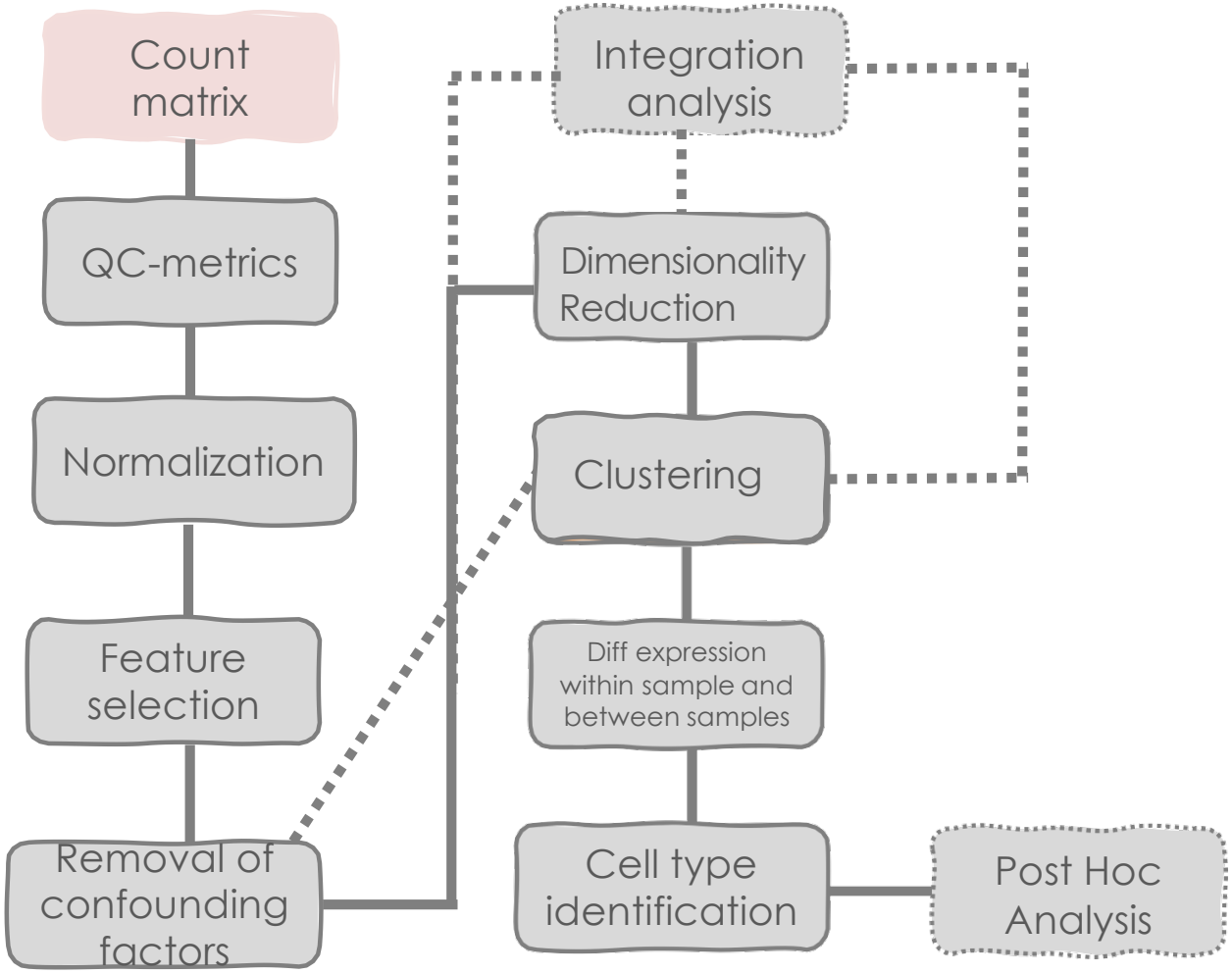

Count matrix
QC-metrics
Normalization
Feature selection
Removal of confounding factors
Integration analysis
Dimensionality Reduction
Clustering
Diff expression within sample and between samples
Cell type identification
Post Hoc Analysis

Post hoc analysis

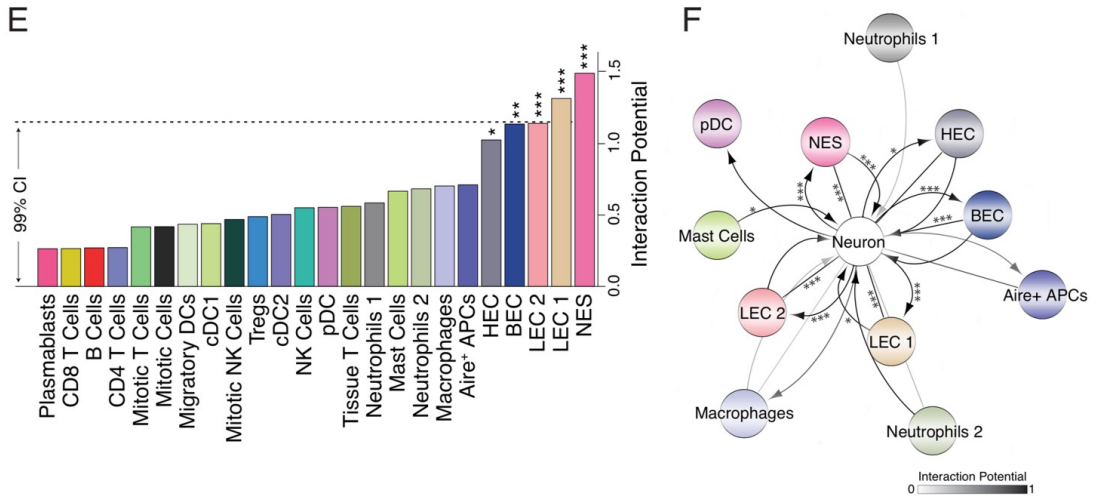
Possible interest after cell classification and DGE

- Cell-cell communication
- Transcription factor activity analysis
- Trajectory Analysis

Cell-cell communication

Cell-cell communications

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

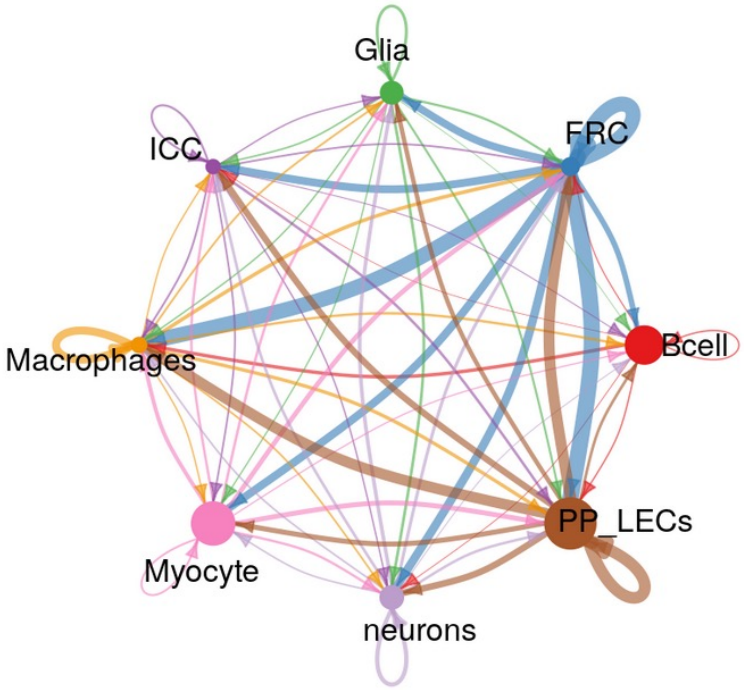


Ligand-receptor interaction potential between LN cells and neurons: LRIP Huang et al, Bioarxiv, https://doi.org/10.1101/833509

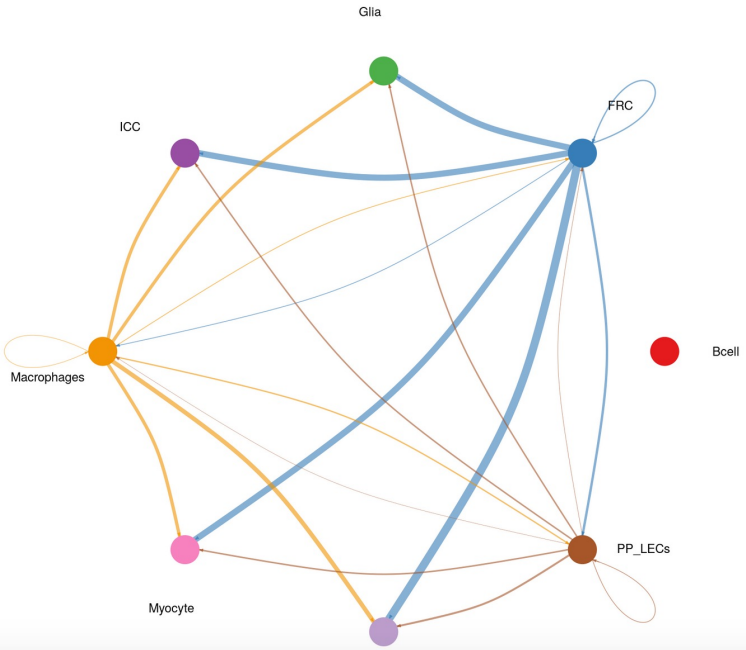
CellChat

CellChat quantifies the signaling communication probability between two cell groups.
Can be used for both scRNAseq and spatial transcriptomics.

Number of ligand-receptor interactions



IGF signaling pathway network



https://github.com/jinworks/CellChat
https://doi.org/10.1101/2023.11.05.565674 BioRxiv update Nov 5 2023, CellChat v2

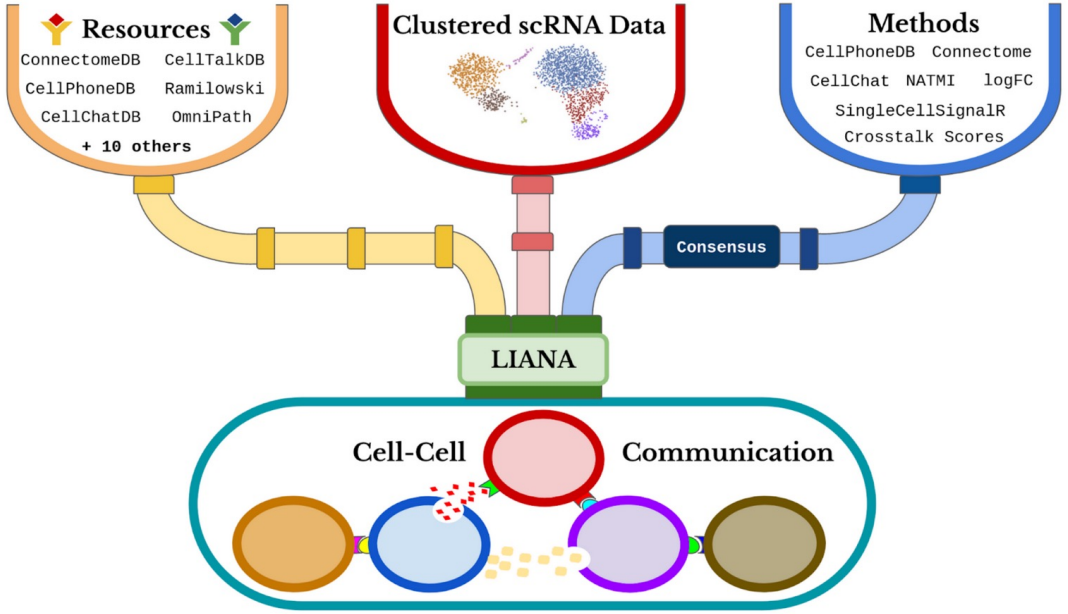
Liana

liana 0.1.12 Reference Articles https://saezlab.github.io/liana/index.html

LIANA: a LIgand-receptor ANalysis frAmework

LIANA enables the use of any combination of ligand-receptor methods and resources, and their consensus. A faster and memory efficient Python implementation is available here.

Importance of the L-R resource

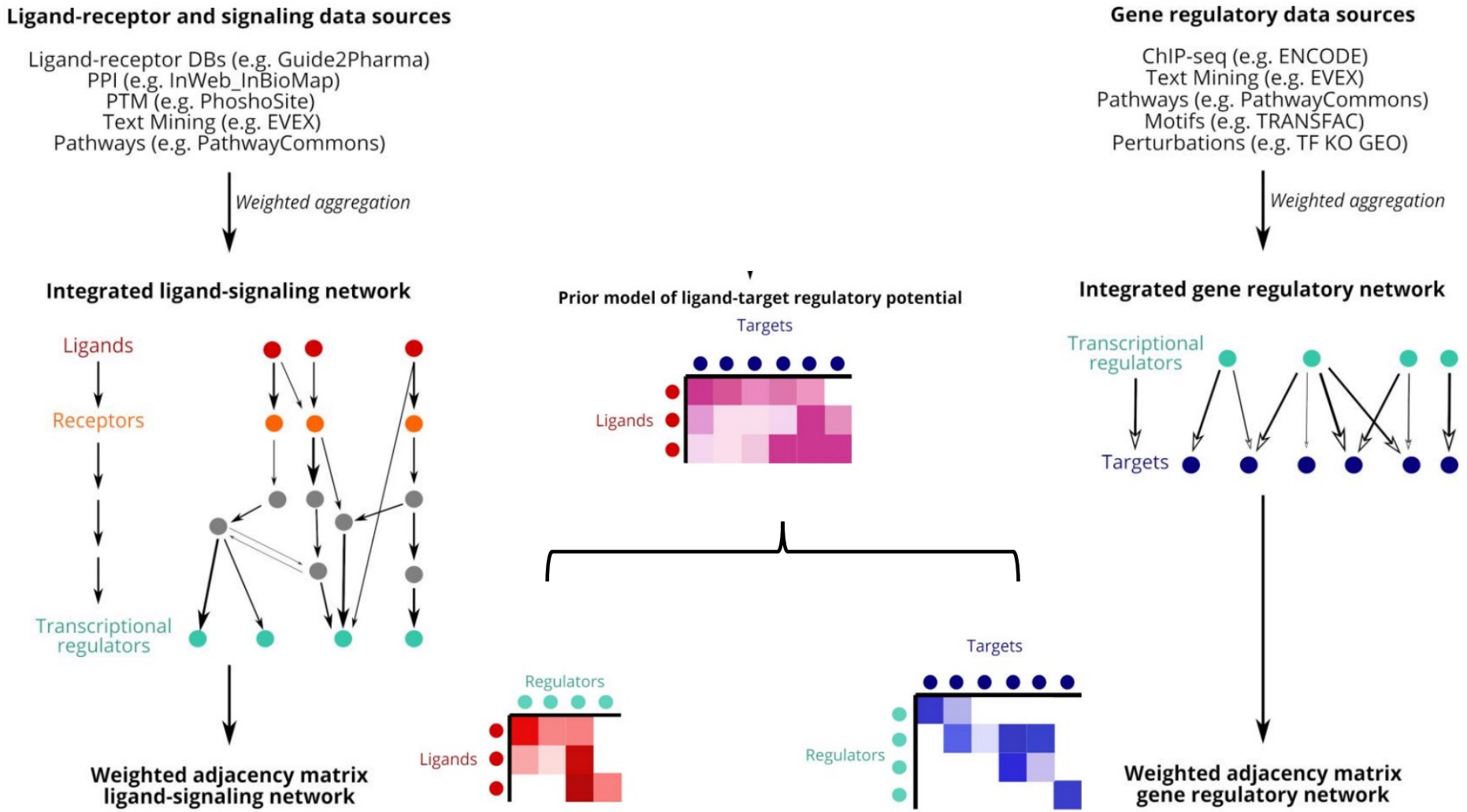


NicheNet- Ligand receptor analysis

- 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 more or less in response to a condition.

NicheNet infers active ligand–target links between interacting cells by combining their expression data with a prior knowledge model on ligand–target links



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

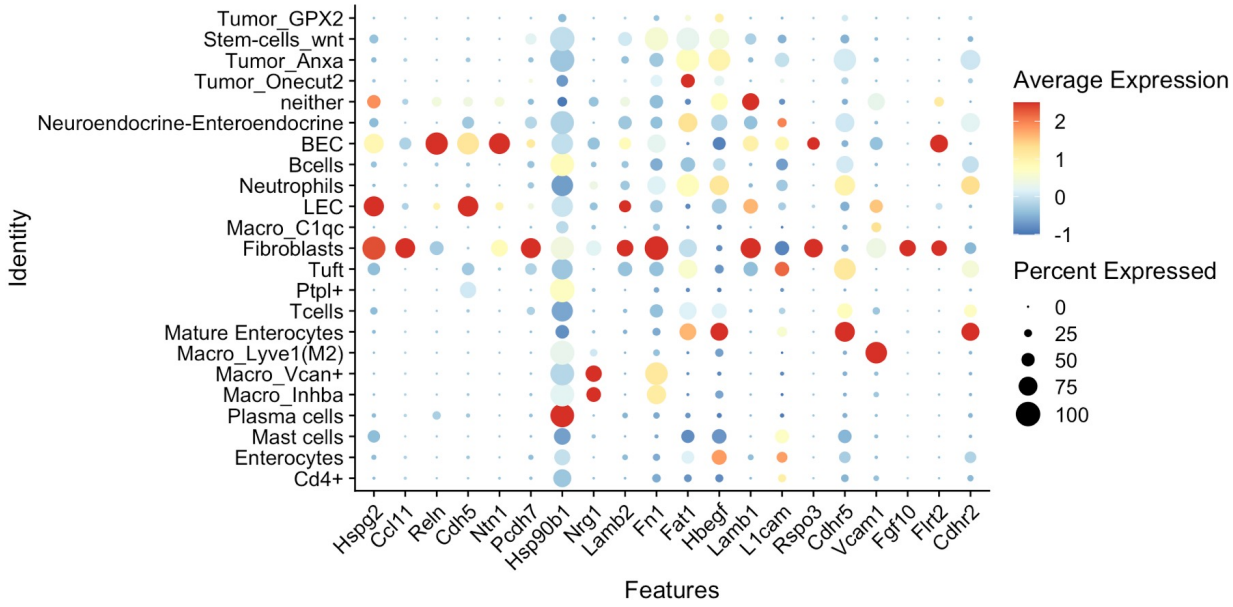
- **Ligand activity scores** :
- AUROC (area under receiver operating characteristic curve)
- AUPR (area under the precision–recall curve)
- **Pearson correlation**
- Spearman's rank correlation
- …

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

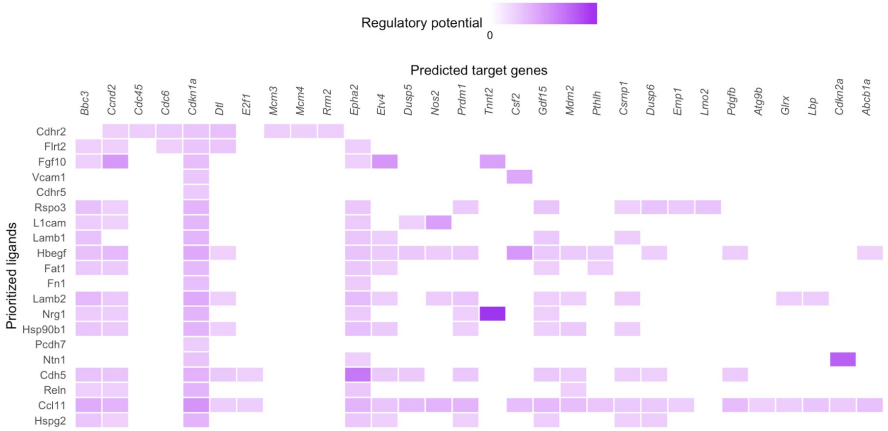


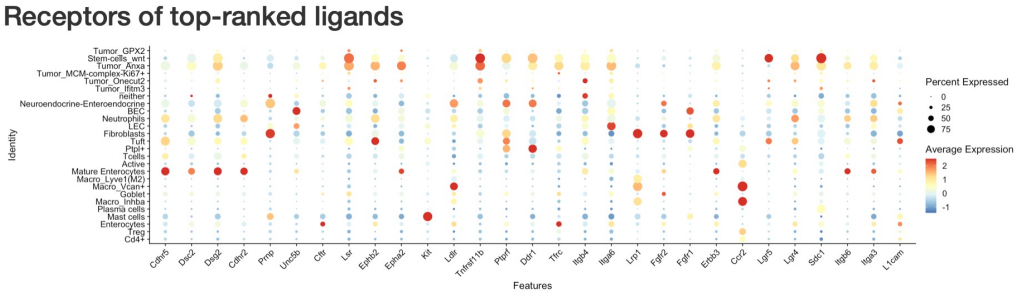
*Courtesy Amber Bowler

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



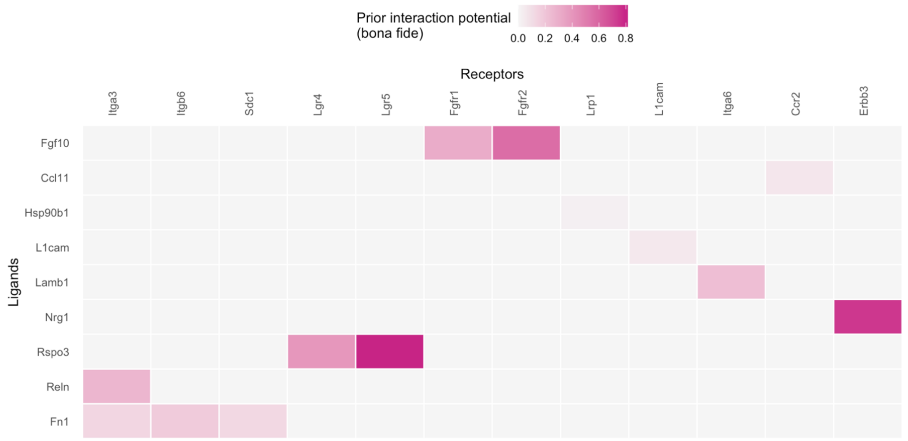


*Courtesy Amber Bowler

NicheNet- Ligand receptor analysis-How it works

List of Ligand Receptors with interaction potential

Bona fide



*Courtesy Amber Bowler

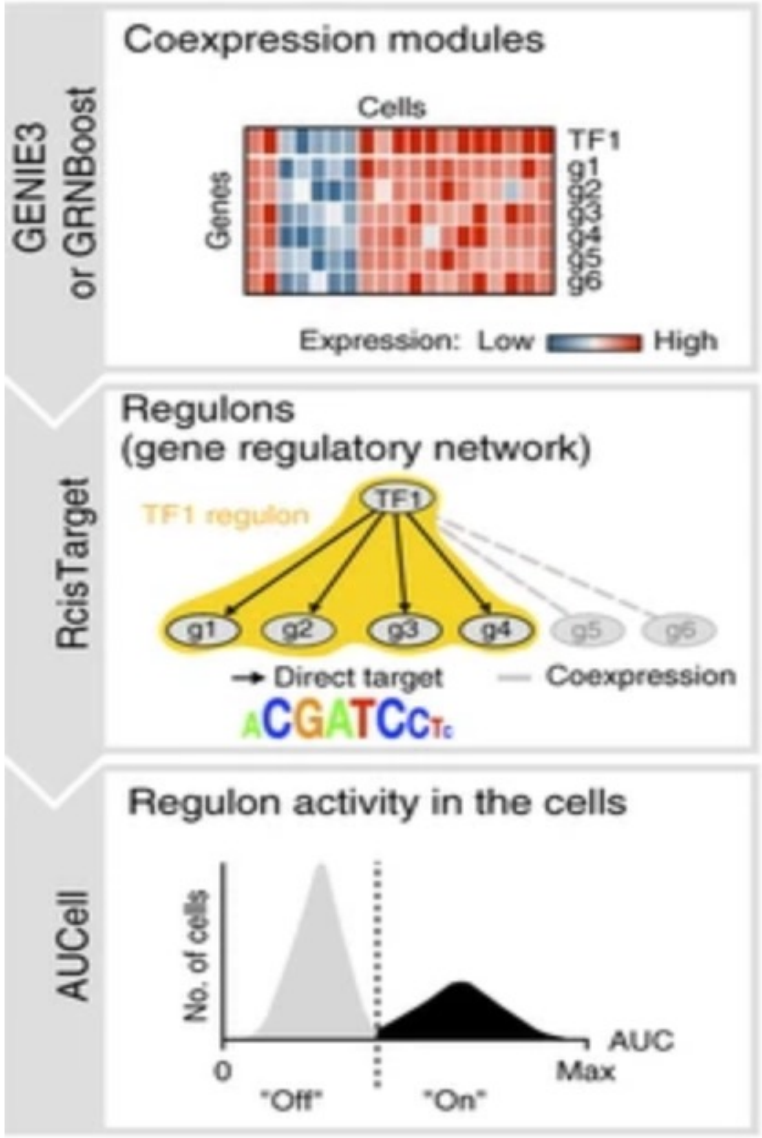
TF activity inference

SCENIC: single-cell regulatory network inference and clustering

- **GENIE3**: Gene Network Inference with Ensemble of trees (modified)
- **RcisTarget**
- **AUCell**

R implementation is computationally slow!

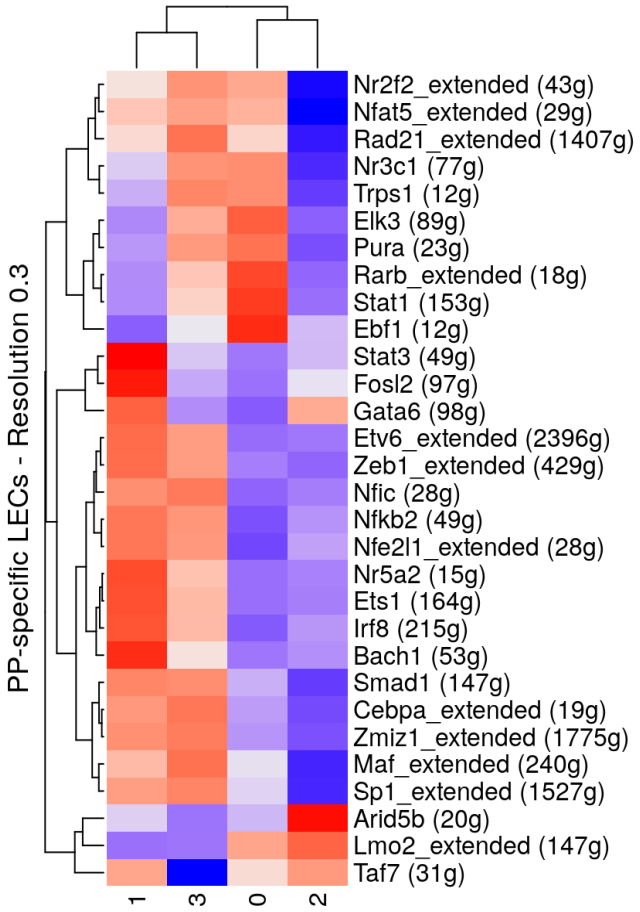
Use python implementation

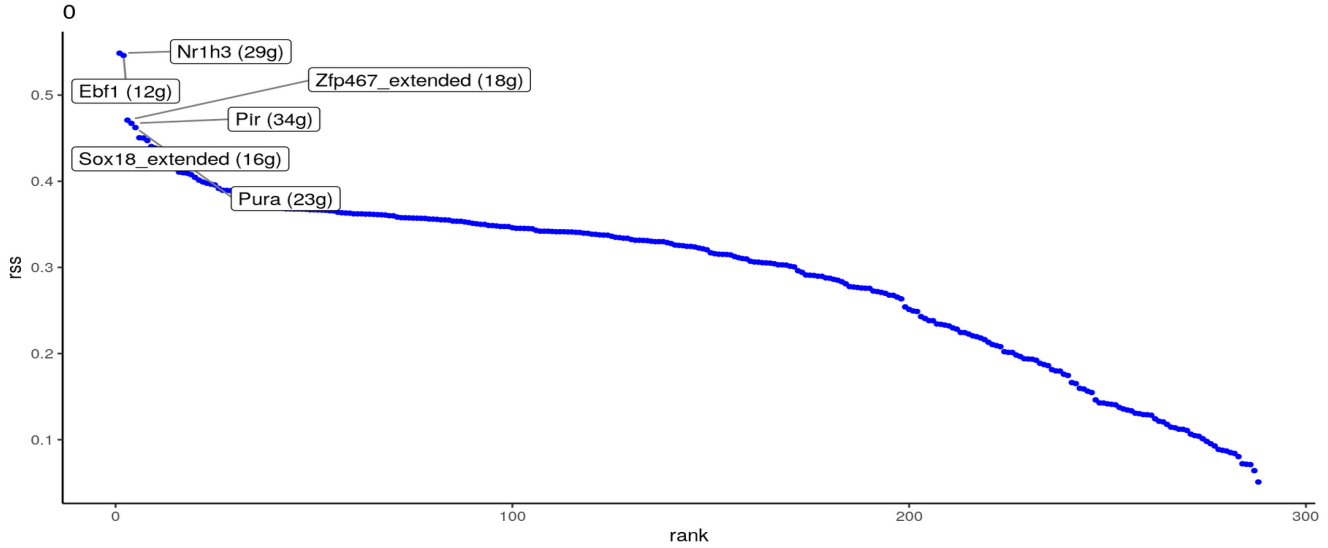


Aibar et al, 2017, Nat. Methods
https://scenic.aertslab.org/

GENIE3: Huynh-Thu et al, PLoS ONE, 2010

Which TFs are active within cell clusters ?





Regulon specificity score (RSS) rank plot

Suo et al, Cell Reports, 2018