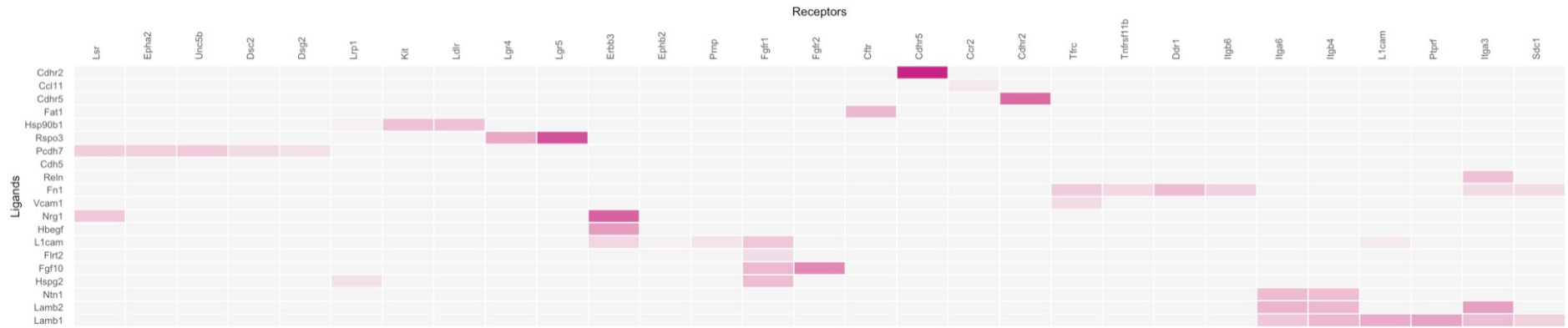# NicheNet- Ligand receptor analysis-How it works

List of Receptors associated to the potential ligands

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

NicheNet- Ligand receptor analysis-How it works
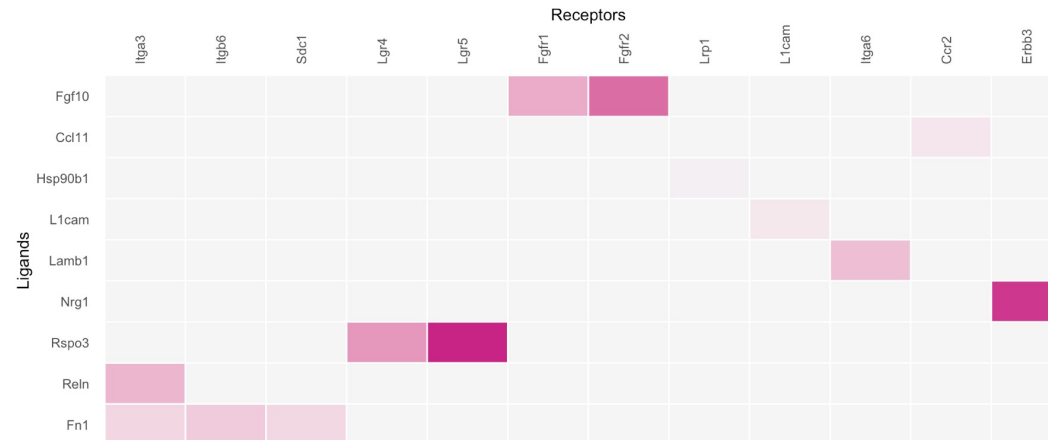
List of Ligand Receptors with interaction potential

Bona fide

In the cluster of cells where the receptor is expressed