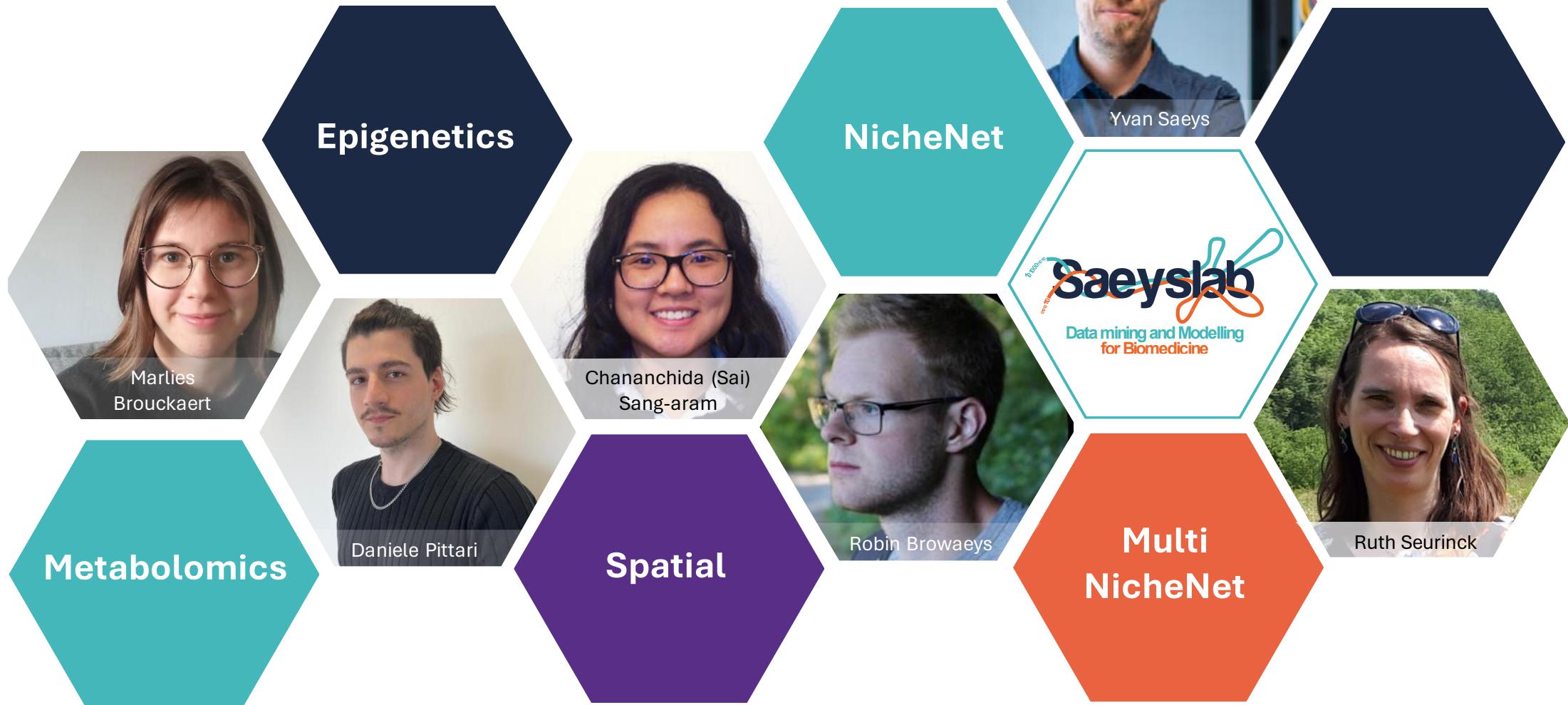


NicheNet training

Program

- 9:30 - 10:30: Introduction to NicheNet
- 10:30 - 10:45 Break
- 10:45 - 12:15: Application of NicheNet
- 12:15 - 13:15: Lunch
- 13:15 - 14:15: Application of NicheNet
- 14:15 - 14:30: Break
- 14:30 - 15:30: MultiNicheNet (introduction & demo)
- 15:30 - 15:45: Break
- 15:45 - 16:15: Q&A

Meet the team



SCIENCE MEETS LIFE

Introduction to NicheNet



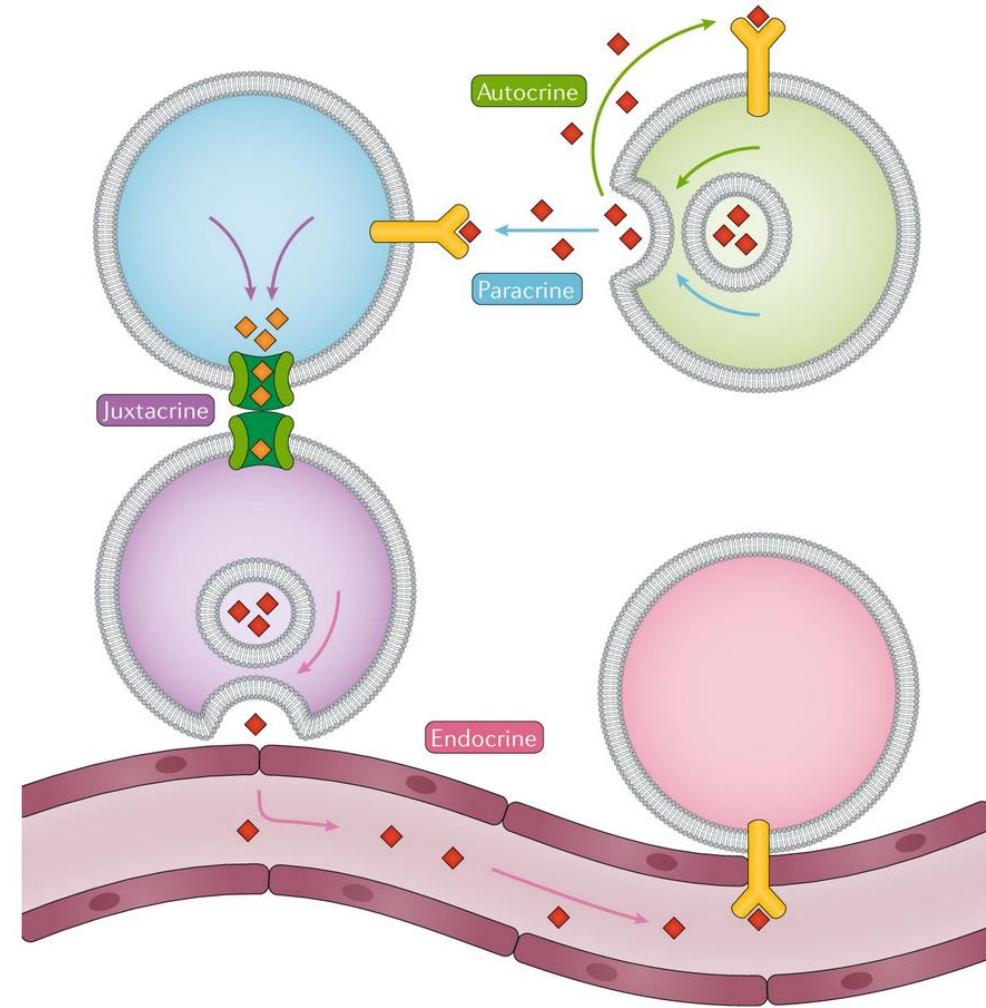
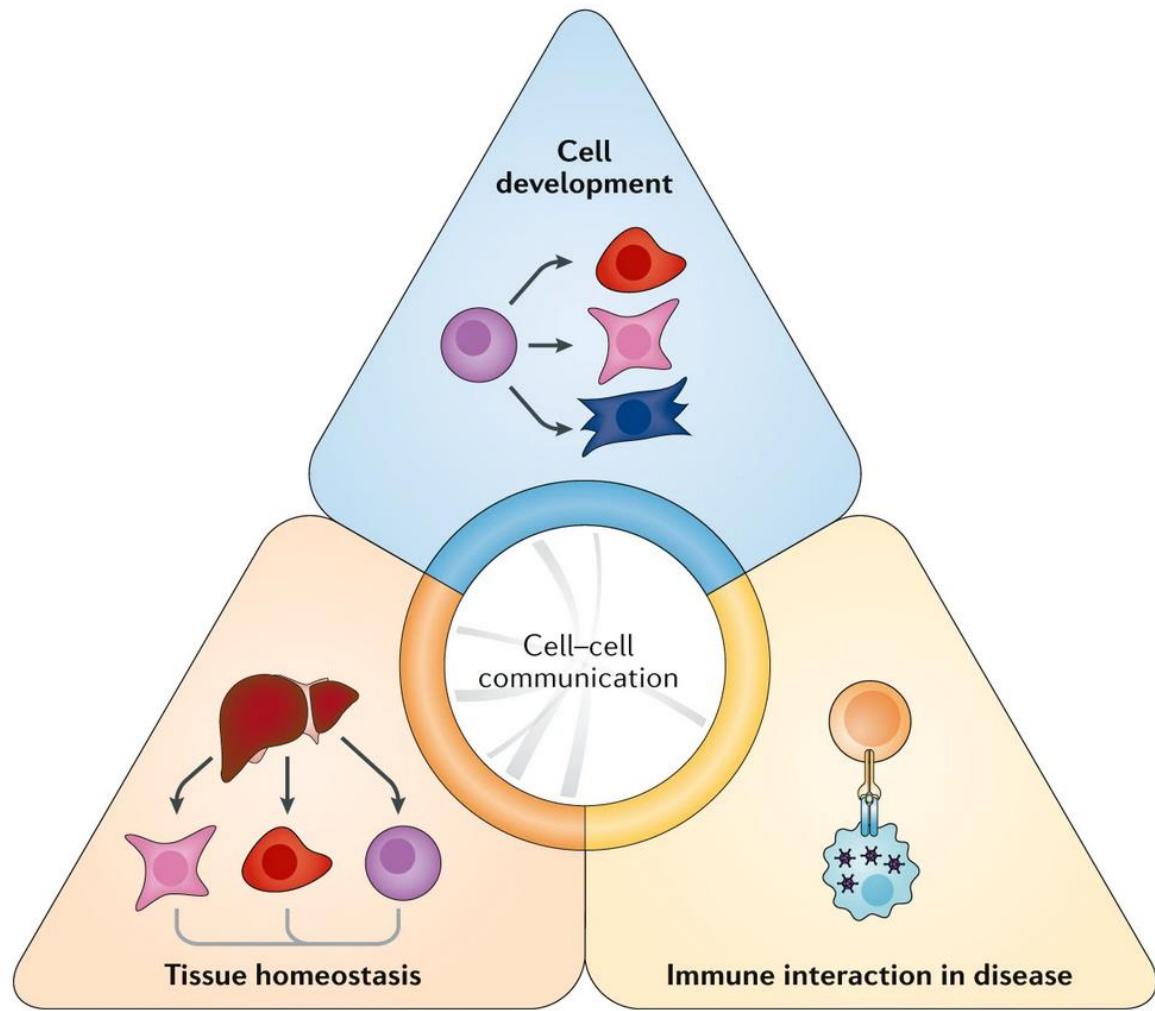
Content

- Cell-Cell Communication (CCC)
 - How to study CCC
 - Ligand-Receptor Inference
- NicheNet
 - Concept
 - Performance
 - Evaluation
 - Limitations
 - How to use

SCIENCE MEETS LIFE

Cell-Cell Communication (CCC)





How to study CCC

- Direct measurement of protein-protein interactions/proteomics

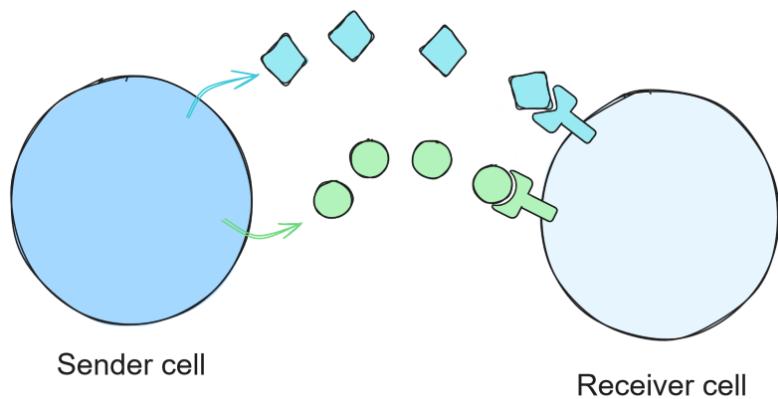
How to study CCC

- Direct measurement of protein-protein interactions/proteomics
- (single-cell) RNA → hypothesis generation

How to study CCC

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LR Tools

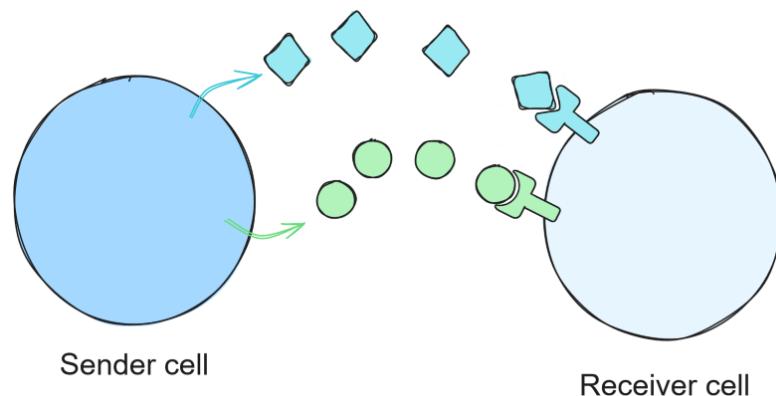


Ligand-receptor inference

How to study CCC

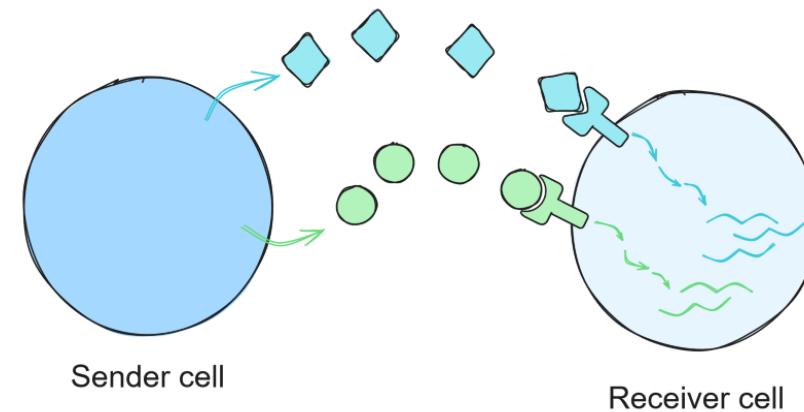
- Direct measurement of protein-protein interactions/proteomics
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LR Tools



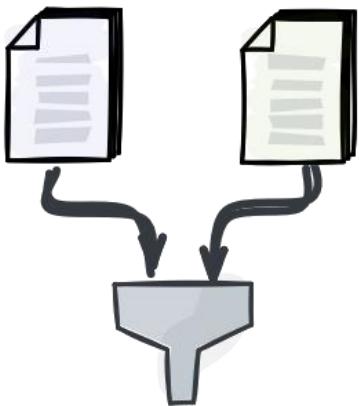
Ligand-receptor inference

NicheNet



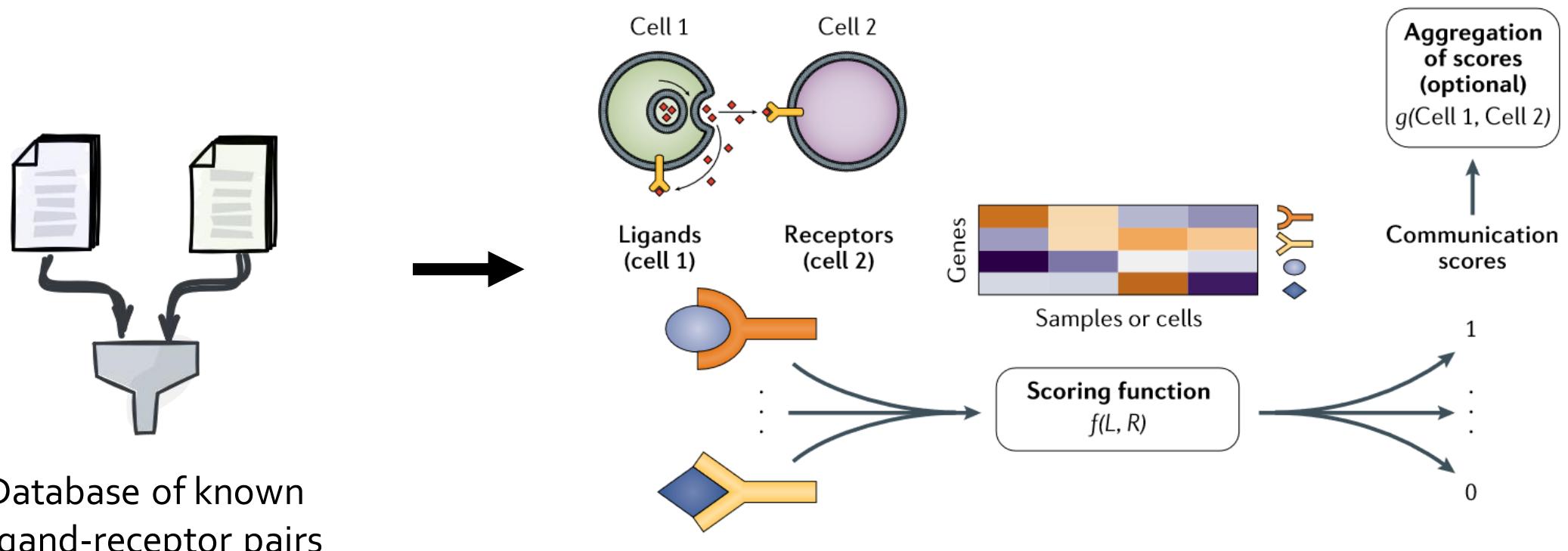
Ligand-target inference

LR inference tools such as CellChat and CellPhoneDB calculate the **co-expression** of LR pairs



Database of known
ligand-receptor pairs

LR inference tools such as CellChat and CellPhoneDB calculate the **co-expression** of LR pairs



Armingol et al. *Nat Rev Genet* 22, 71–88 (2021)

LR inference tools

- Advantages
 - Easy to use
 - Extensive overview of all expressed LR pairs

LR inference tools

- Advantages
 - Easy to use
 - Extensive overview of all expressed LR pairs
- Limitations
 - Ignores downstream effect in receiver cell
 - Co-expression of LR pairs \neq physical interaction
 - RNA might not reflect protein secretion of ligands and receptors
 - Extensive list of potential interactions

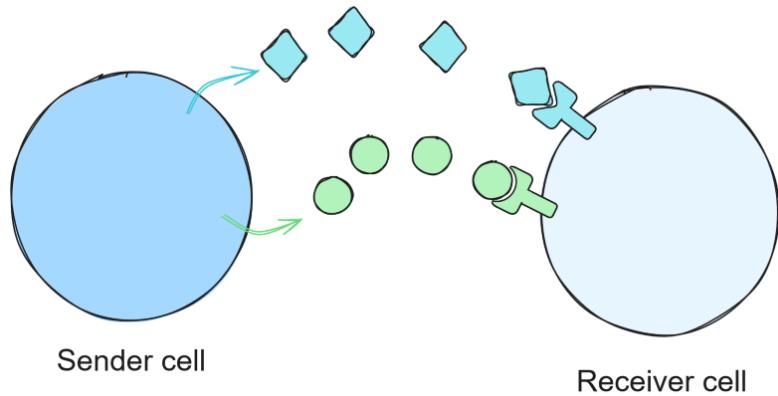
NicheNet

<https://github.com/saeyslab/nichennetr>



Browaeys, R., Saelens, W. & Saeys, Y. NicheNet: modeling intercellular communication by linking ligands to target genes. *Nat Methods* **17**, 159–162 (2020). <https://doi.org/10.1038/s41592-019-0667-5>

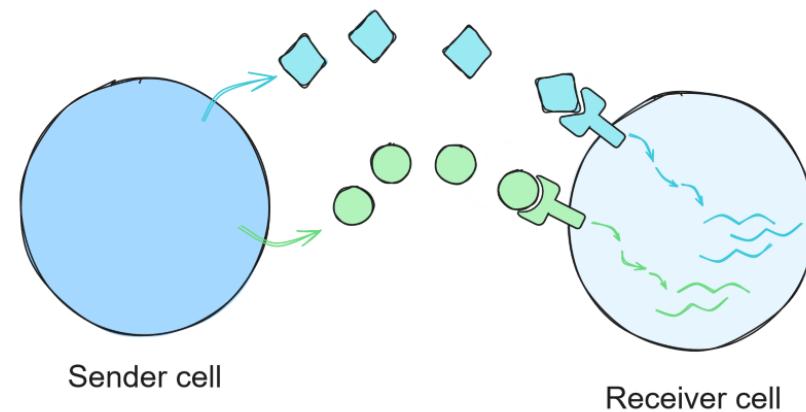
LR Tools



Ligand-receptor inference

Which known LR pairs are expressed?

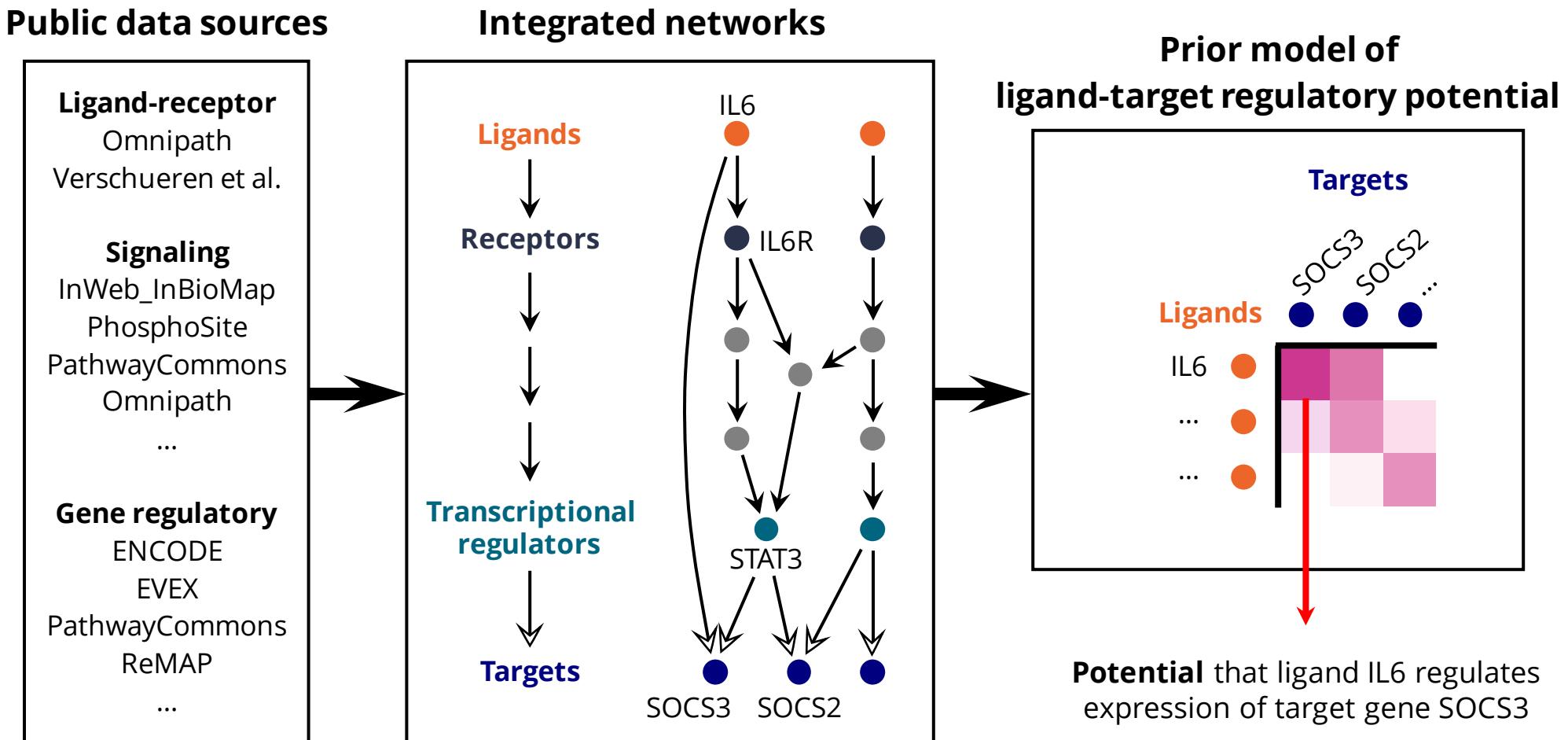
NicheNet



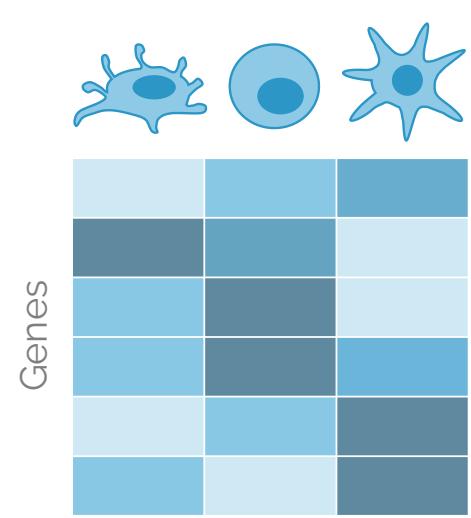
Ligand-target inference

Which known LR pairs show signaling and target gene enrichment in receivers?

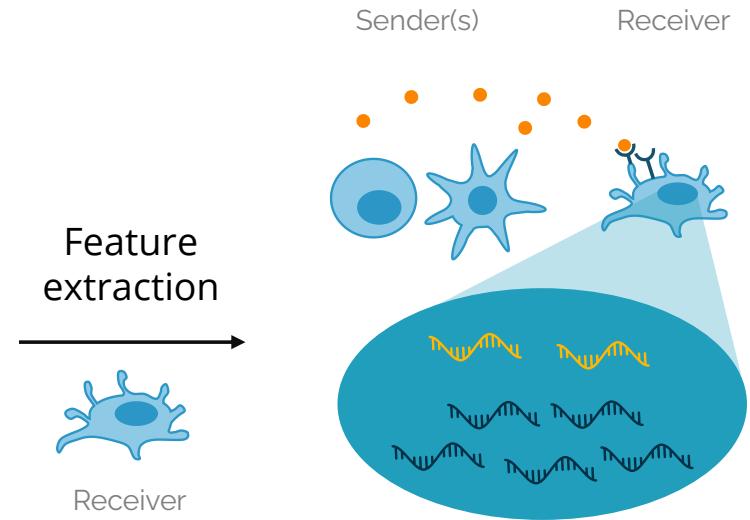
Target genes are probabilistically linked to **ligands** based on **data-integration** of public data sources



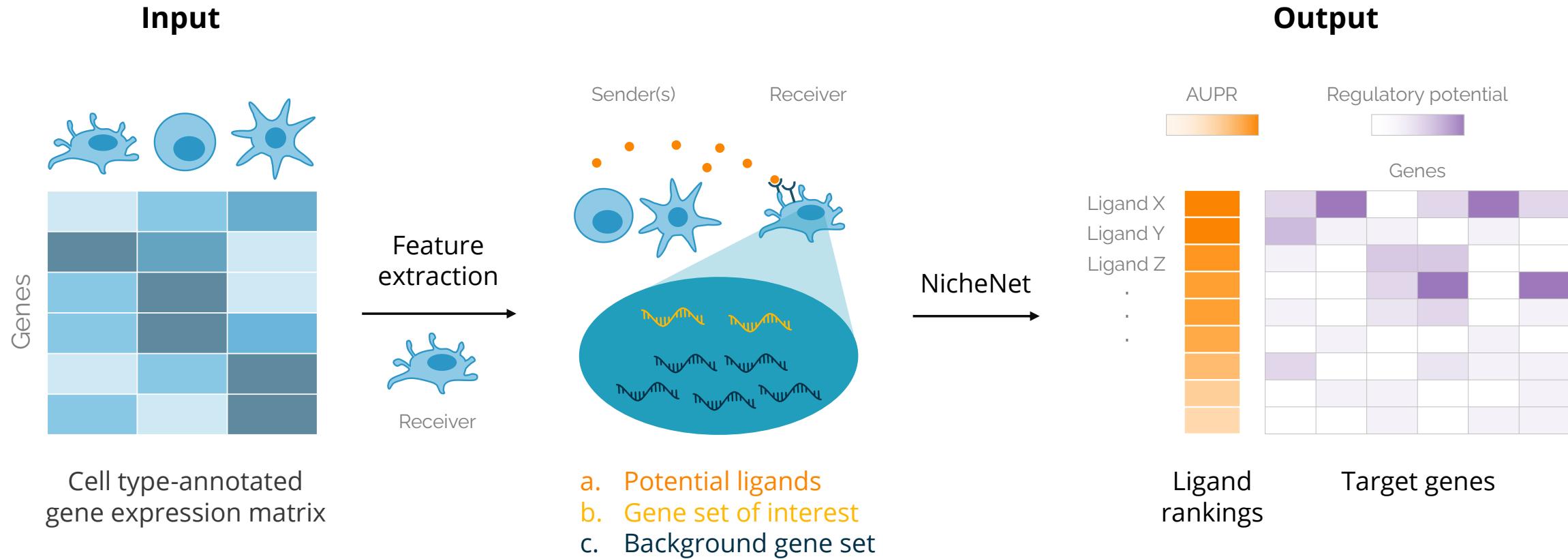
Input

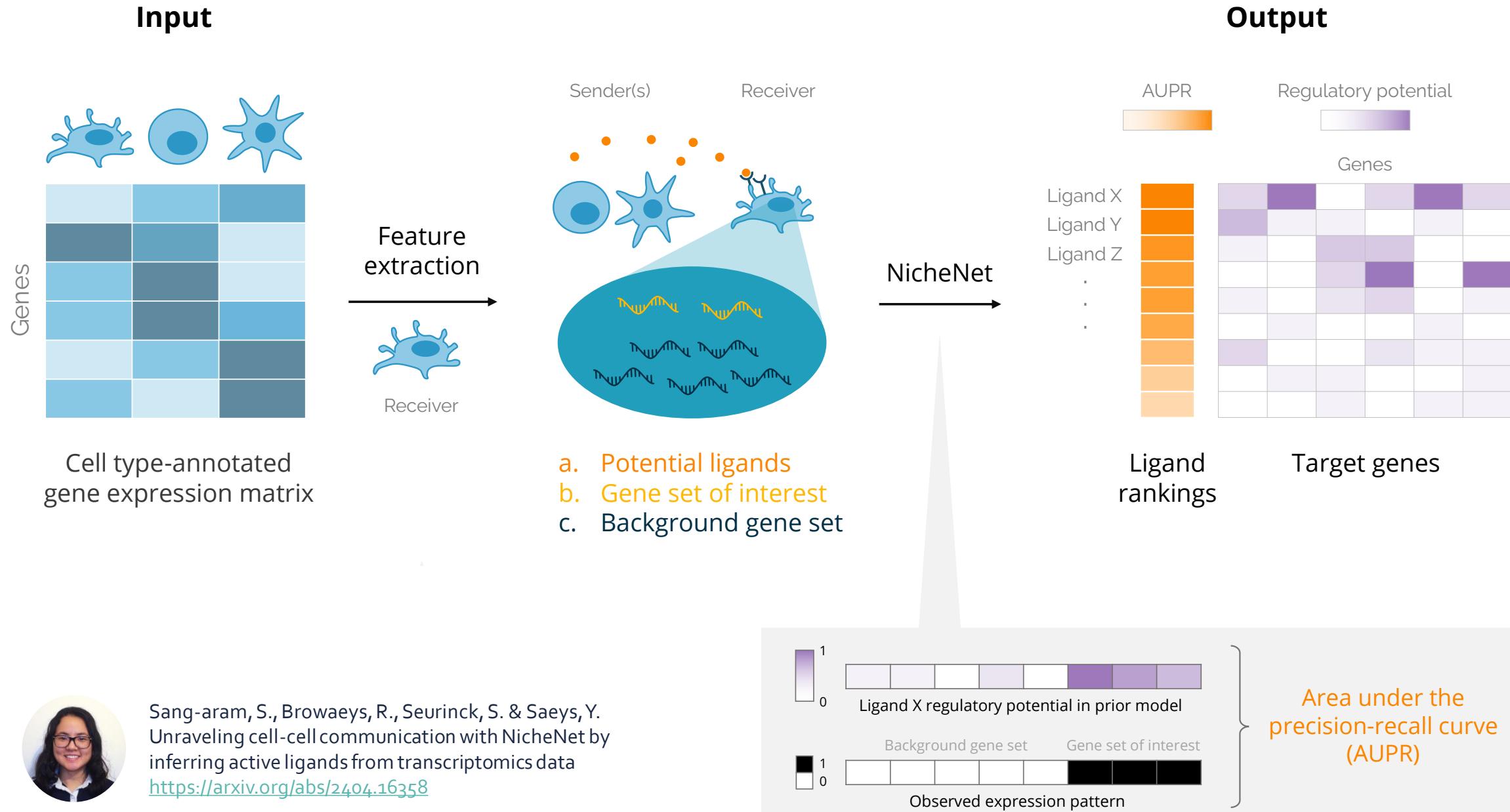


Cell type-annotated
gene expression matrix



- a. Potential ligands
b. Gene set of interest
c. Background gene set





NicheNet is ...

NicheNet is ...

... an enrichment tool!

NicheNet is ...

... an enrichment tool!

Is the Gene set of Interest (GSOI) enriched
in the (sc)RNA-seq data compared to the
background gene set?

What affects the ligand-target potential?

The ligand-target potential is the supporting evidence in the prior model that a specific **ligand** can regulate a given **target gene**

What affects the ligand-target potential?

Literature

e.g. Pubmed abstract:
ligand A regulates
expression of gene B

What affects the ligand-target potential?

Literature

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**Ligand treatment
databases**

CytoSig

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Ligand treatment databases

CytoSig

Signal propagation

- $L \rightarrow R \rightarrow$ TF path
- Genes regulated by the TF
(e.g. from ChIP-seq or
TF perturbation experiments)

What affects the ligand-target potential?

Literature

e.g. Pubmed abstract:
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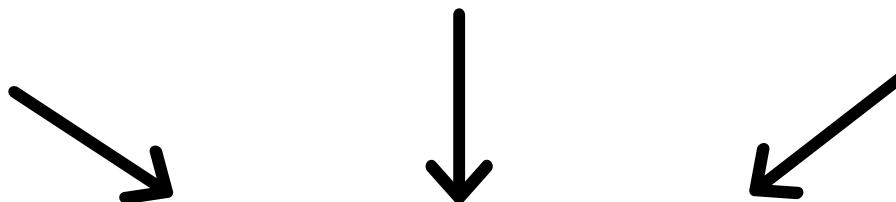
Ligand treatment databases

CytoSig

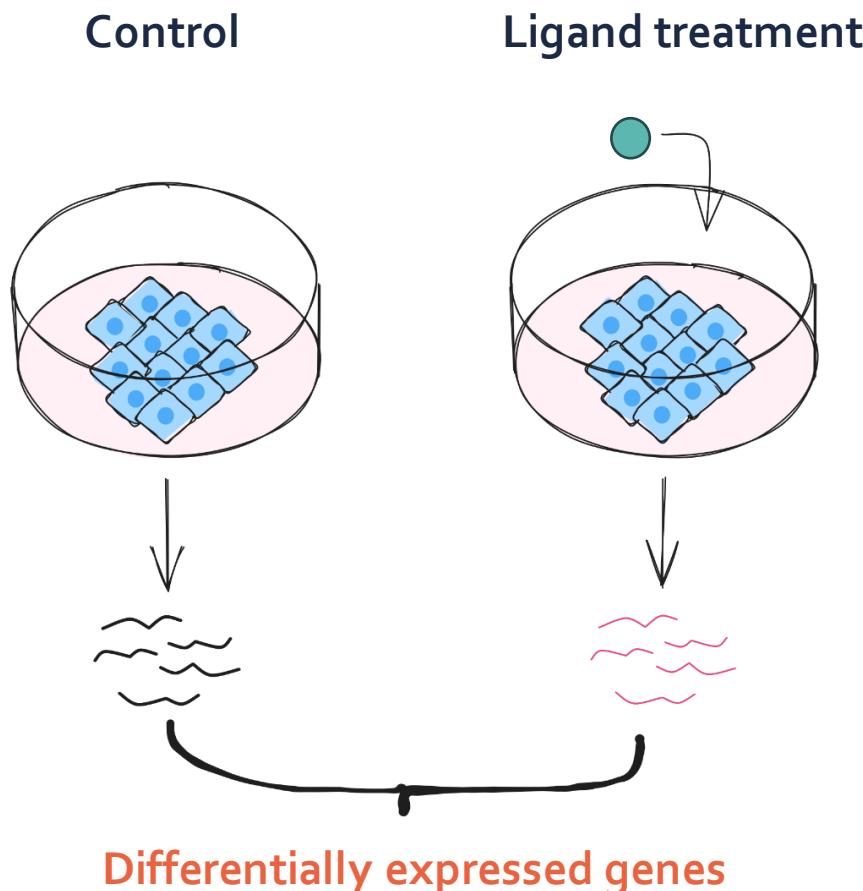
Signal propagation

- $L \rightarrow R \rightarrow$ TF path
- Genes regulated by the TF
(e.g. from ChIP-seq or
TF perturbation experiments)

Combinations will score highest



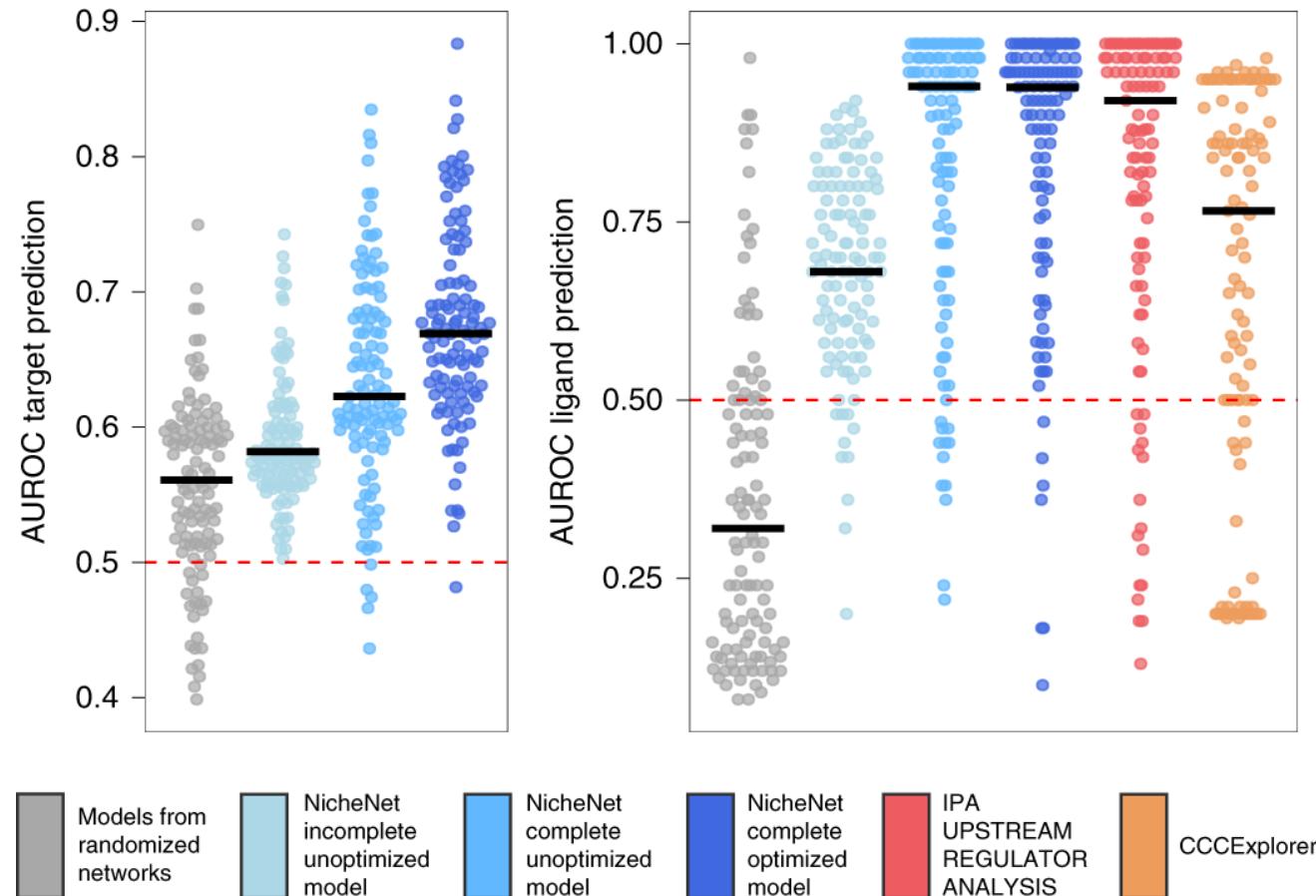
How well does NicheNet work?



Evaluation metrics

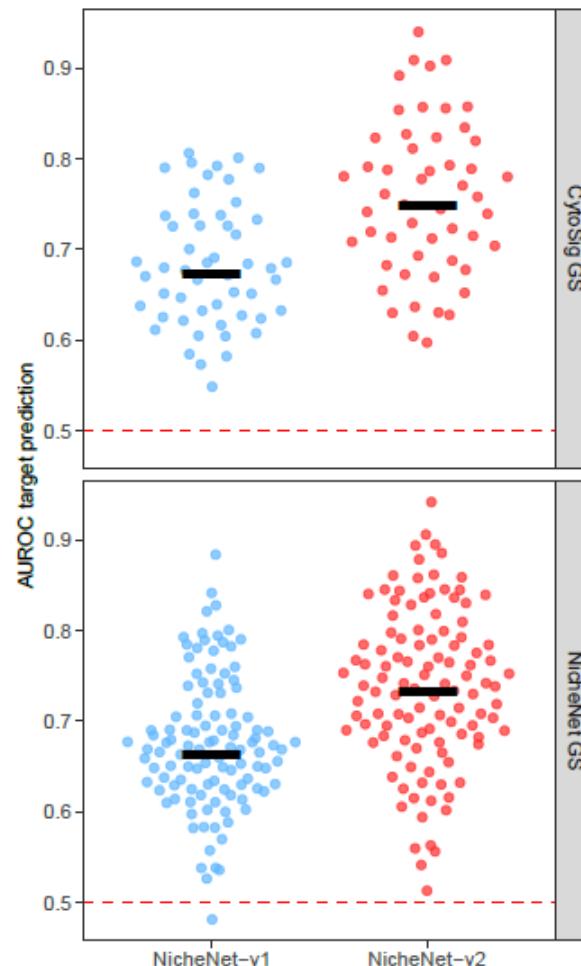
1. **Target prediction:** Given the **ligand**, can NicheNet predict the **DE genes**?
2. **Ligand prediction:** Given the **DE genes**, can NicheNet predict the correct **ligand**?

How well does NicheNet work?

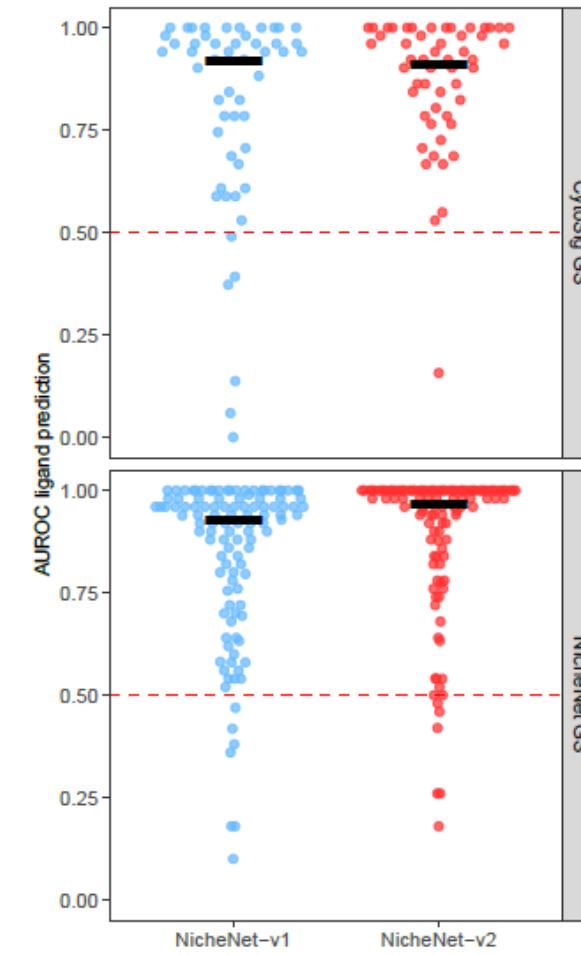


How well does NicheNet work?

a) Target gene prediction performance



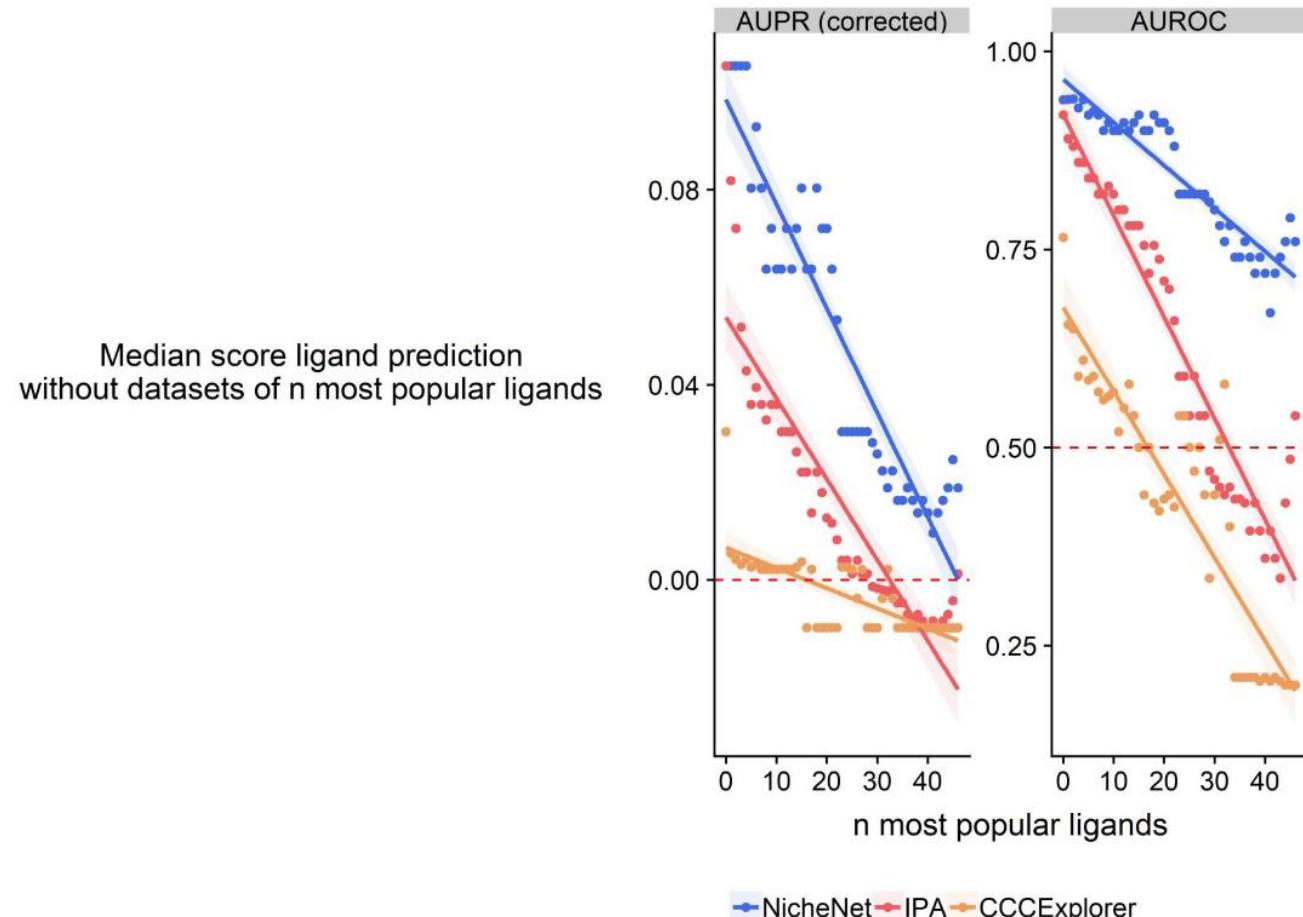
b) Ligand activity prediction performance



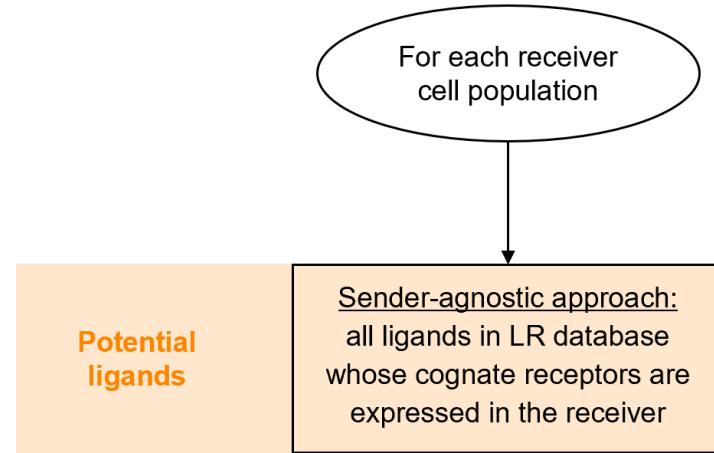
Limitations

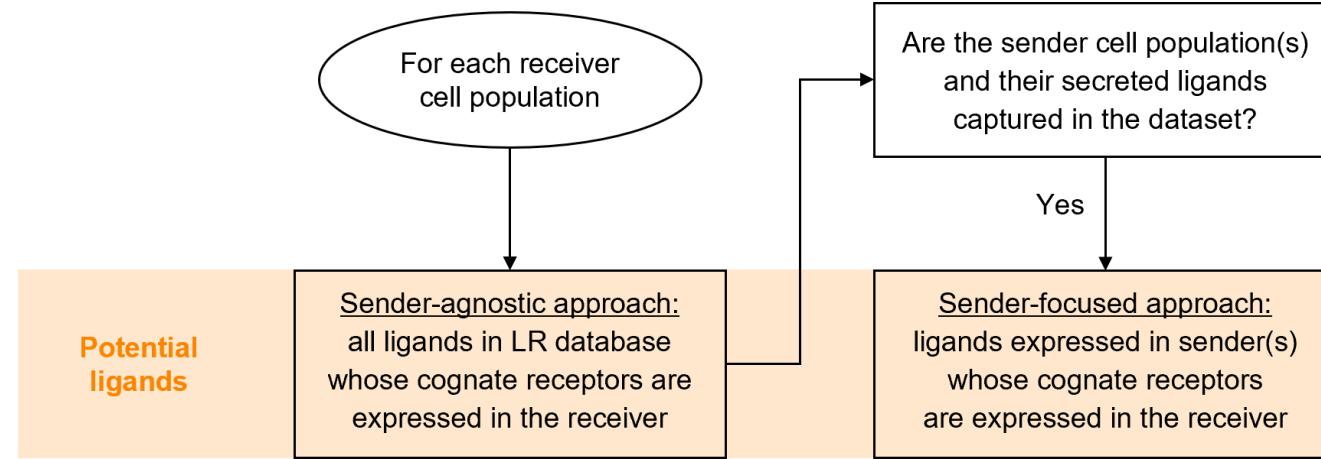
- No context-specificity – general database
- Pathway crosstalk, combinatorial effects,...
- Popularity bias

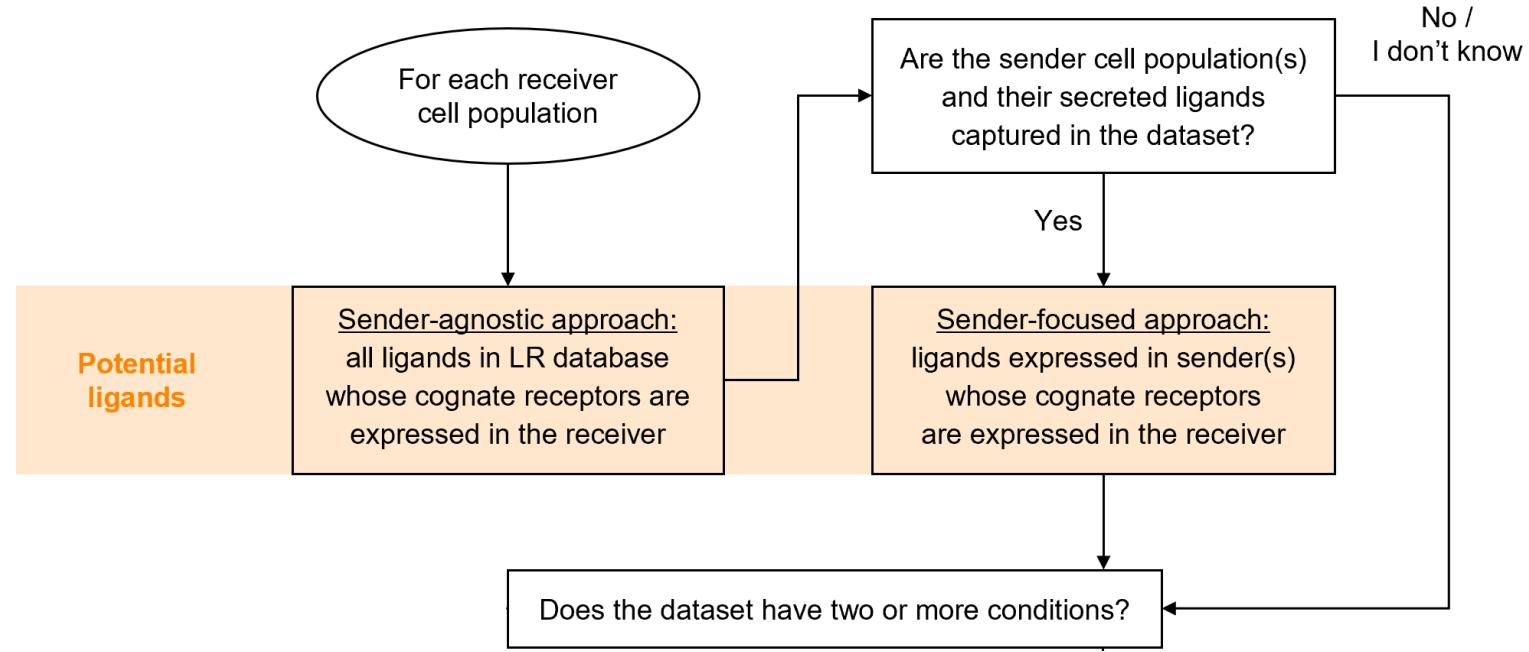
Popularity bias

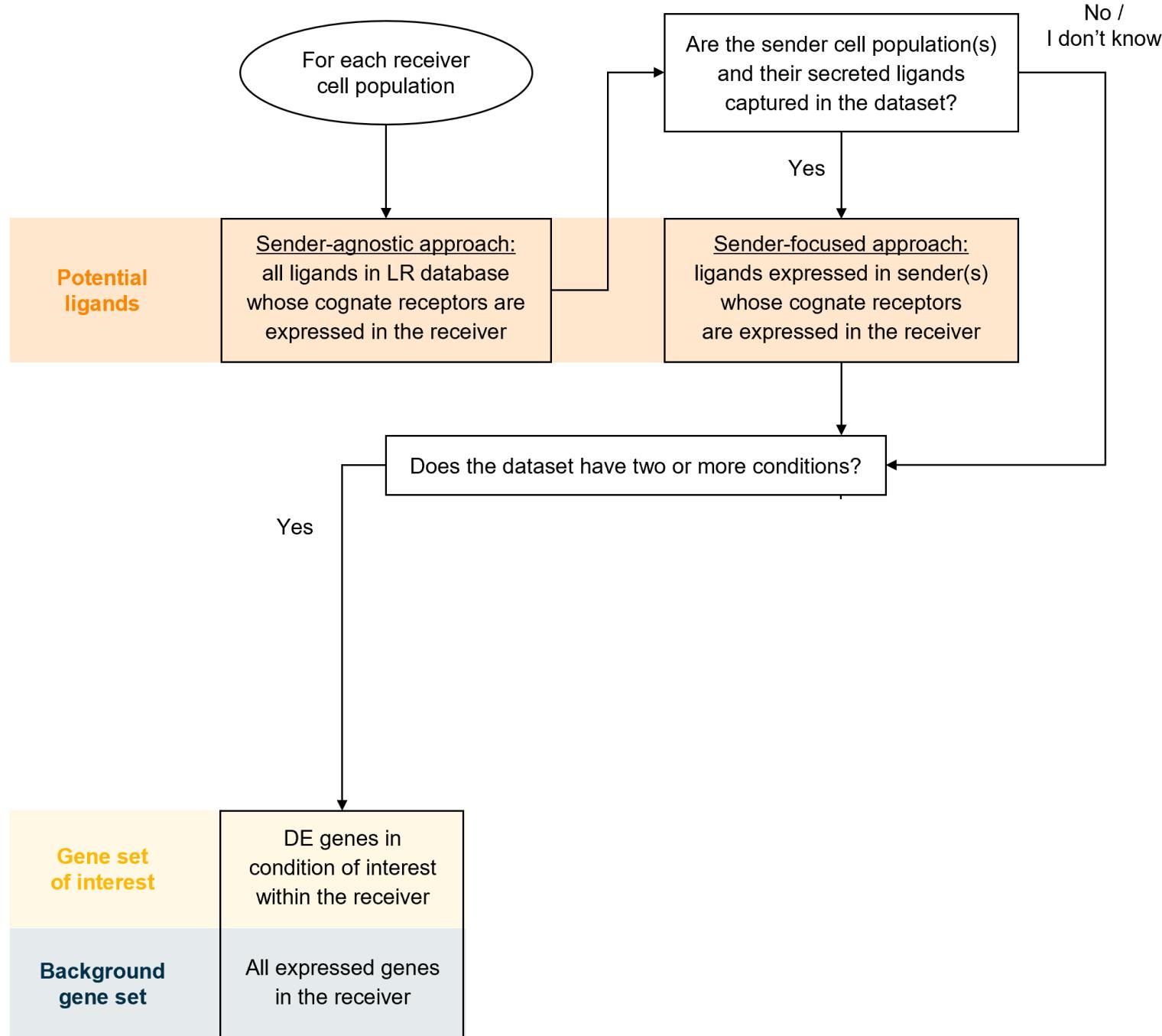


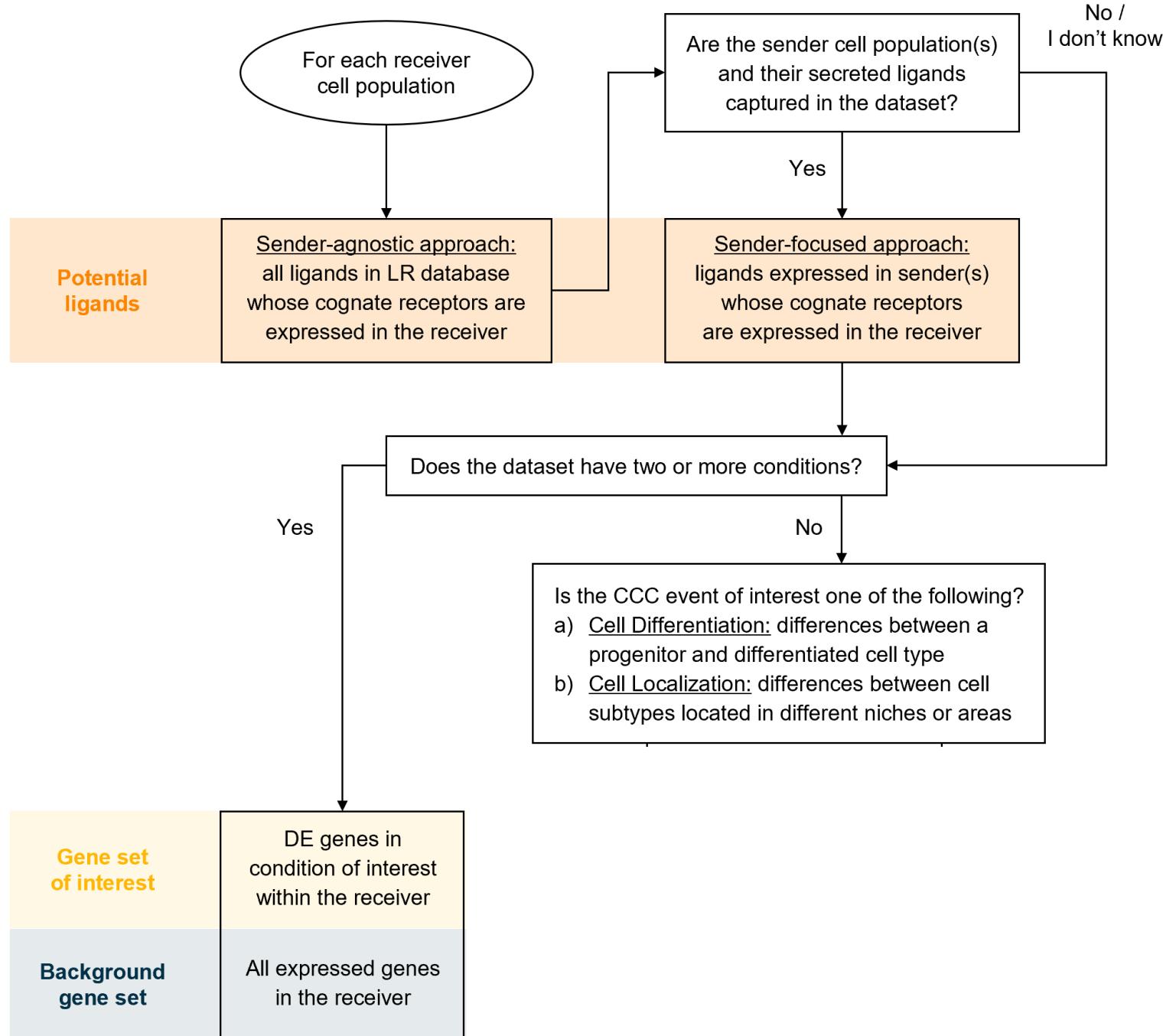
How and when to use NicheNet

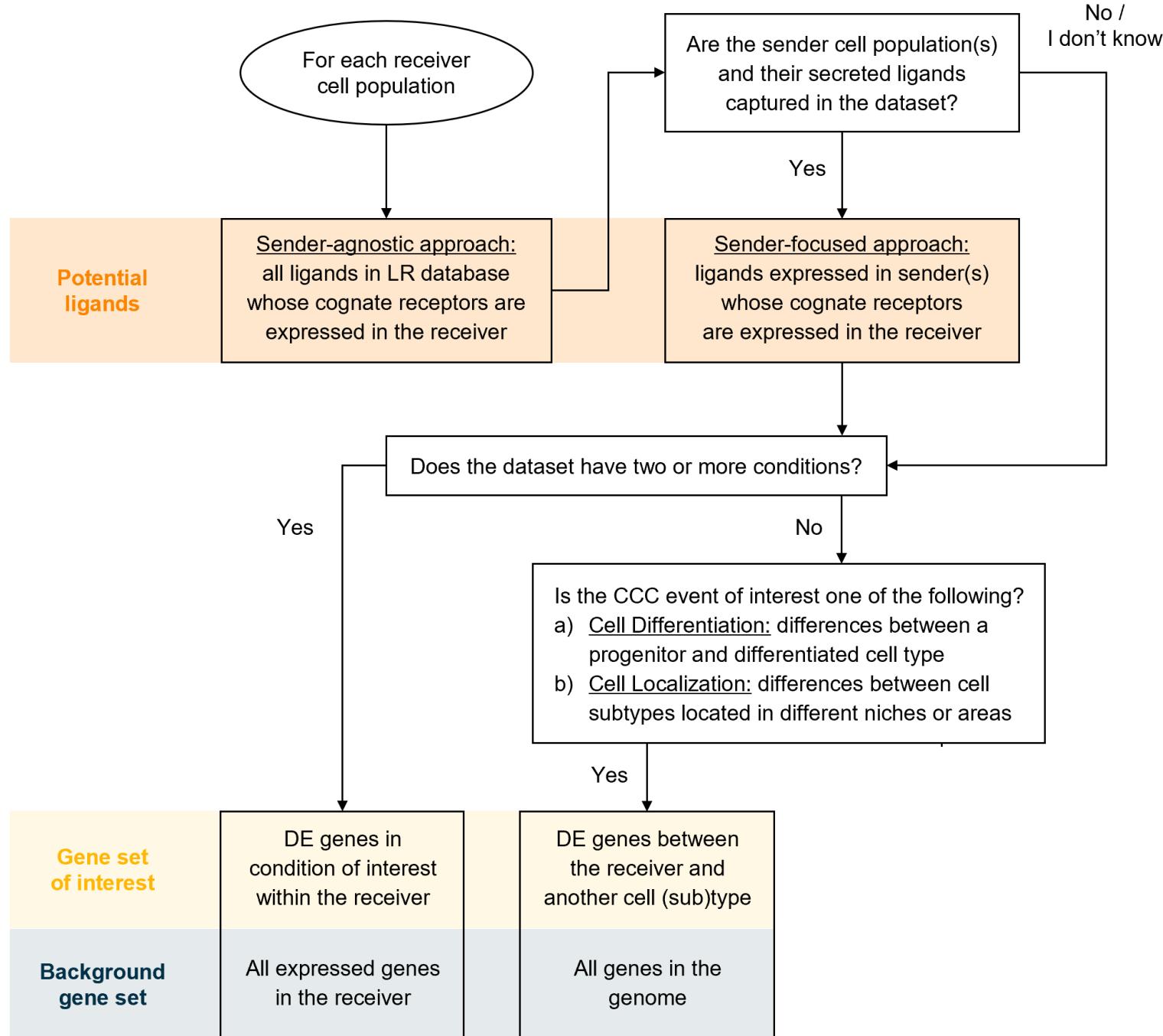


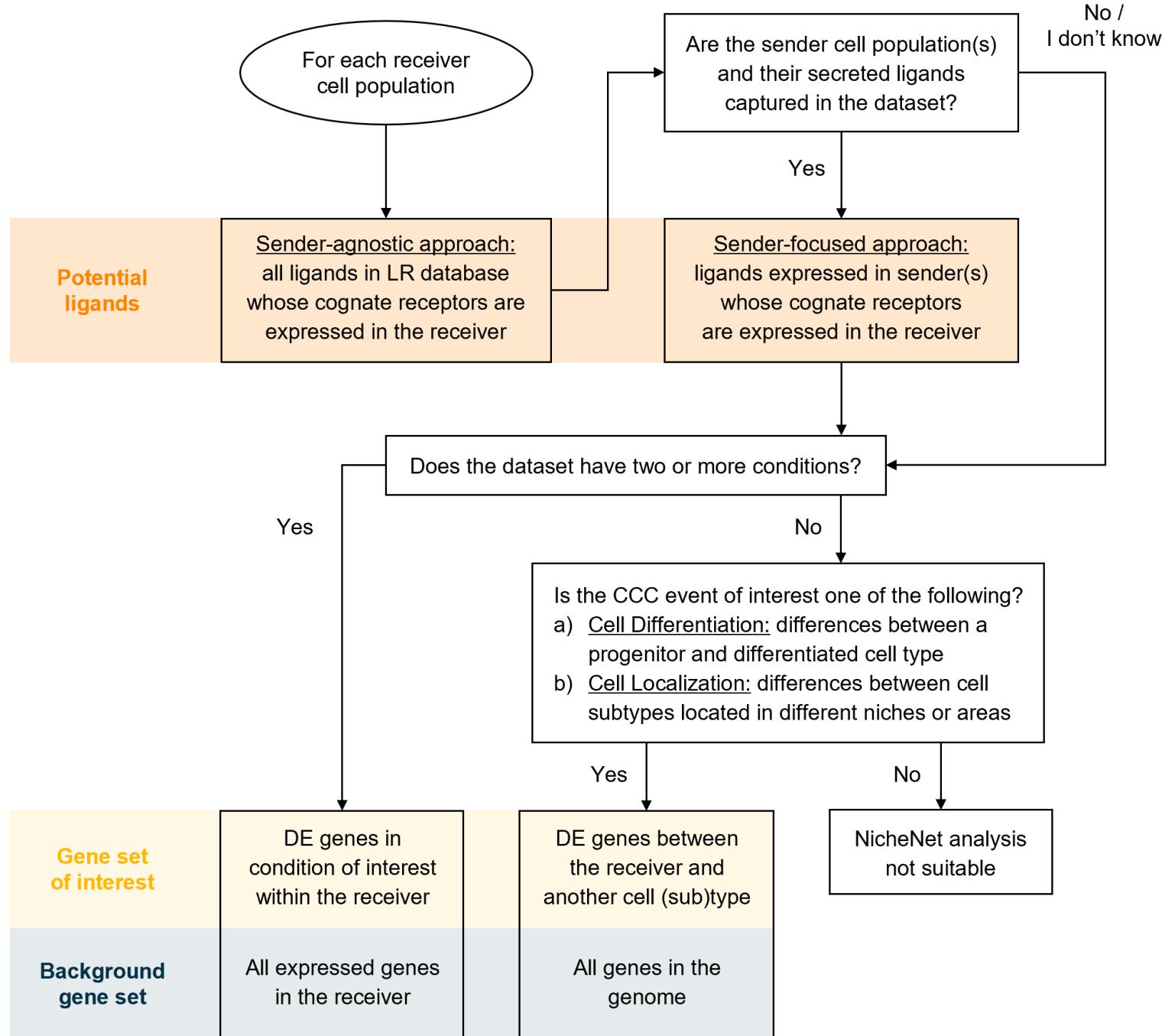












When will NicheNet (not) work?

- Does the prior model cover your signaling pathway of interest?

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- How precise can you define the GSOI using the RNA profile?
Preferably 0,05% to 10% of the background gene set

When will NicheNet (not) work?

- Does the prior model cover your signaling pathway of interest?
- Is (sc)RNA a reasonable proxy for protein secretion of potential LR pairs? Sender-agnostic model?
- How precise can you define the GSOI using the RNA profile?
Preferably 0,05% to 10% of the background gene set
- How much noise (e.g. doublets, ambient RNA, ...) does your data contain?

What do we hope to teach you?

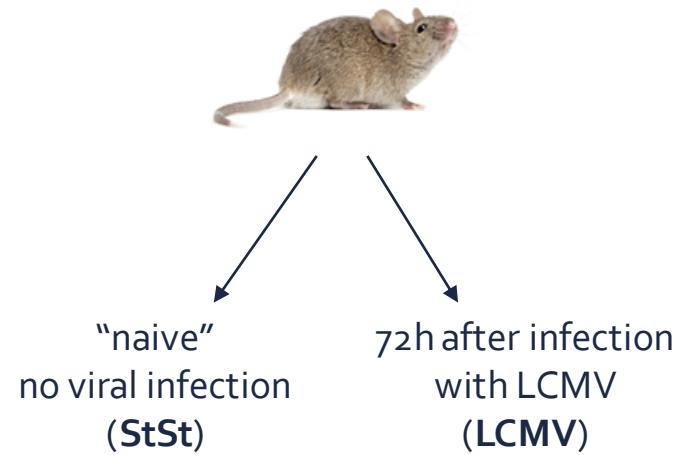
What you hopefully take home ...

-  You understand how NicheNet works
-  You can assess if NicheNet is suited to study CCC in your data
-  You can adjust the NicheNet workflow to accommodate your research question,
 - Potential ligands?
 - Gene set of interest?

Application: Case-control

*Studying immune cell interactions in
lymph nodes after viral infection*

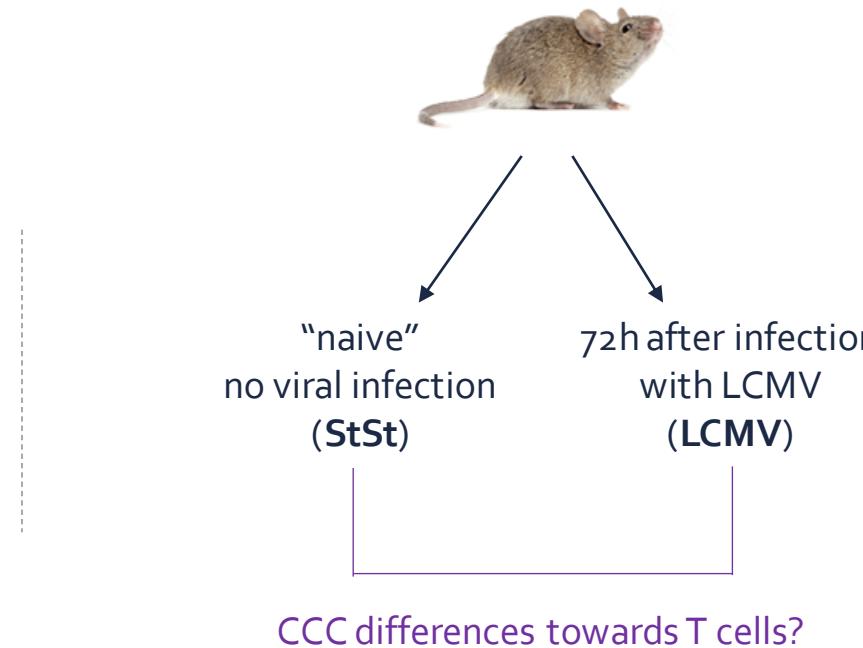
scRNAseq data
from T cell area in
inguinal lymph node



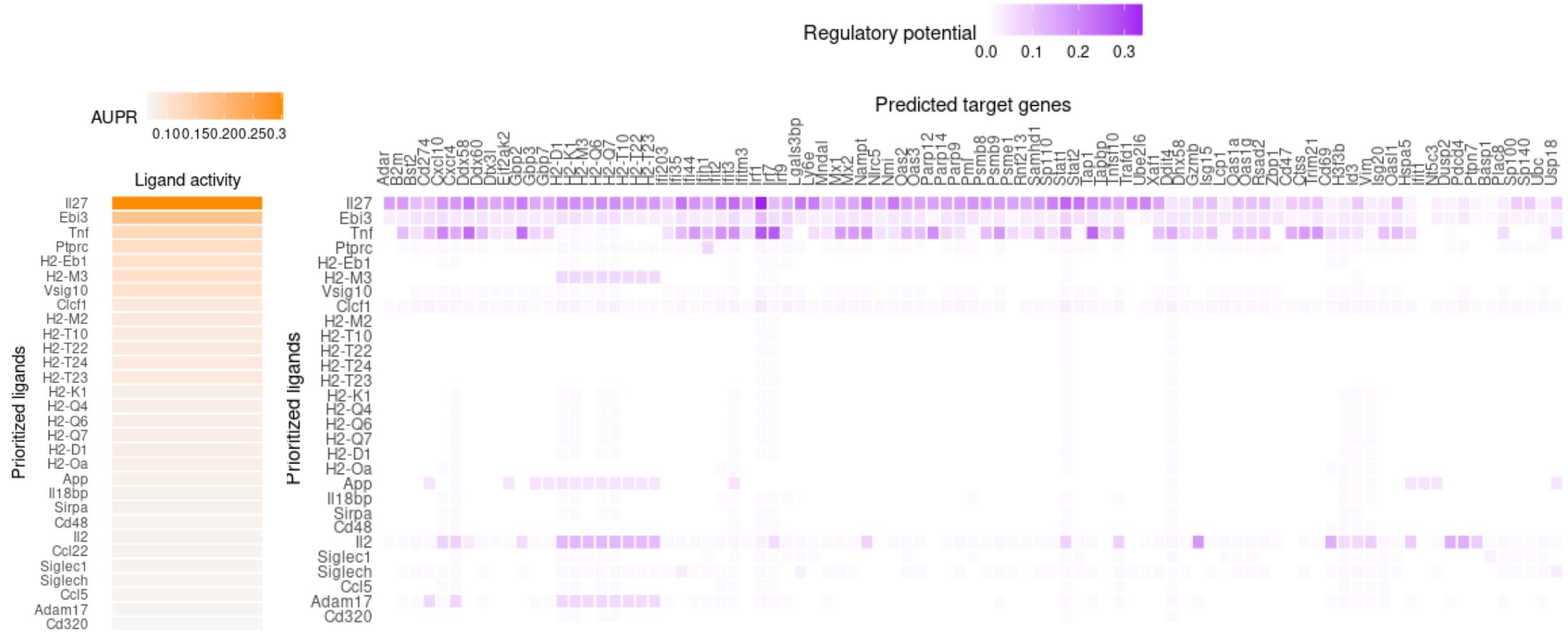
Research question:

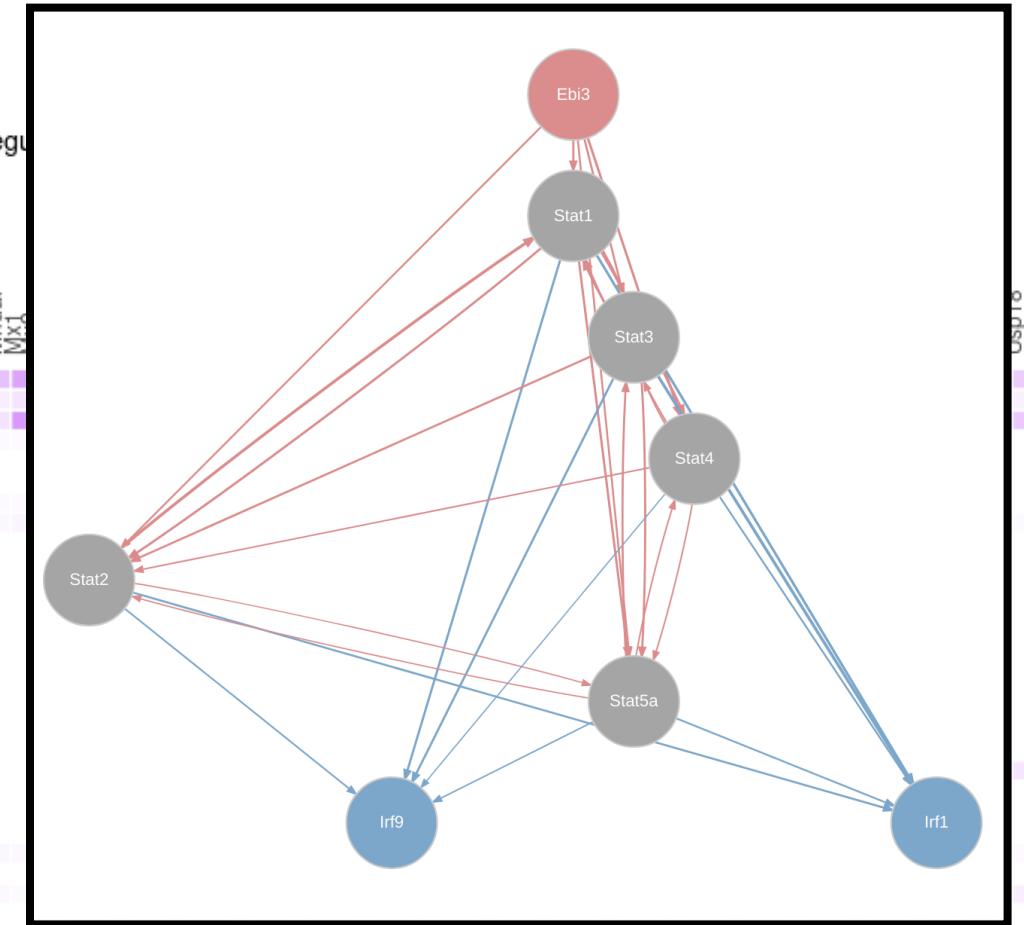
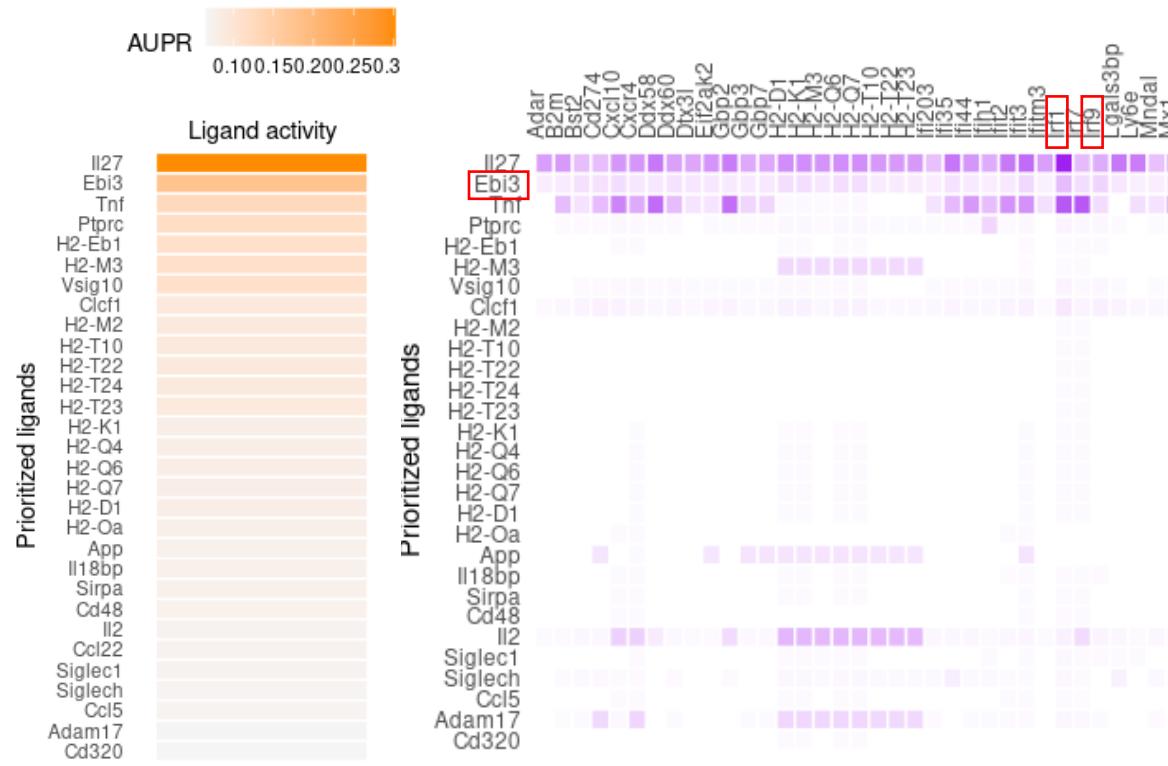
What are the most important interactions driving gene expression differences in T cells after LCMV infection?

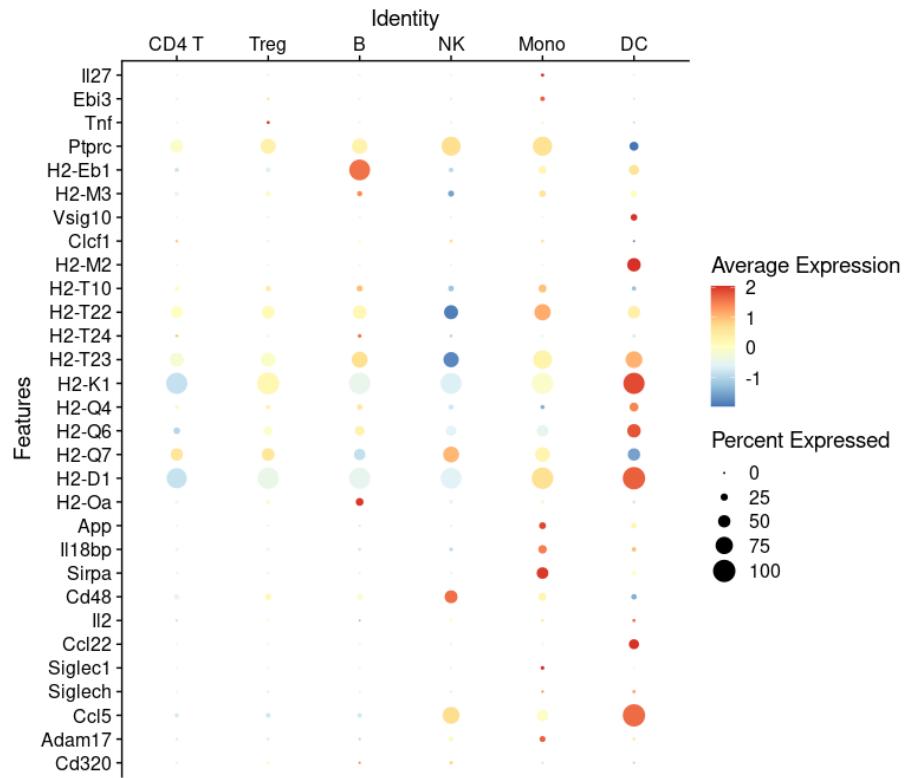
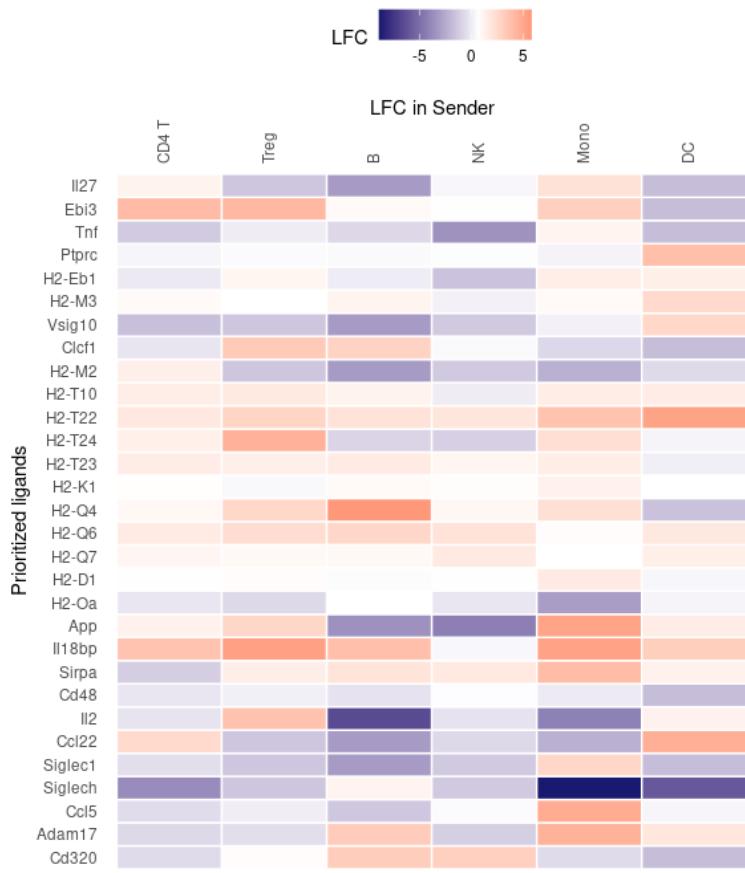
scRNAseq data
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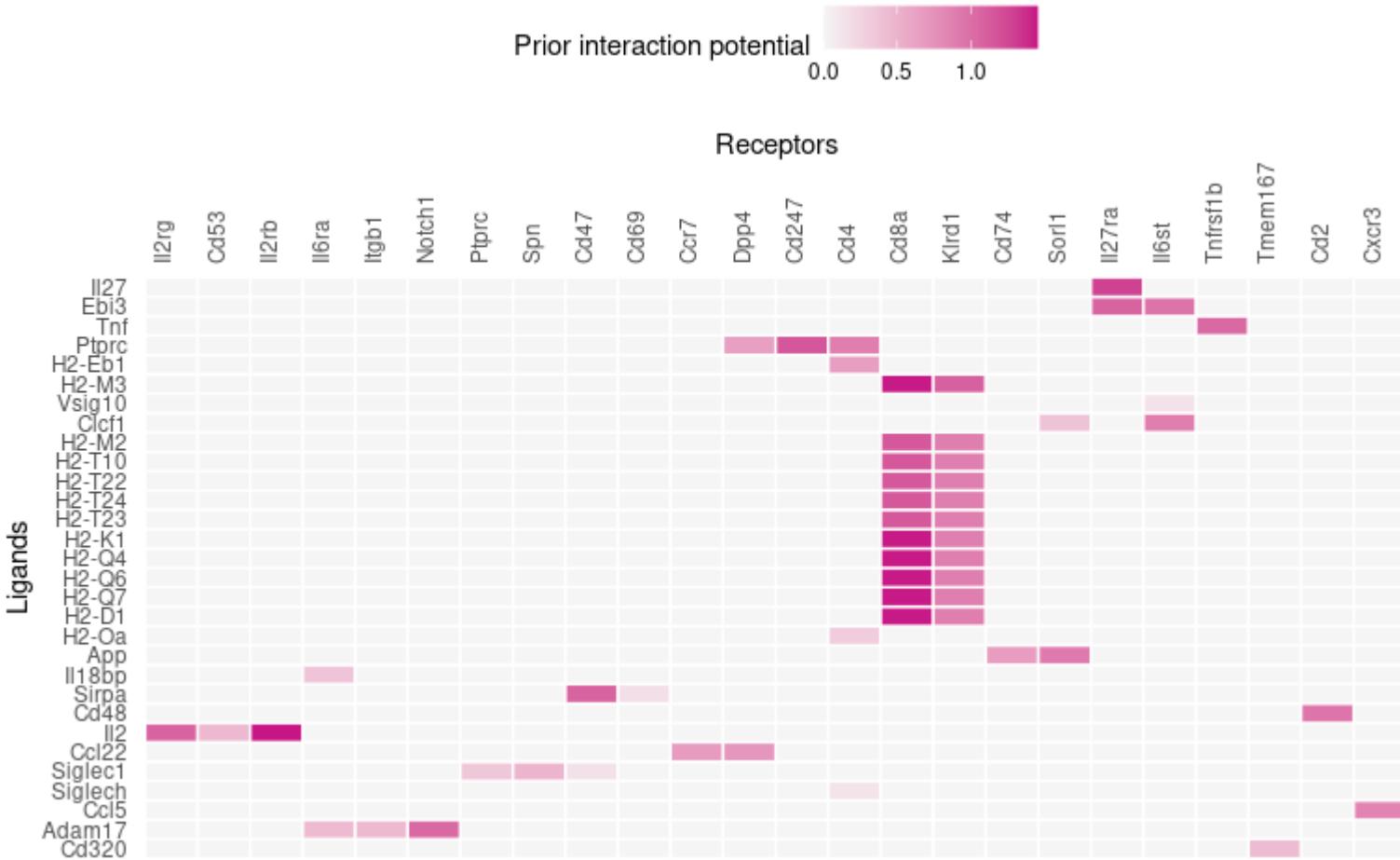


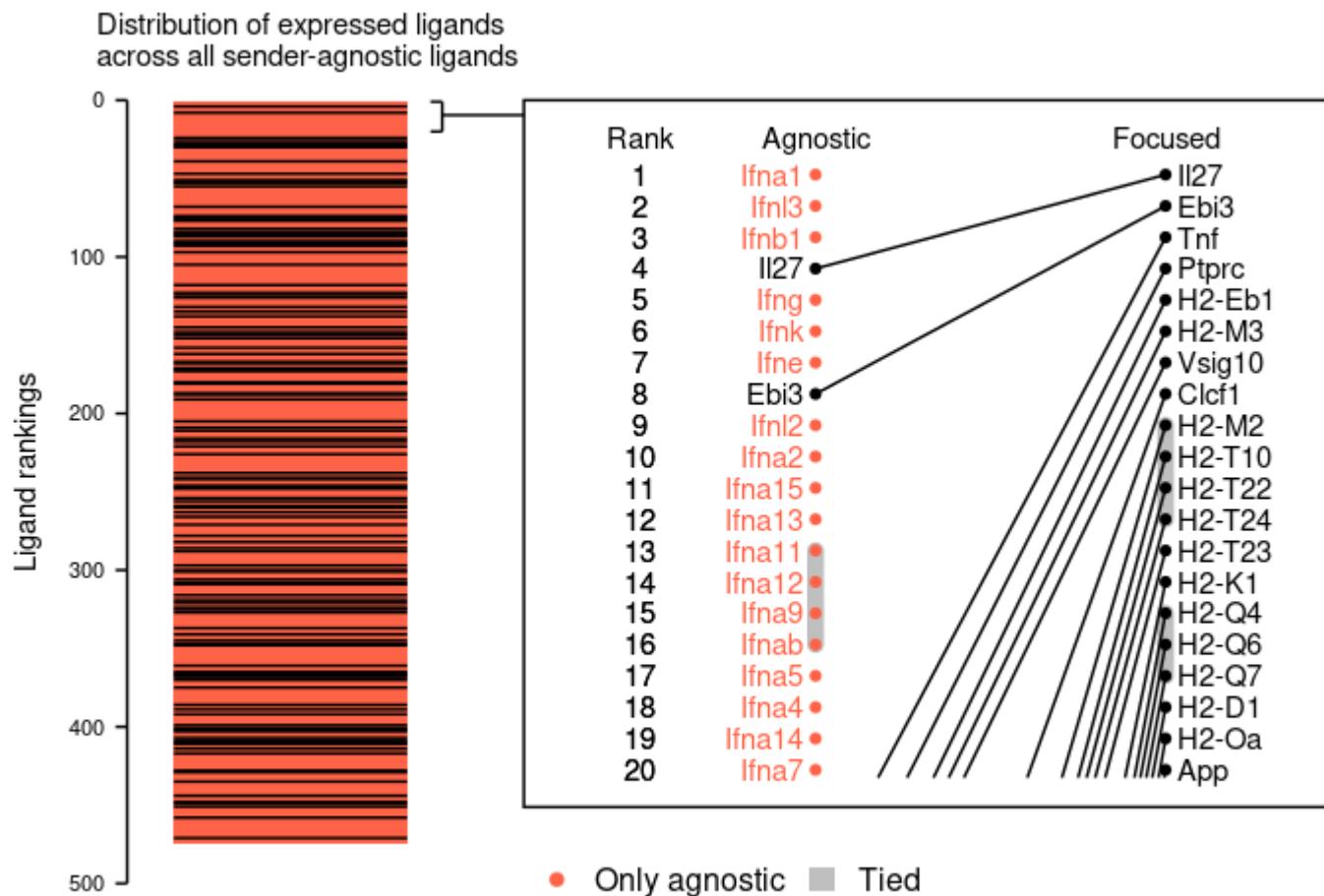
Results & Visualization

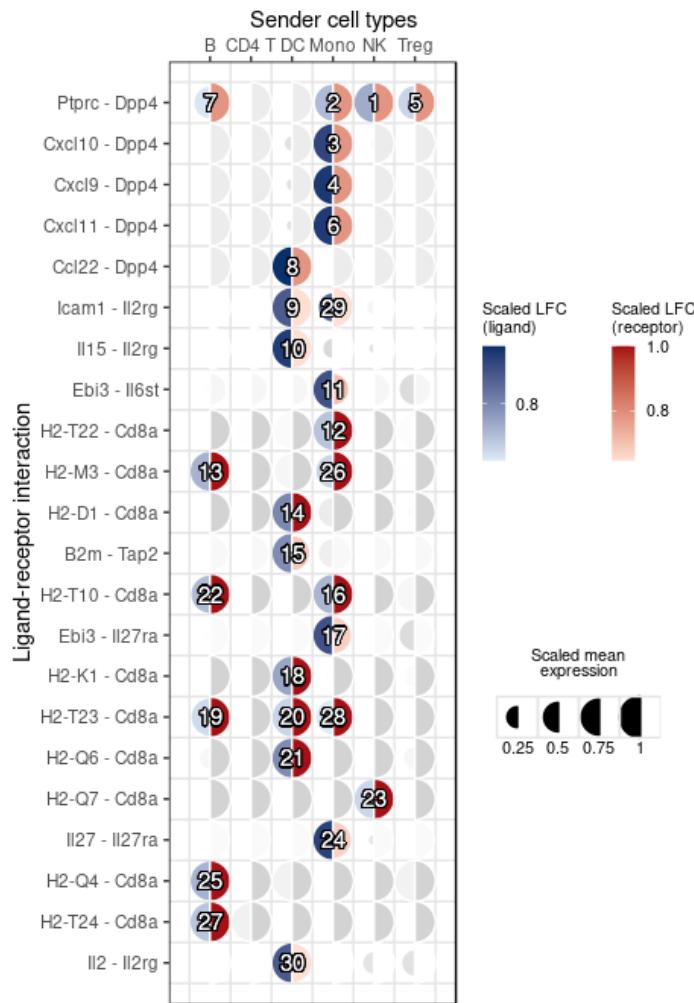








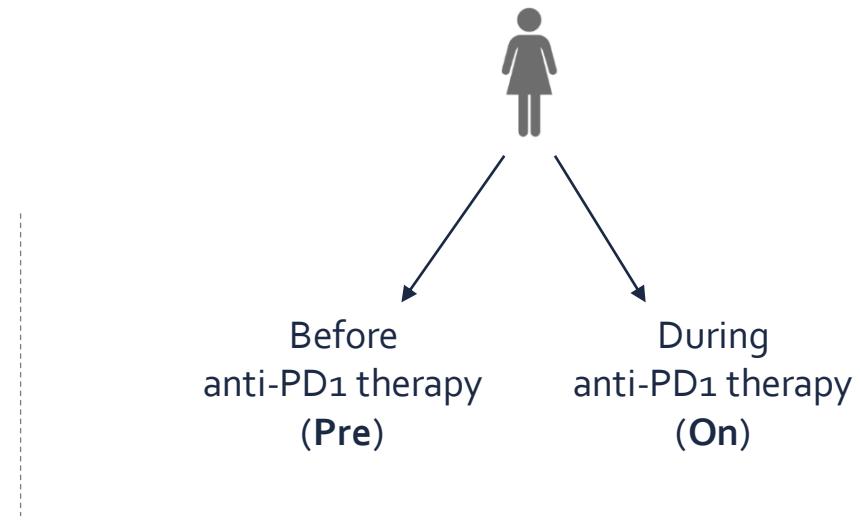




Application: Case-control

*Studying TME interactions in the context of
anti-PD1 immunotherapy*

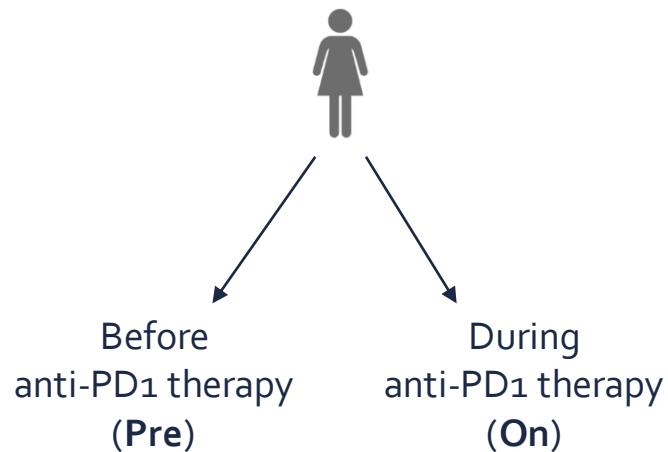
scRNAseq data:
Point of sampling



Patient group:
Anti-PD1
therapy response

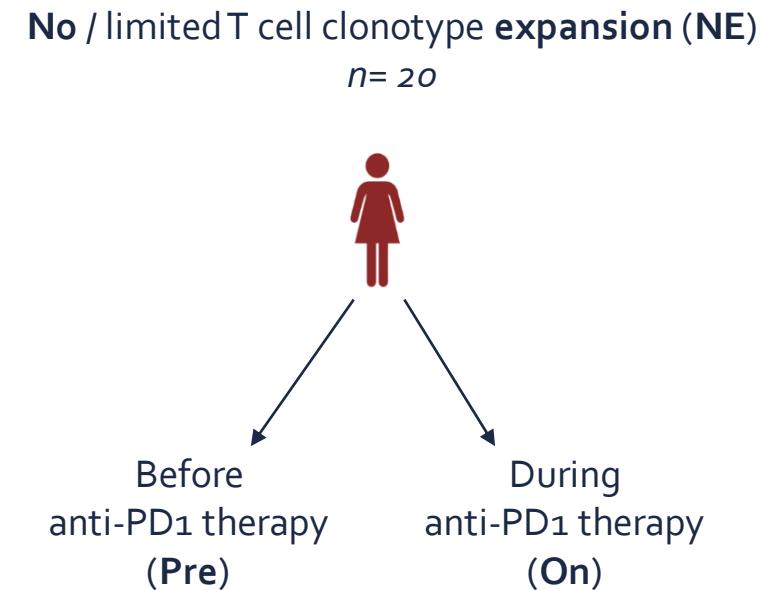
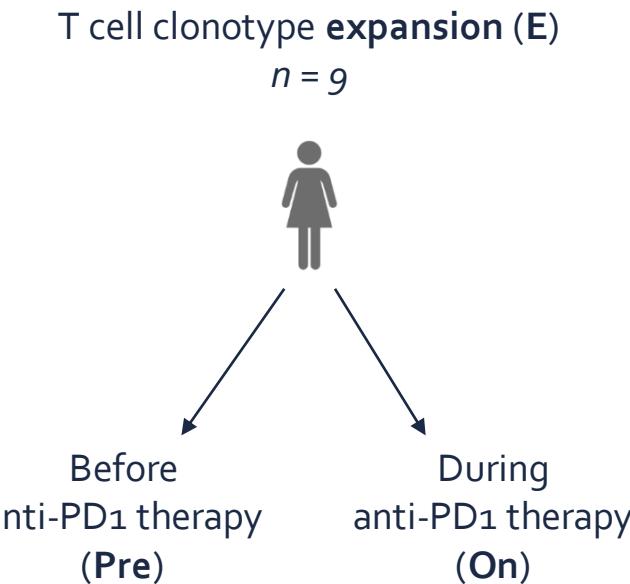
scRNAseq data:
Point of sampling

T cell clonotype expansion (E)



Patient group:
Anti-PD1
therapy response

scRNAseq data:
Point of sampling



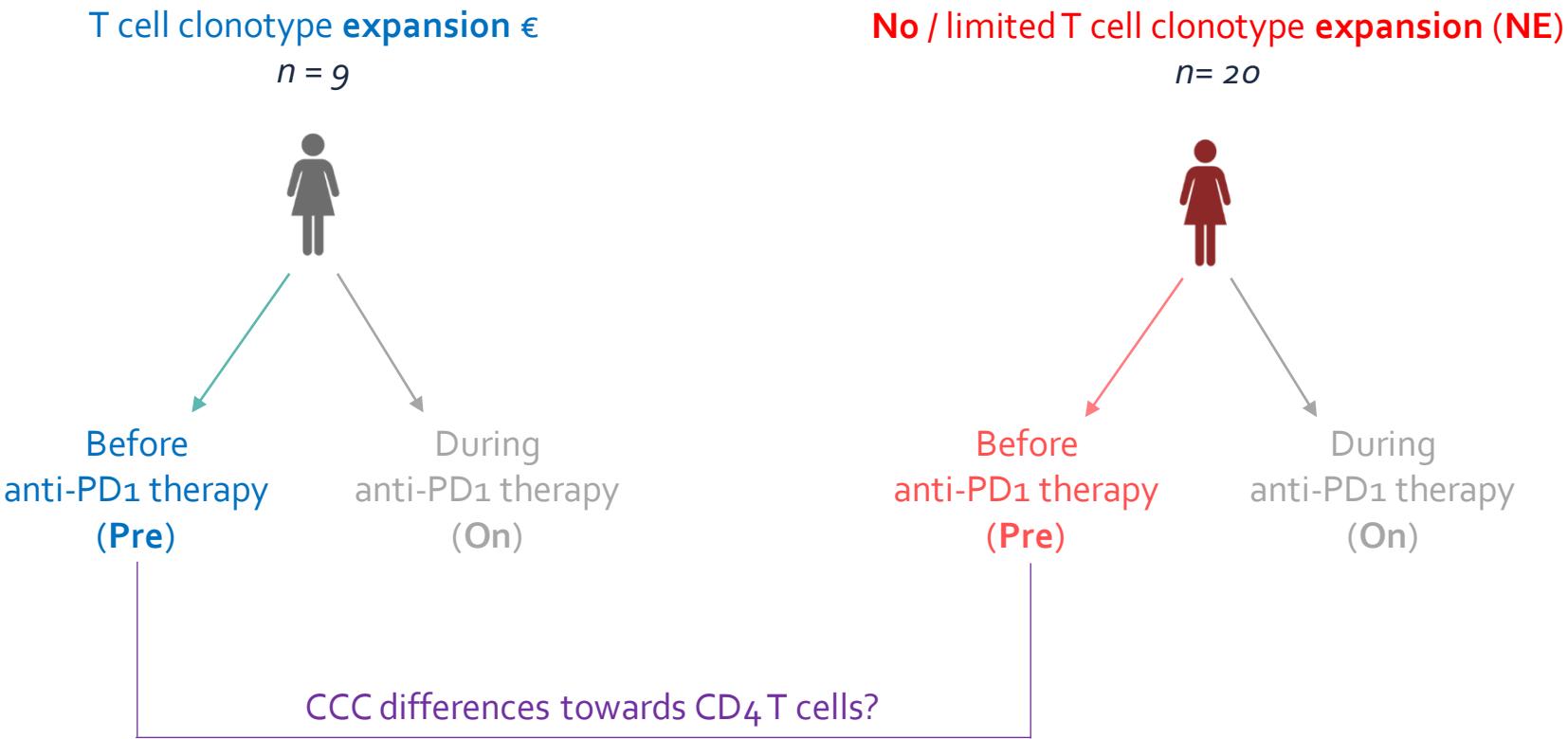
Research question:

What are the most important interactions driving pre-therapy differences in CD4 T cells between expander and non-expander patients?

Patient group:
Anti-PD1
therapy response

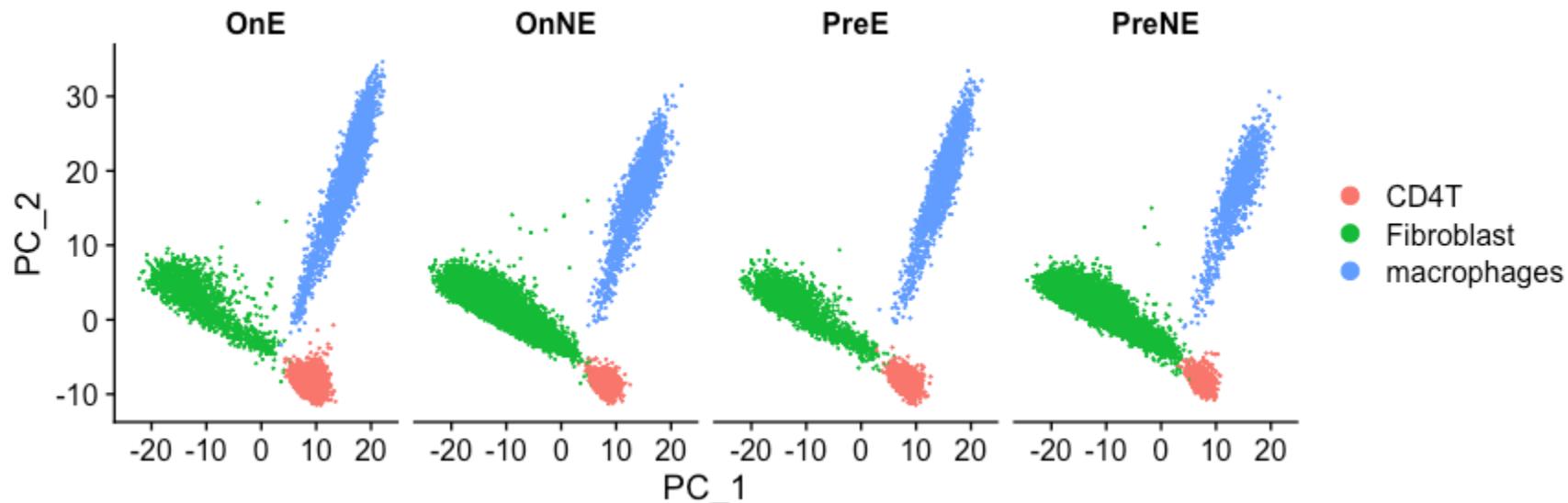
scRNAseq data:
Point of sampling

contrast:
PreE - PreNE



Dataset details

- <https://zenodo.org/records/11400203>
- Meta data columns
 - ▶ Cell type ID: "subType"
 - ▶ Condition ID: "expansion_timepoint"

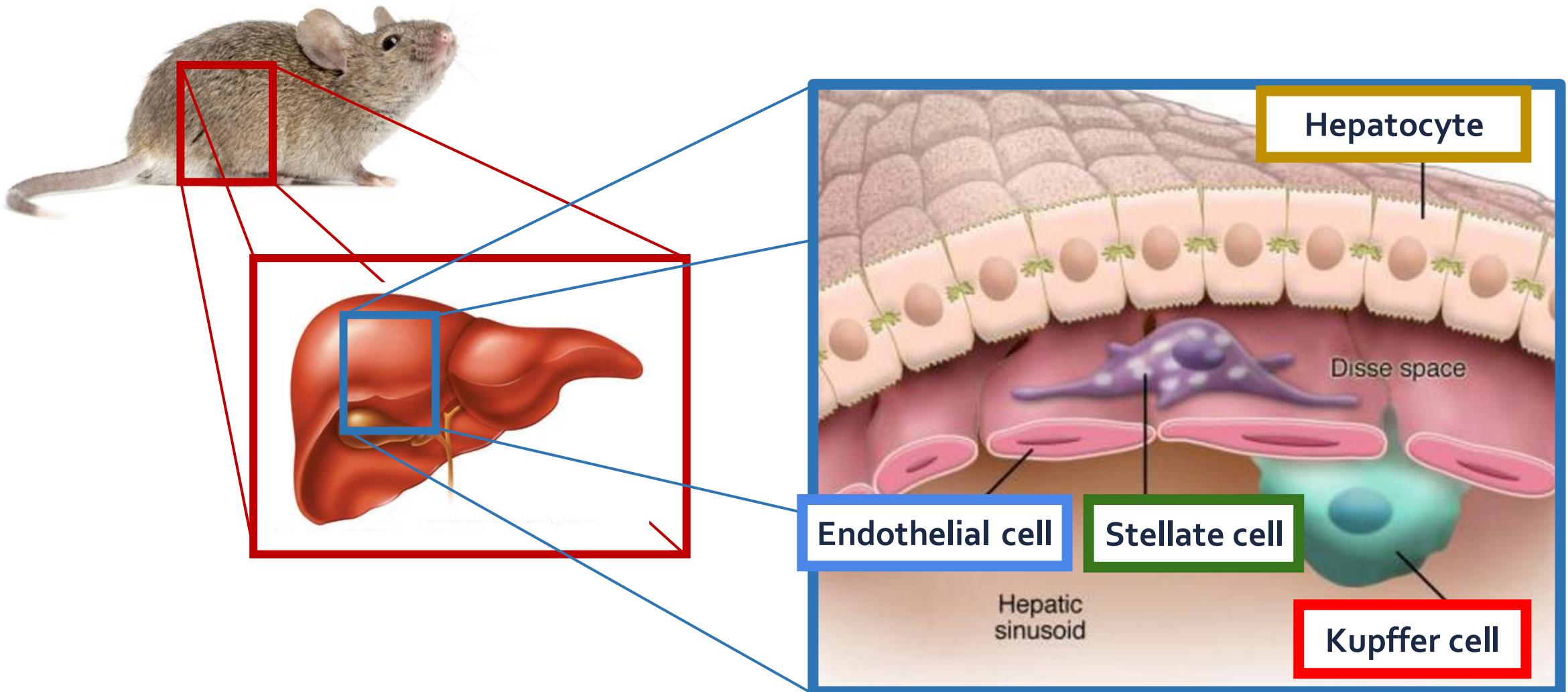


Application:

Cell localization / differentiation

Studying the Kupffer cell niche

Kupffer cell niche in the mouse liver



Known biology:

- Kupffer cell (KC) is a tissue-resident macrophage
- The gene expression profile of tissue-resident macrophages depends on the cells interacting with them

Research question:

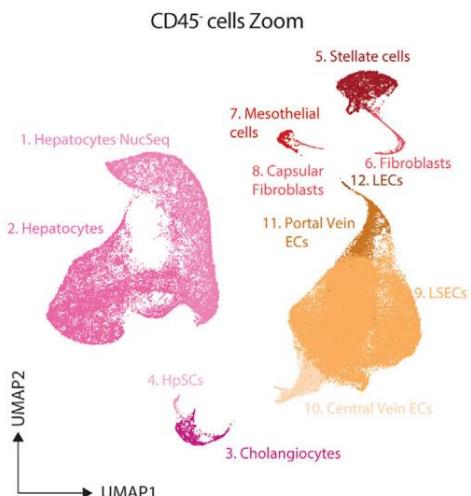
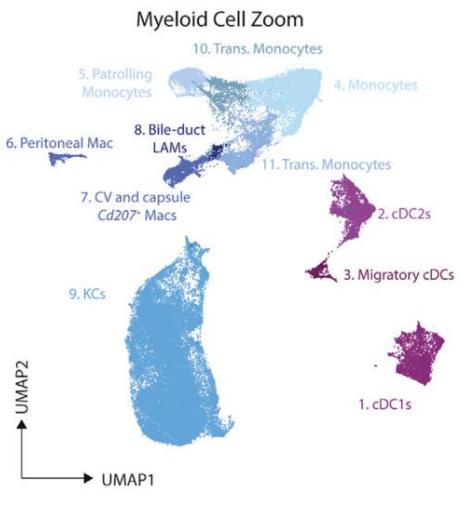
- **Which cell-cell communication signals determine KC identity?**

Available data:

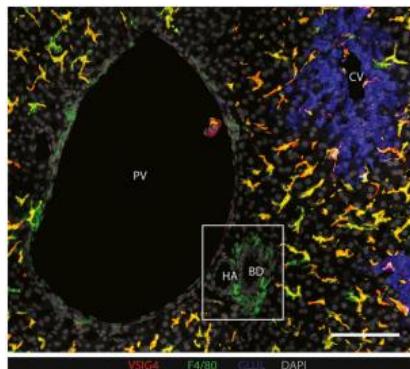
- “spatial proteo-genomics liver atlas”

Spatial proteo-genomics liver atlas

CITE-seq & NUC-seq

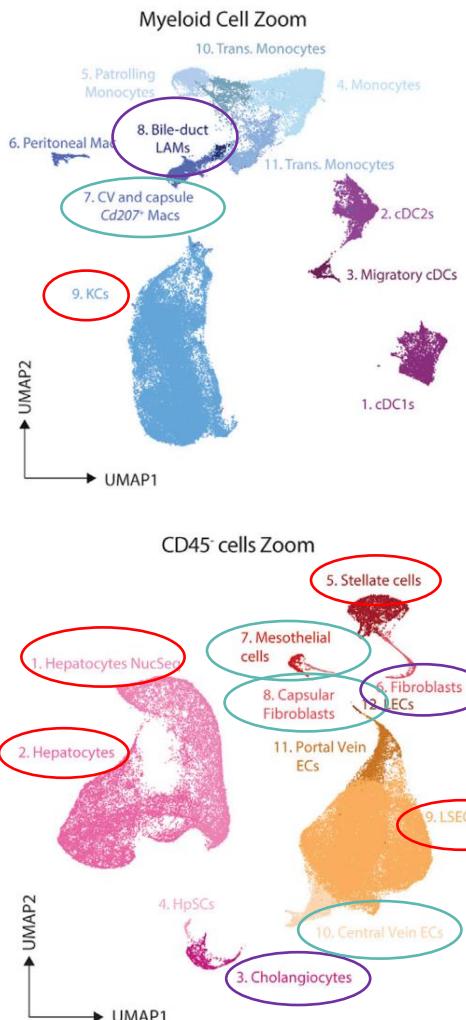


Spatial
transcriptomics
& proteomics

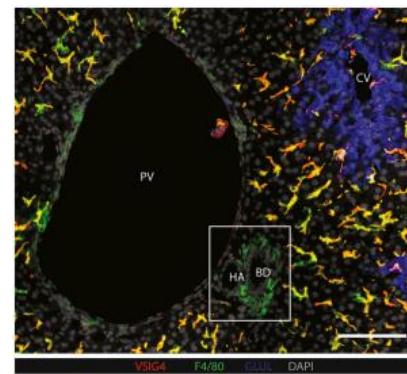


Spatial proteo-genomics liver atlas → distinct liver macrophage niches

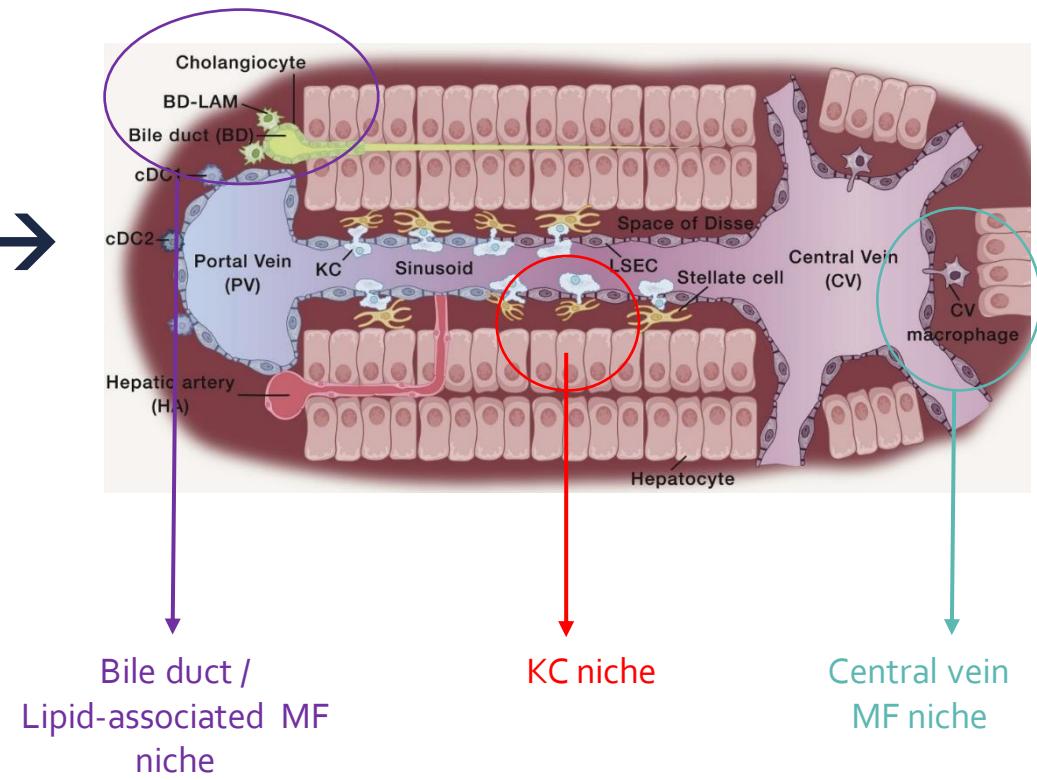
CITE-seq & NUC-seq



Spatial
transcriptomics
& proteomics



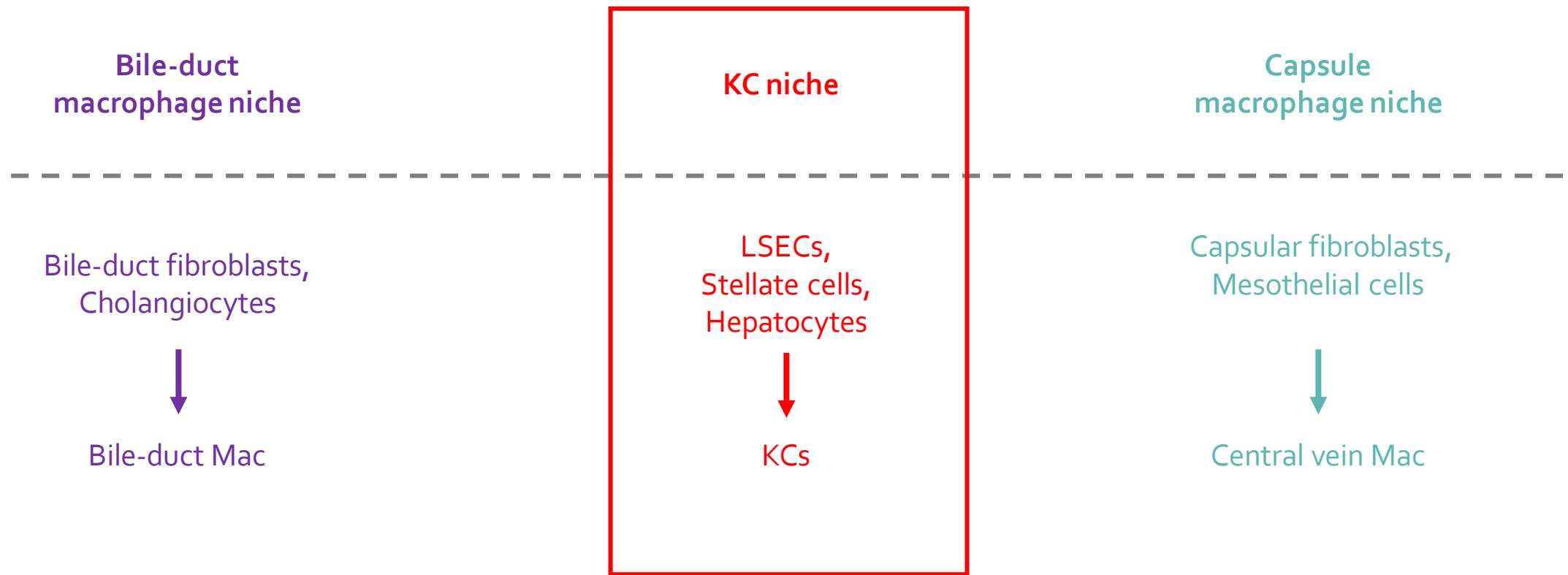
Discovery of KC & non-KC
liver macrophages and
their putative niche cells



This liver atlas gives us an idea about...

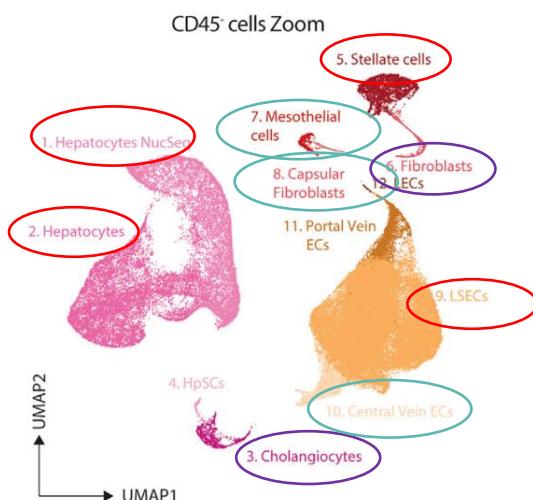
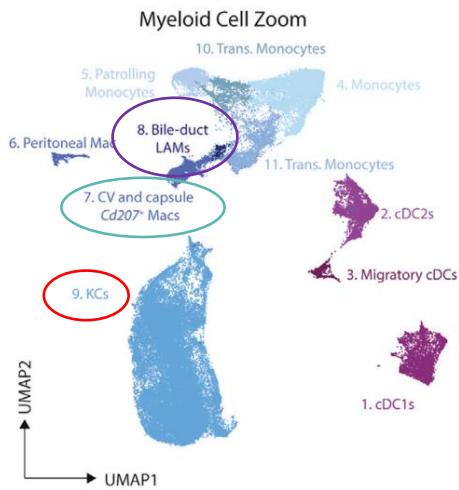
- ... How KCs and other macrophages in the liver differ in gene expression
- ... How KCs and other macrophages reside in different niches / spatial locations
- ... How the different structural cells in these niches differ in gene expression

NicheNet: Which cell-cell communication signals determine KC identity?



NicheNet: Which cell-cell communication signals determine KC identity?

CITE-seq & NUC-seq



How would you define the several “variable” elements in the NicheNet analysis to tackle this question?

- ▶ *Potential ligands*
- ▶ *Gene set of interest*
- ▶ *Other elements*

Dataset details

- Meta data columns
 - ▶ Cell type ID: celltype

NicheNet take-aways

Attention points for output interpretation

- The inferred *ligand activity* of a ligand is independent from its *expression*
- Ligands are ranked according to inferred activity - NicheNet does not determine "significant" ligands!
- Results of NicheNet will sometimes be quite different from results of the classic ligand-receptor inference tools.
 - This is because both approaches do something completely differently.
 - This is not necessarily an indication that NicheNet and/or the other tools did not work well on your data.

Most frequent mistakes

- Gene set of interest contains too many genes not affected by cell-cell communication
- Too few or too many genes in the gene set of interest compared to the background
- Running multiple NicheNet analyses for the same receiver with each time another sender cell type
 - Unnecessary since ligand activity does not depend on sender cell type expression
- Inappropriate cell type hierarchy level
 - looking for signals driving changes within Th17 CD4 T cells? --> *receiver* = "Th17"
 - looking for signals driving Th17 skewing within CD4 T cells? --> *receiver* = "CD4T"

Take-home messages

The vignettes demonstrate a standard analysis workflow that will be reasonable for most datasets, but they will not always be the best approach for a specific dataset

So...

1. Think before you run and take time to consider the different analysis options
2. Communicate these choices and their consequences when presenting the results

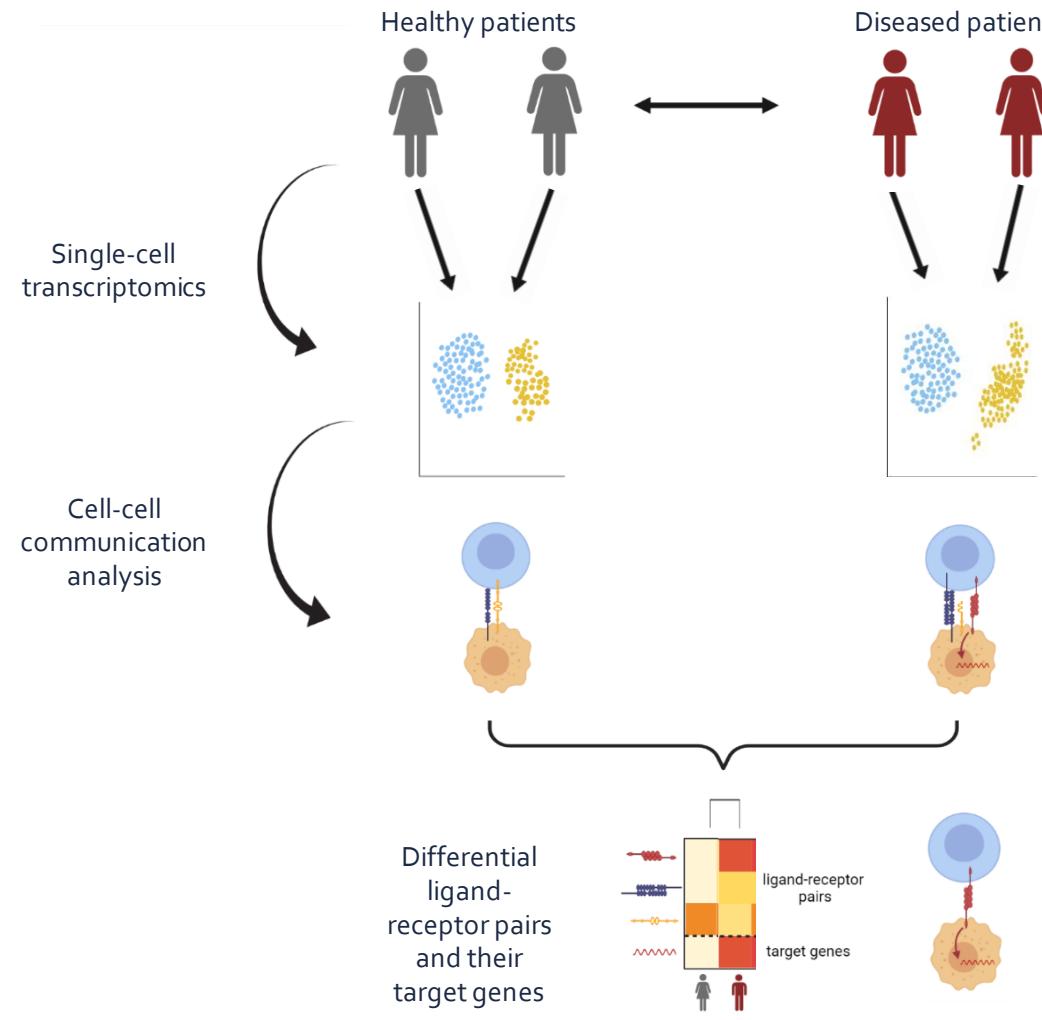
MultiNicheNet

a novel tool for **differential cell-cell communication** inference
from **multi-sample multi-condition scRNA-seq** data



**Limitations of NicheNet
(and other CCC tools)
when applied to multi-sample data?**

Classic cell-cell communication algorithms don't handle inter-patient variation properly when comparing different conditions



Classic cell-cell communication algorithms don't handle inter-patient variation properly when comparing different conditions

Biological problems associated with pooling of cells

cell-cell communication
happens within one patient

Statistical problems associated with pooling of cells

Variation between samples should
be taken into account

How to handle batch effects?

How to correct for covariates?

How to handle differences in cell
numbers between patients?

Solution:

MultiNicheNet

a novel tool for differential cell-cell communication inference
from **multi-sample multi-condition scRNA-seq data**

<https://www.biorxiv.org/content/10.1101/2023.06.13.544751v1>

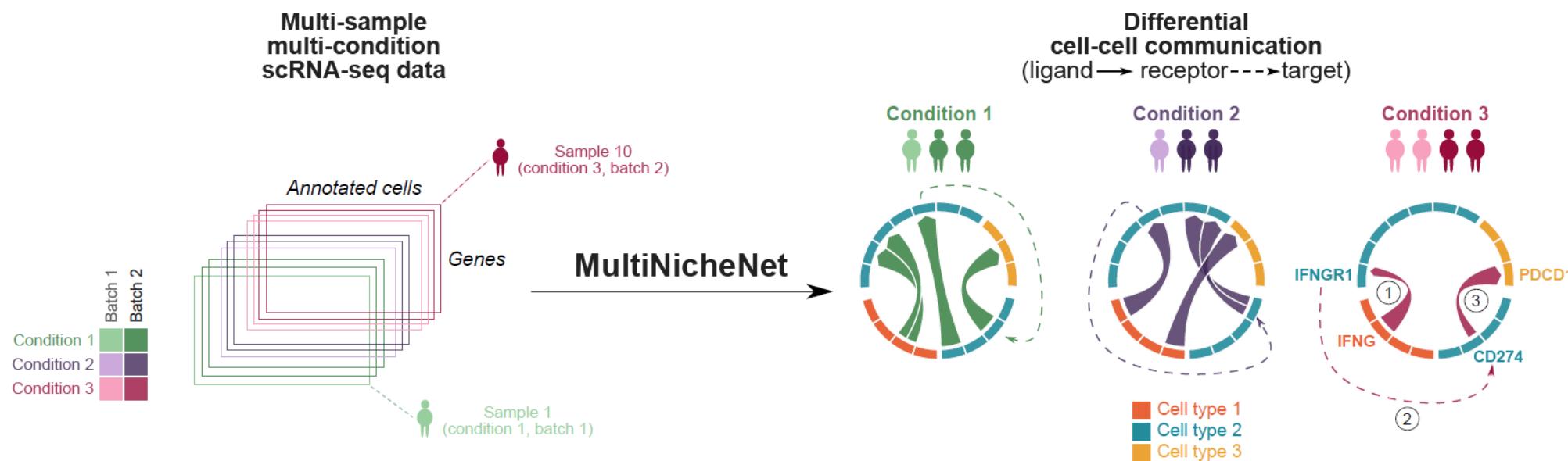
MultiNicheNet

= multi-criteria prioritization framework

- MultiNicheNet = tool/aid
- Given single-cell transcriptome profiles of several cell types: try to get an idea of the most important interactions
- How? By ranking interactions based on properties we think...
 - ▶ ... are relevant for cell-cell communication
 - ▶ ... can be estimated from single-cell transcriptomics data

Unique benefits of MultiNicheNet

1. **Multi-sample multi-condition prioritization:** prioritize condition-driving CCC events - while appropriately handling sample-to-sample variability & complex experimental designs



Unique benefits of MultiNicheNet

1. Multi-sample multi-condition prioritization:

prioritize condition-driving CCC events - while appropriately handling sample-to-sample variability & complex experimental designs

<https://doi.org/10.1038/s41467-020-19894-4>

OPEN

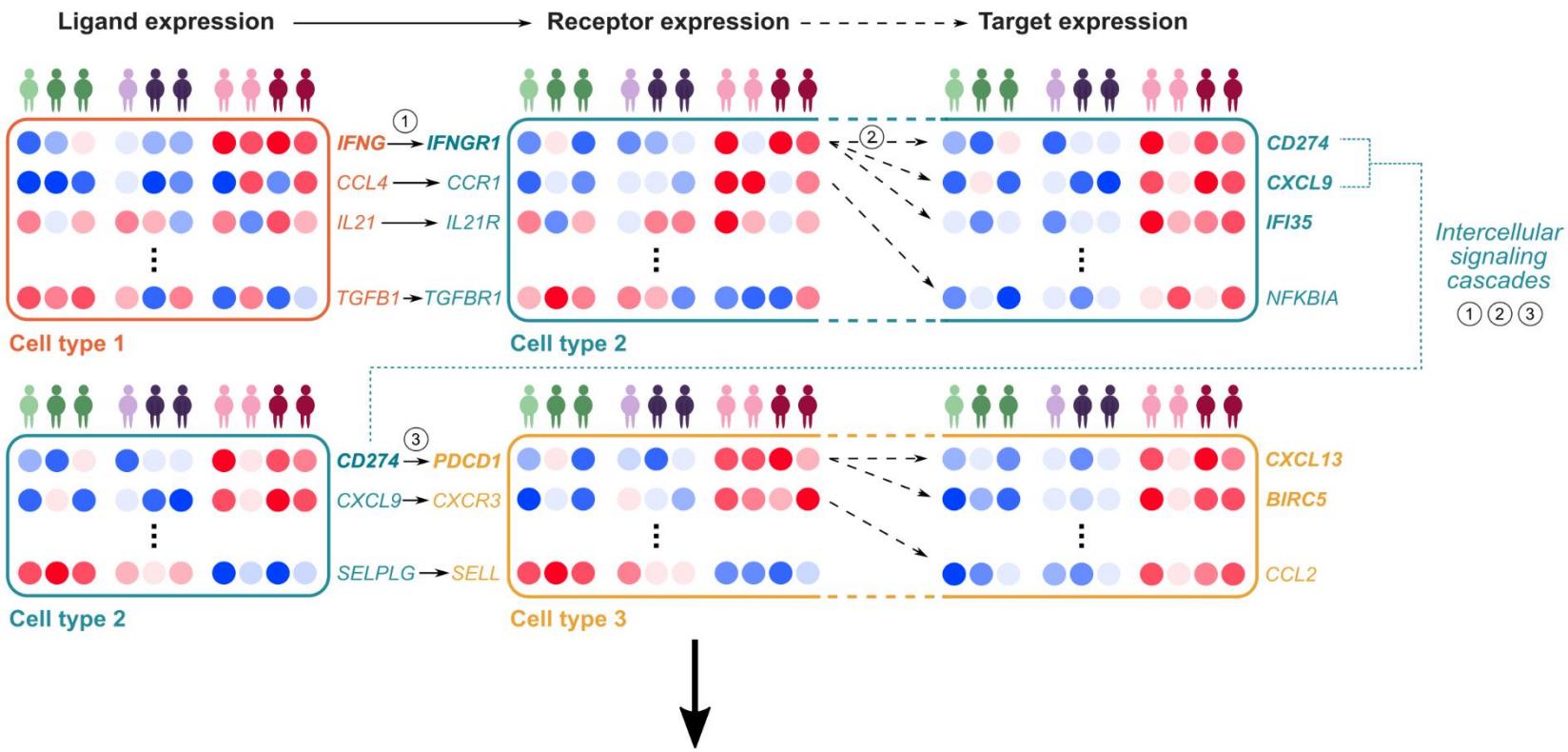
muscat detects subpopulation-specific state transitions from multi-sample multi-condition single-cell transcriptomics data

Helena L. Crowell^{1,2}, Charlotte Soneson^{1,2,3,6}, Pierre-Luc Germain^{1,4,6}, Daniela Calini⁵, Ludovic Collin⁵, Catarina Raposo⁵, Dheeraj Malhotra⁵ & Mark D. Robinson^{1,2✉}

Unique benefits of MultiNicheNet

- 1. Multi-sample multi-condition prioritization:**
prioritize condition-driving CCC events - while appropriately handling sample-to-sample variability & complex experimental designs

- 2. Enhanced multi-criteria prioritization:**
score interactions for both **expression and activity** of ligand-receptor pairs (= extracellular and intracellular aspects of CCC).



Prioritization of differential ligand-receptor pairs

example comparison: Condition 3 – (Condition 1 + Condition 2)

Ranked ligand-receptor pairs	DE score ligand	DE score receptor	Cell-type specificity ligand	Cell-type specificity receptor	Downstream signaling activity	Fraction samples with ligand present	Fraction samples with receptor present	Aggregated prioritization score
<i>IFNG</i> → <i>IFNGR1</i>	█	█	█	█	█	█	█	█
<i>CD274</i> → <i>PDCD1</i>	█	█	█	█	█	█	█	█
<i>CXCL9</i> → <i>CXCR3</i>	█	█	█	█	█	█	█	█
<i>CCL4</i> → <i>CCR1</i>	█	█	█	█	█	█	█	█
<i>IL21</i> → <i>IL21R</i>	█	█	█	█	█	█	█	█
⋮								
<i>TGFB1</i> → <i>TGFBR1</i>	█	█	█	█	█	█	█	█
<i>SELPLG</i> → <i>SELL</i>	█	█	█	█	█	█	█	█

User-defined cutoff for visualizations

Cf:
prioritization
scheme
NicheNet-v2

Unique benefits of MultiNicheNet

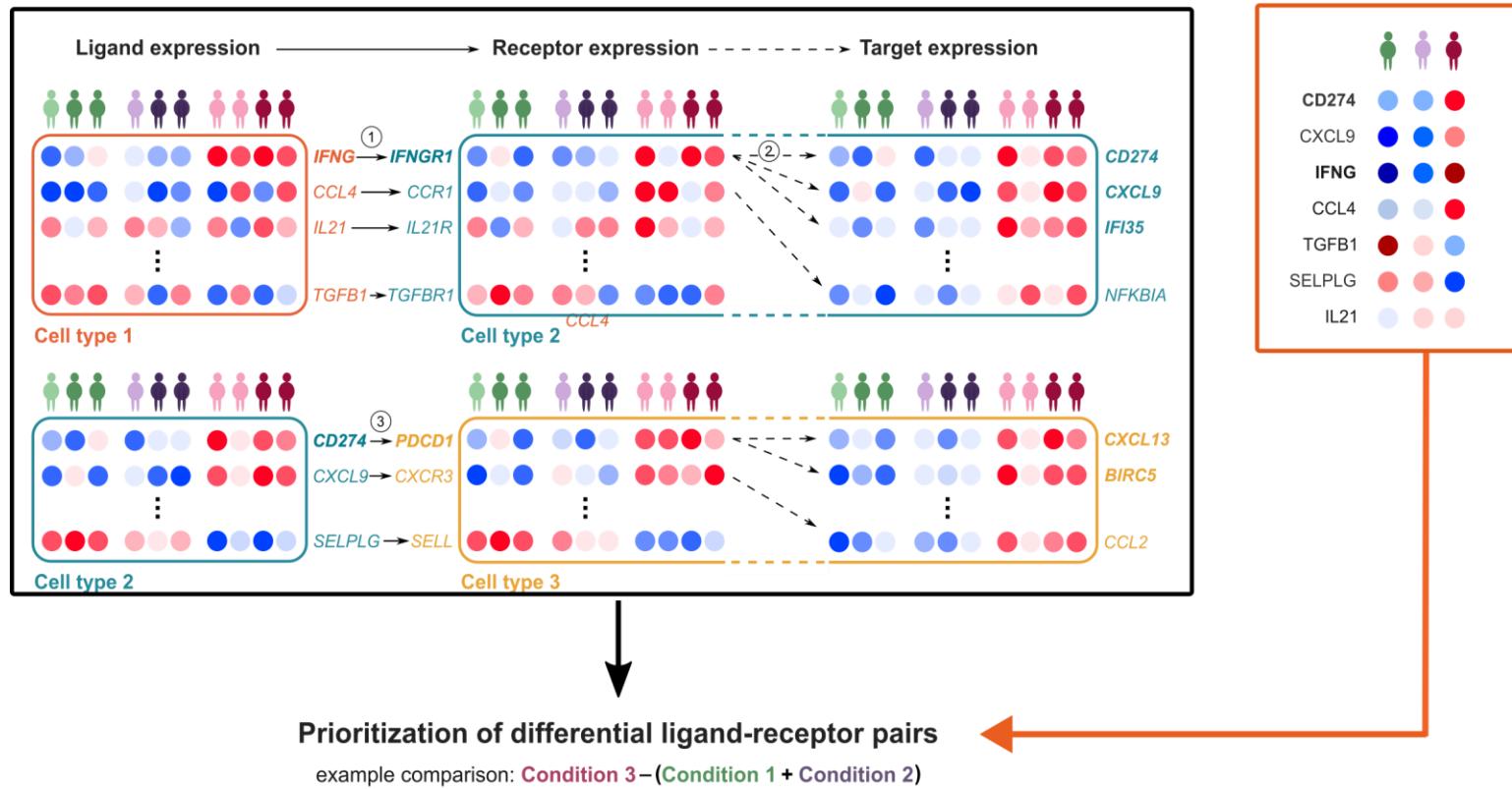
- 1. Multi-sample multi-condition prioritization:**
prioritize condition-driving CCC events - while appropriately handling sample-to-sample variability & complex experimental designs

- 2. Enhanced multi-criteria prioritization:**
score interactions for both expression and activity of ligand-receptor pairs (= extracellular and intracellular aspects of CCC).

- 3. Extendable prioritization:**
possible to **integrate complementary data** (e.g. proteomics)

Single-cell transcriptomics

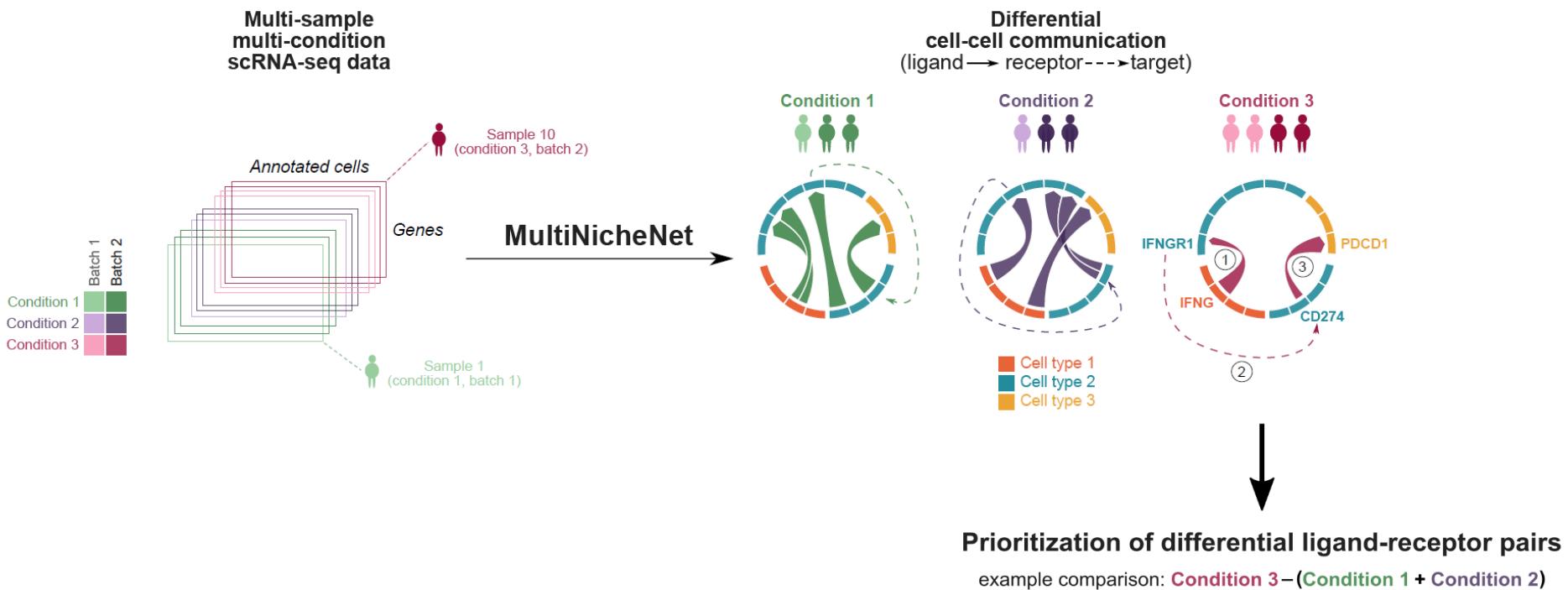
Serum proteomics



Ranked ligand-receptor pairs	DE score ligand	DE score receptor	Cell-type specificity ligand	Cell-type specificity receptor	Downstream signaling activity	Fraction samples with ligand present	Fraction samples with receptor present	Protein-level DE score ligand	Aggregated prioritization score
<i>IFNG</i> → <i>IFNGR1</i>	■	■	■	■	■	■	■	■	■
<i>CD274</i> → <i>PDCD1</i>	■	■	■	■	■	■	■	■	■
<i>CXCL9</i> → <i>CXCR3</i>	■	■	■	■	■	■	■	■	■
<i>CCL4</i> → <i>CCR1</i>	■	■	■	■	■	■	■	■	■
<i>IL21</i> → <i>IL21R</i>	■	■	■	■	■	■	■	■	■
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
<i>TGFB1</i> → <i>TGFBR1</i>	□	□	□	□	□	□	□	□	□
<i>SELPLG</i> → <i>SELL</i>	□	□	□	□	□	□	□	□	□

User-defined cutoff for visualizations

MultiNicheNet performs multi-criteria prioritization



Visualization and prioritization explicitly

- Consider inter-patient heterogeneity
- Enable batch effect correction
- Address complex experimental designs

Ranked ligand-receptor pairs	DE score ligand	DE score receptor	Cell-type specificity ligand	Cell-type specificity receptor	Downstream signaling activity	Fraction samples with ligand present	Fraction samples with receptor present	Aggregated prioritization score
<i>IFNG</i> → <i>IFNGR1</i>	█	█	█	█	█	█	█	█
<i>CD274</i> → <i>PDCD1</i>	█	█	█	█	█	█	█	█
<i>CXCL9</i> → <i>CXCR3</i>	█	█	█	█	█	█	█	█
<i>CCL4</i> → <i>CCR1</i>	█	█	█	█	█	█	█	█
<i>IL21</i> → <i>IL21R</i>	█	█	█	█	█	█	█	█
⋮								
<i>TGFB1</i> → <i>TGFBR1</i>	█	█	█	█	█	█	█	█
<i>SELPLG</i> → <i>SELL</i>	█	█	█	█	█	█	█	█

User-defined cutoff for visualizations

When to use MultiNicheNet?

Differential CCC question: interest in differences between conditions

Single-cell/nuclei data with ≥ 3 samples in the smallest group

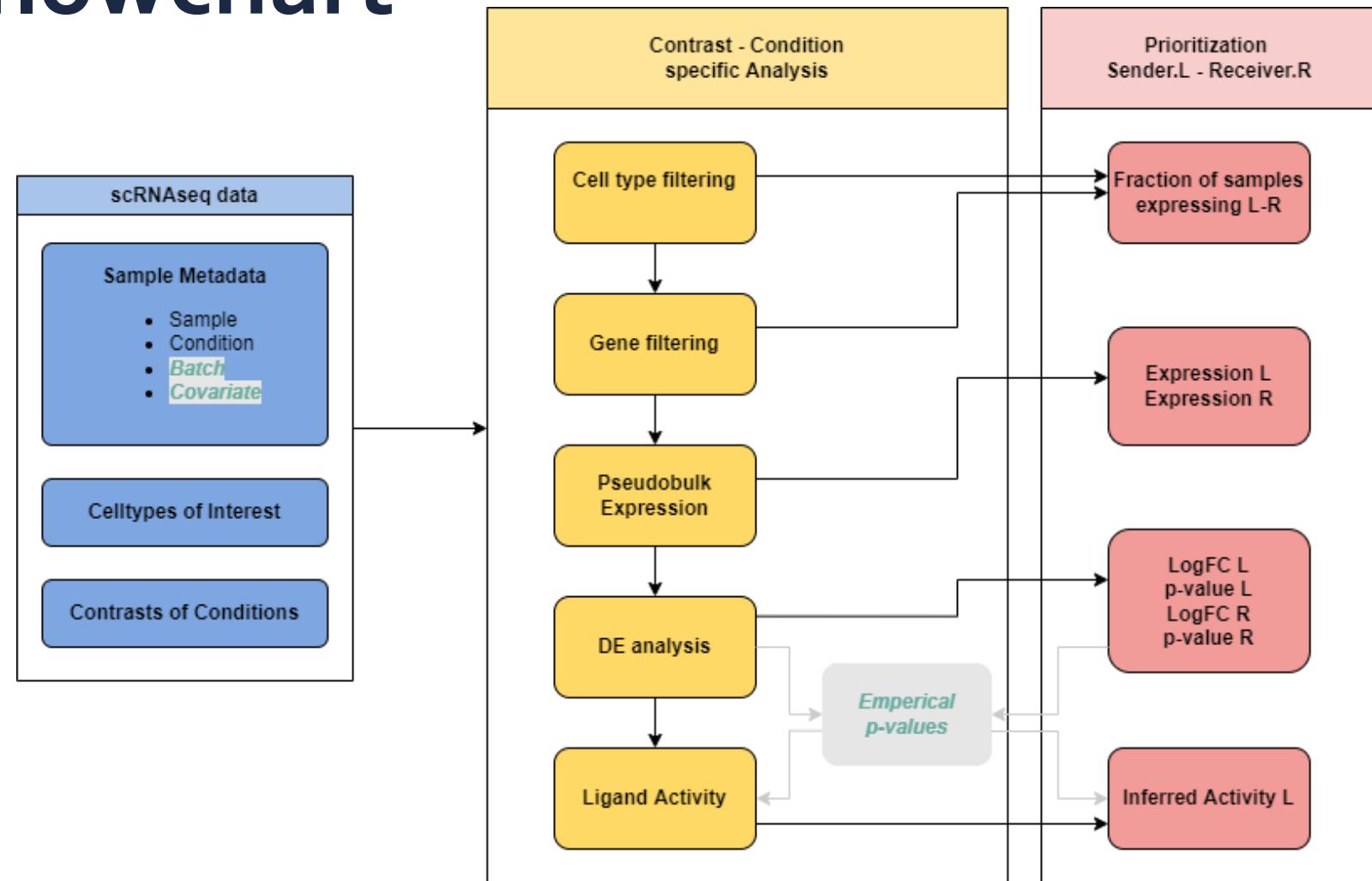
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Using MultiNicheNet

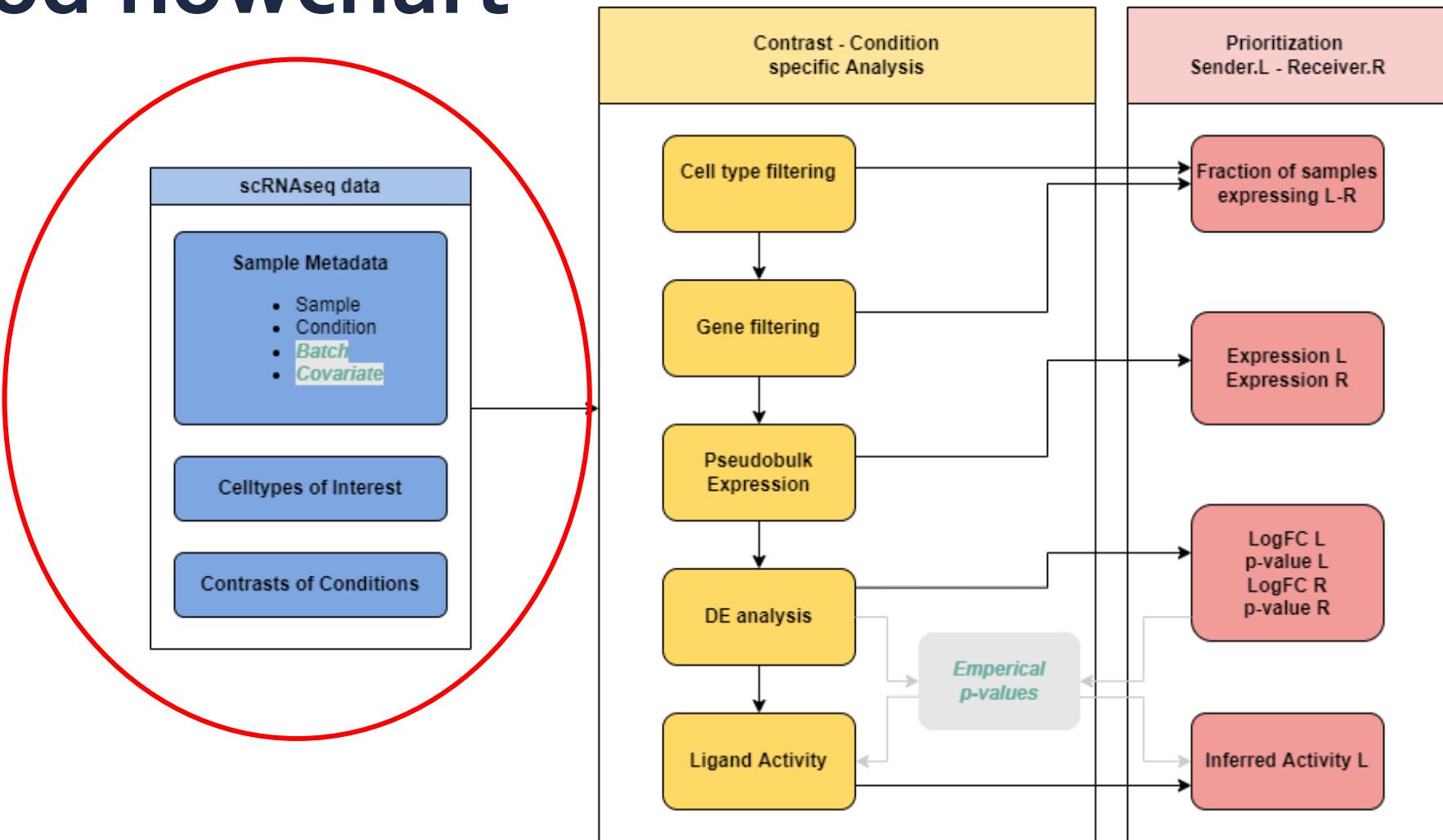
github.com/saeyslab/multinichenet



Method flowchart



Method flowchart



Input dataset requirements

- SingleCellExperiment object

- ▶ Raw counts

- ▶ Meta data

- Sample ID
 - Cell type ID
 - Condition ID

```
seurat_obj <- Seurat::DietSeurat(seurat_obj)
sce <- Seurat::as.SingleCellExperiment(seurat_obj, assay = "RNA")
```

Data should have been preprocessed adequately:
proper cell filtering, doublet removal, ambient RNA correction

Cell types of interest

- Do you know which cell types are localized together → focus on these
- If you don't know this: consider all cell types as senders and receivers during the analysis
 - Better for calculation of the cell-type specificity criteria
 - Still possible to zoom in on specific cell types during downstream analysis

Contrasts of conditions

- Formalize your research question
 - ▶ Example: "What are CCC differences between M-patients and S-patients?"
- Contrasts for such classic case-control design:

```
```{r}
contrasts_oi = c("M-S", "S-M")
contrast_tbl = tibble(
 contrast = c("M-S", "S-M"),
 group = c("M", "S")
)
...```

```

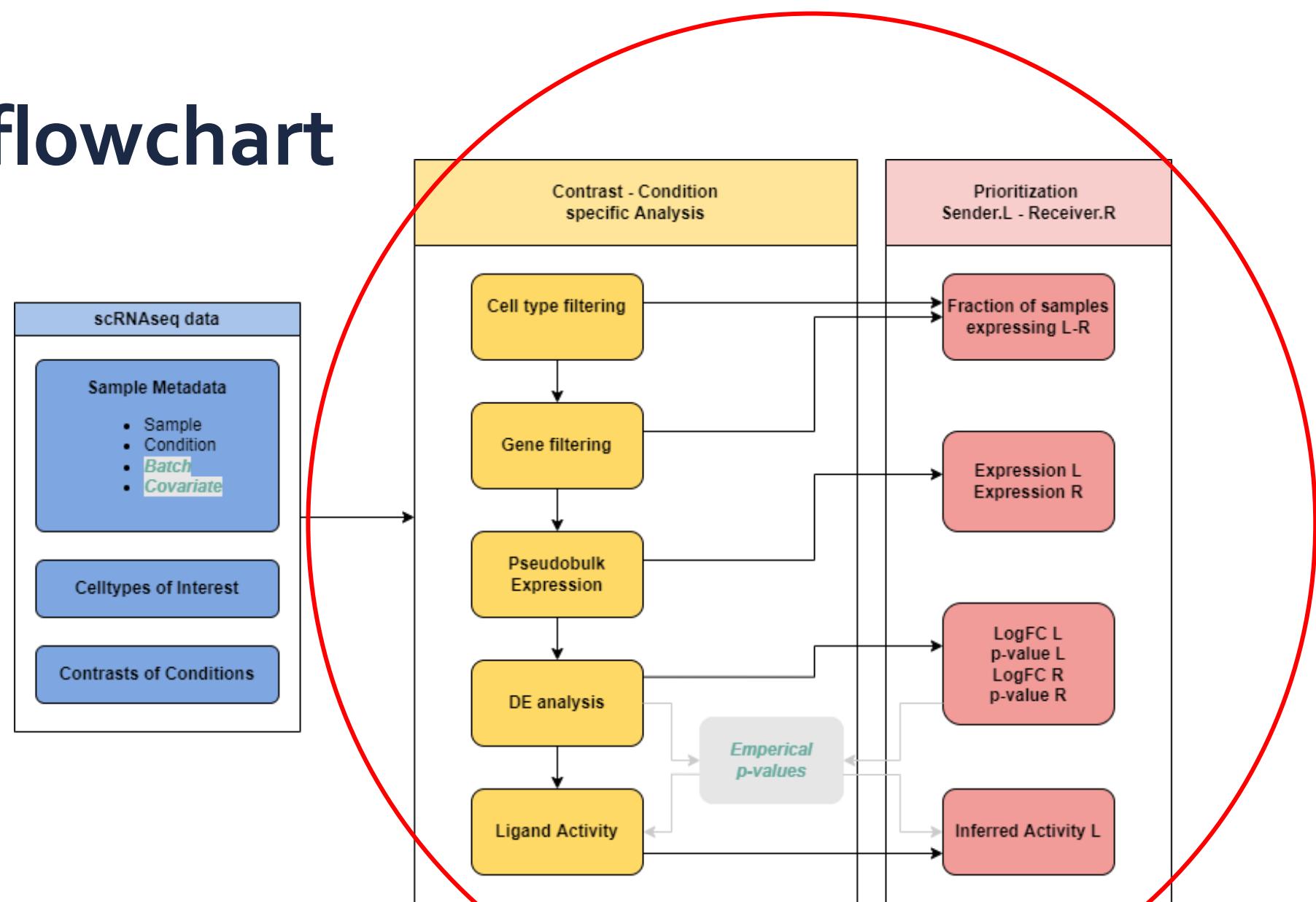
# Contrasts of conditions

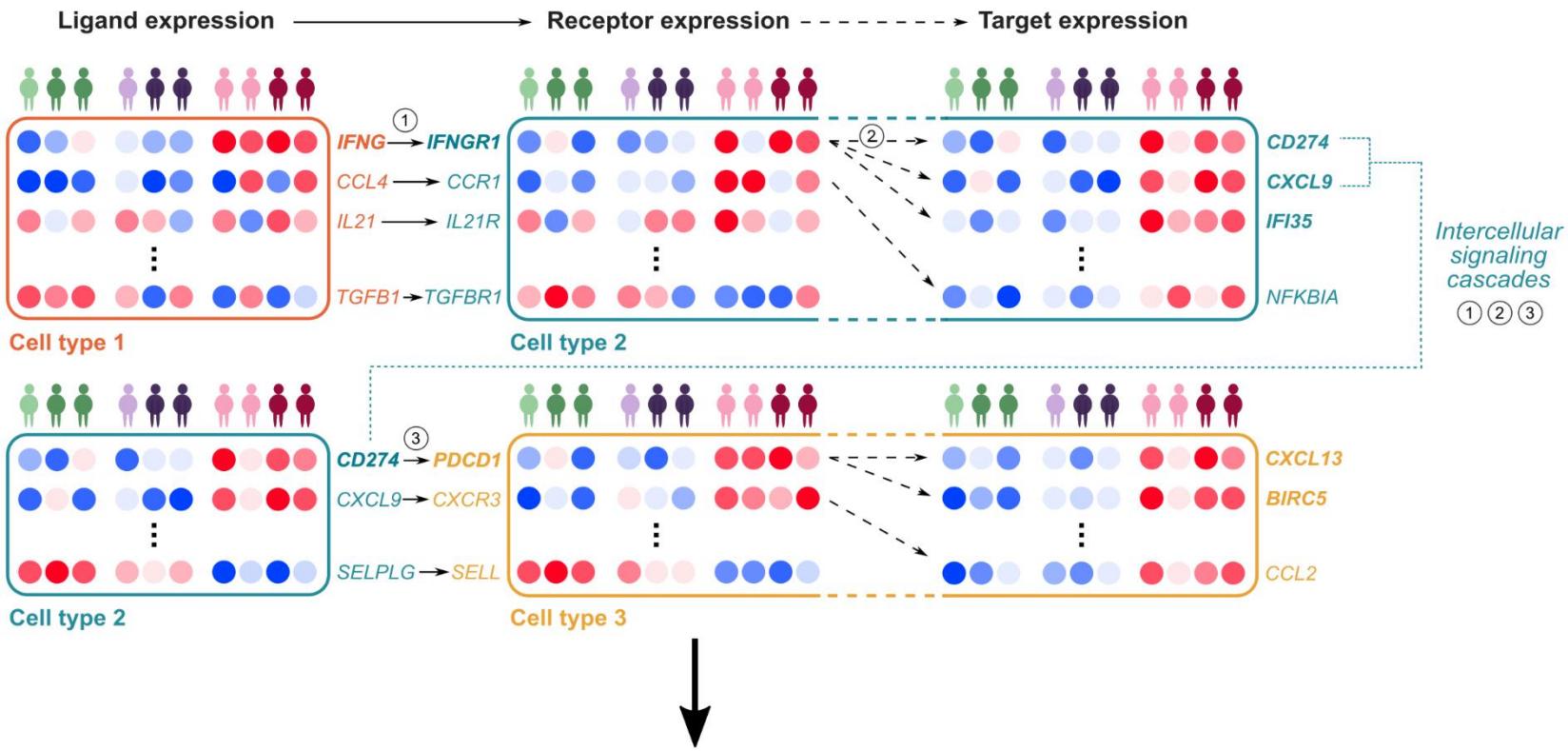
- Formalize your research question = most important part of analysis
  - ▶ Example: "What are CCC differences between M-patients and S-patients?"
- Contrasts for such classic case-control design:

```
```{r}
contrasts_oi = c("M-S", "S-M")
contrast_tbl = tibble(
  contrast = c("M-S", "S-M"),
  group = c("M", "S")
)
...```

```

Method flowchart





Prioritization of differential ligand-receptor pairs

example comparison: Condition 3 – (Condition 1 + Condition 2)

Ranked ligand-receptor pairs	DE score ligand	DE score receptor	Cell-type specificity ligand	Cell-type specificity receptor	Downstream signaling activity	Fraction samples with ligand present	Fraction samples with receptor present	Aggregated prioritization score
<i>IFNG</i> → <i>IFNGR1</i>	█	█	█	█	█	█	█	█
<i>CD274</i> → <i>PDCD1</i>	█	█	█	█	█	█	█	█
<i>CXCL9</i> → <i>CXCR3</i>	█	█	█	█	█	█	█	█
<i>CCL4</i> → <i>CCR1</i>	█	█	█	█	█	█	█	█
<i>IL21</i> → <i>IL21R</i>	█	█	█	█	█	█	█	█
⋮								
<i>TGFB1</i> → <i>TGFBR1</i>	█	█	█	█	█	█	█	█
<i>SELPLG</i> → <i>SELL</i>	█	█	█	█	█	█	█	█

User-defined cutoff for visualizations

Demo MultiNicheNet analysis

Case study:

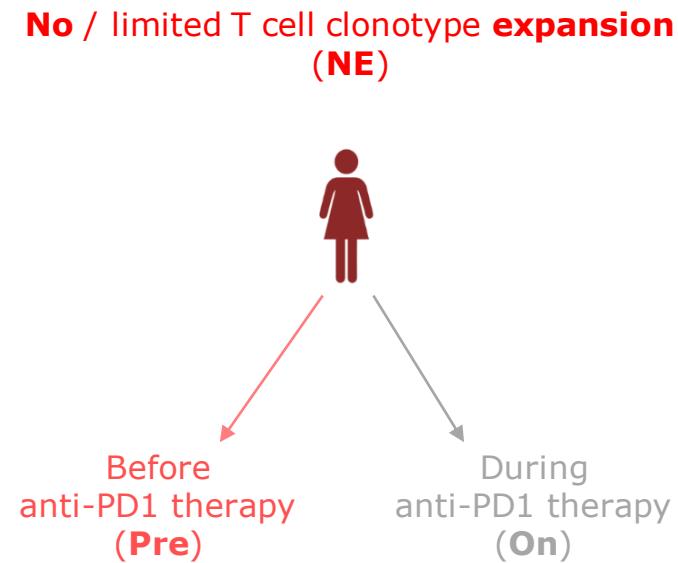
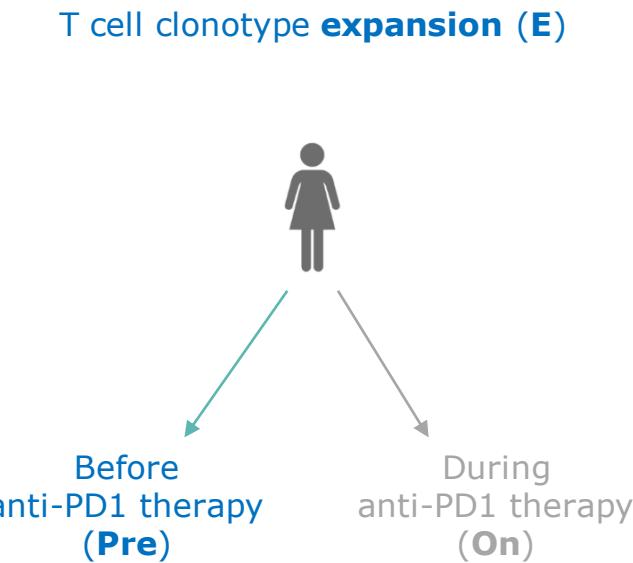
**using MultiNicheNet to study
tumor microenvironment interactions in breast
cancer in context of anti-PD1 immunotherapy**

Which pre-therapy cell-cell signaling patterns are different between therapy responders and non-responders?

Patient group:
Anti-PD1
therapy response

scRNAseq data:
Point of sampling

contrast:
PreE - PreNE



Differentially expressed & active LR pairs?

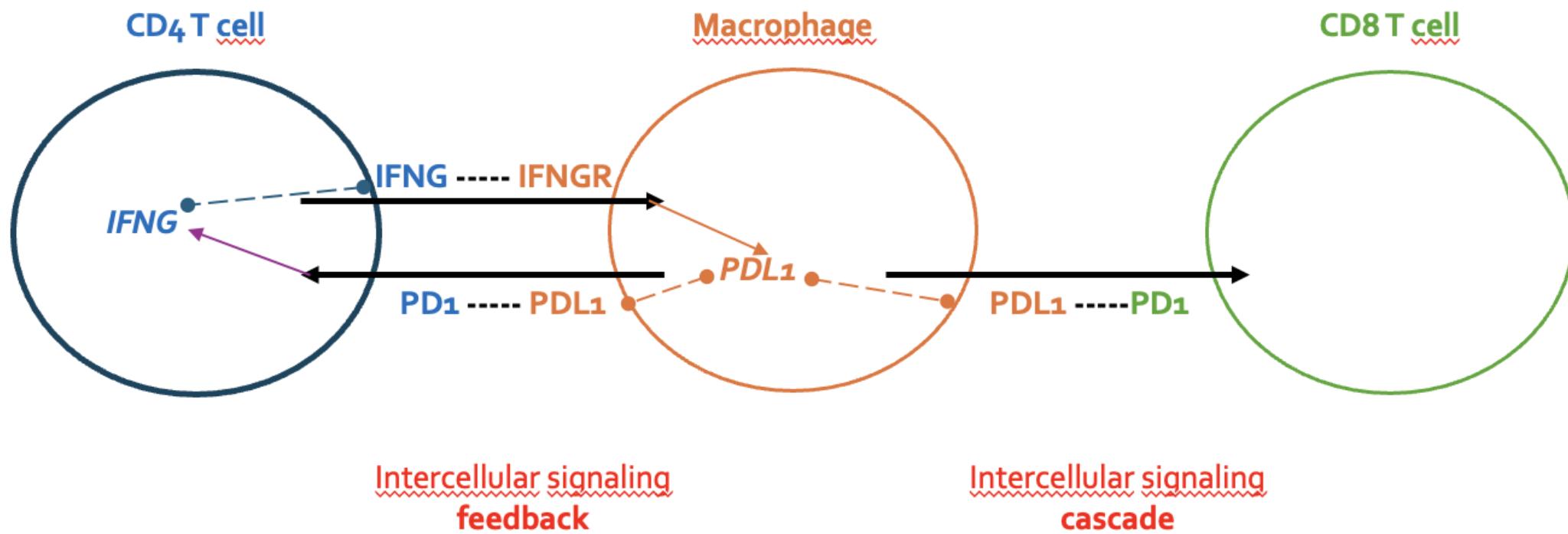
Demo MultiNicheNet analysis

Go over HTML file tutorial to illustrate these steps and their parameters

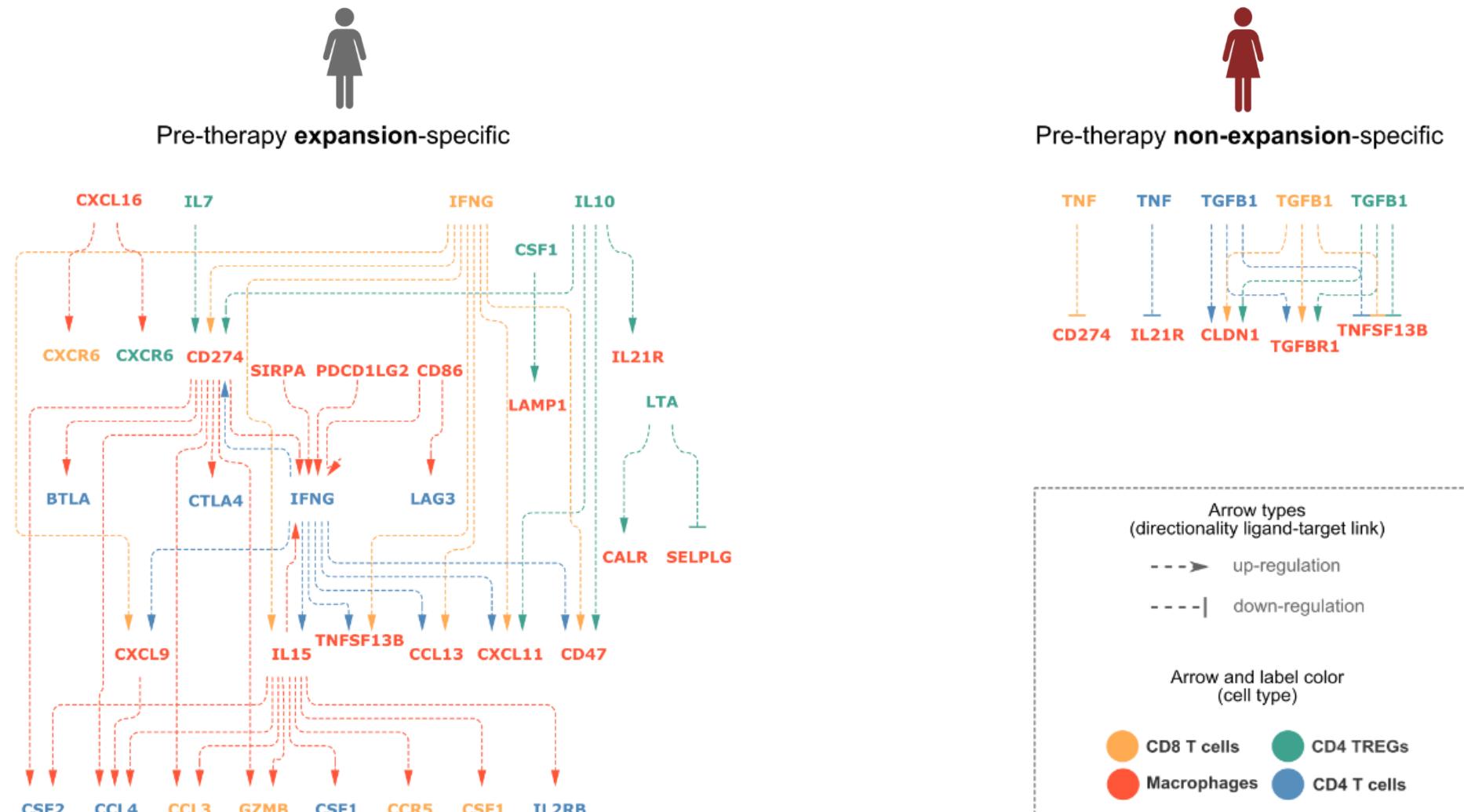
https://github.com/saeyslab/nichenet_training/blob/main/basic_analysis_steps_BRCA.html

Demo MultiNicheNet analysis: extra explanation slides

Ligand-receptor pairs regulate expression of ligands/receptors in other cell types...
→ Intercellular signaling feedback and cascade mechanisms

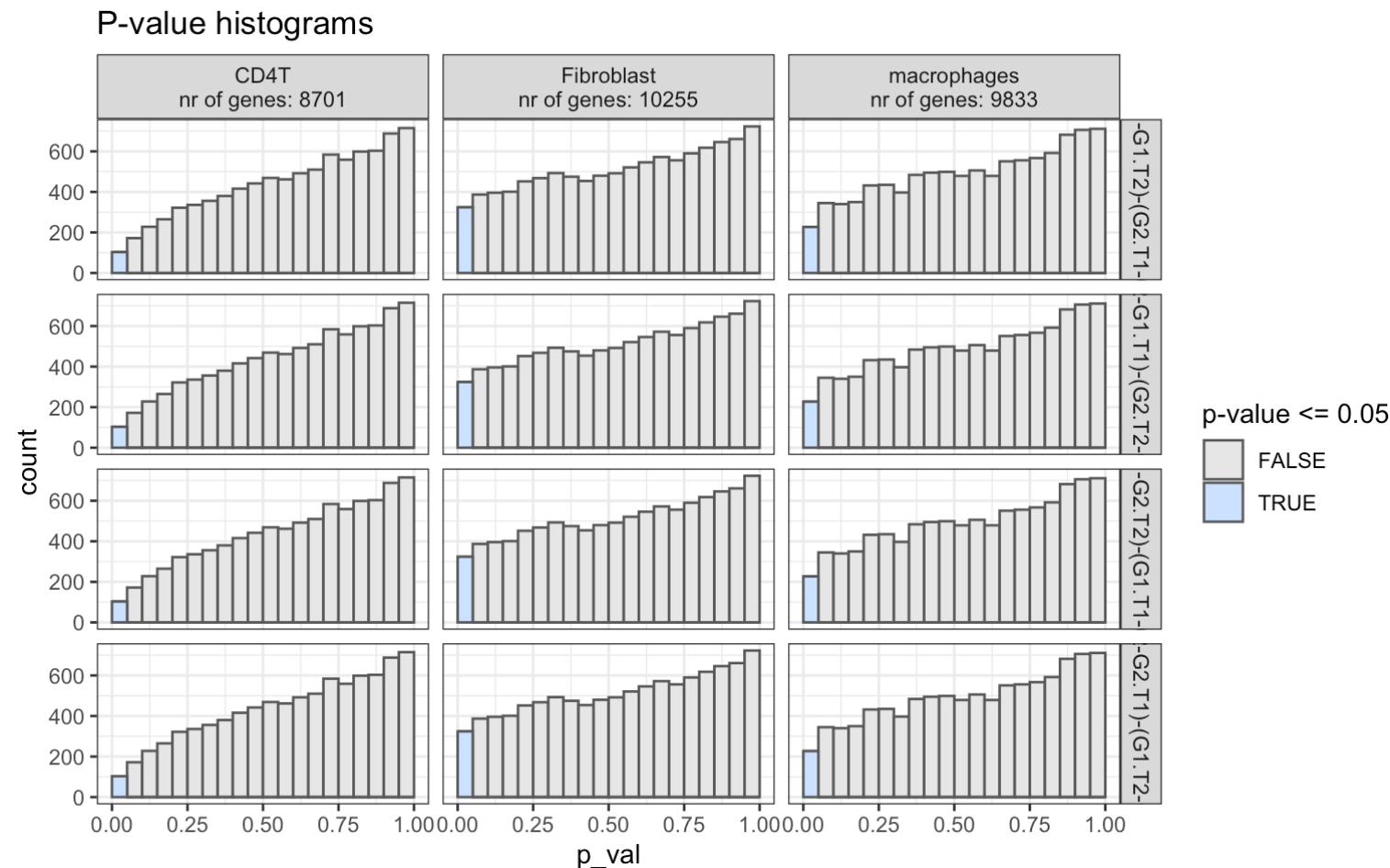


Differential intercellular signaling cascades between macrophages and T cells



When to use the empirical p-values?

- When p-value histograms are not uniform/uniform+peak at 0.05 → violation DE model assumptions



Possible analysis designs

- Case-control
- Case-control with repeated samples of same subjects
- 3 conditions
- Condition-differences in treatment effects
- Batch effects / study effects in integrated atlas data

Case-control:

```
```{r}
contrasts_oi = c("M-S", "S-M")
contrast_tbl = tibble(
 contrast = c("M-S", "S-M"),
 group = c("M", "S")
)
````
```

Case-control: repeated samples of same subject

```
```{r}
covariates = "patient"
contrasts_oi = c("'Tumor-Normal','Normal-Tumor'")
contrast_tbl = tibble(contrast =
 c("Tumor-Normal", "Normal-Tumor"),
 group = c("Tumor", "Normal"))
```

```

3 conditions: threewise comparison

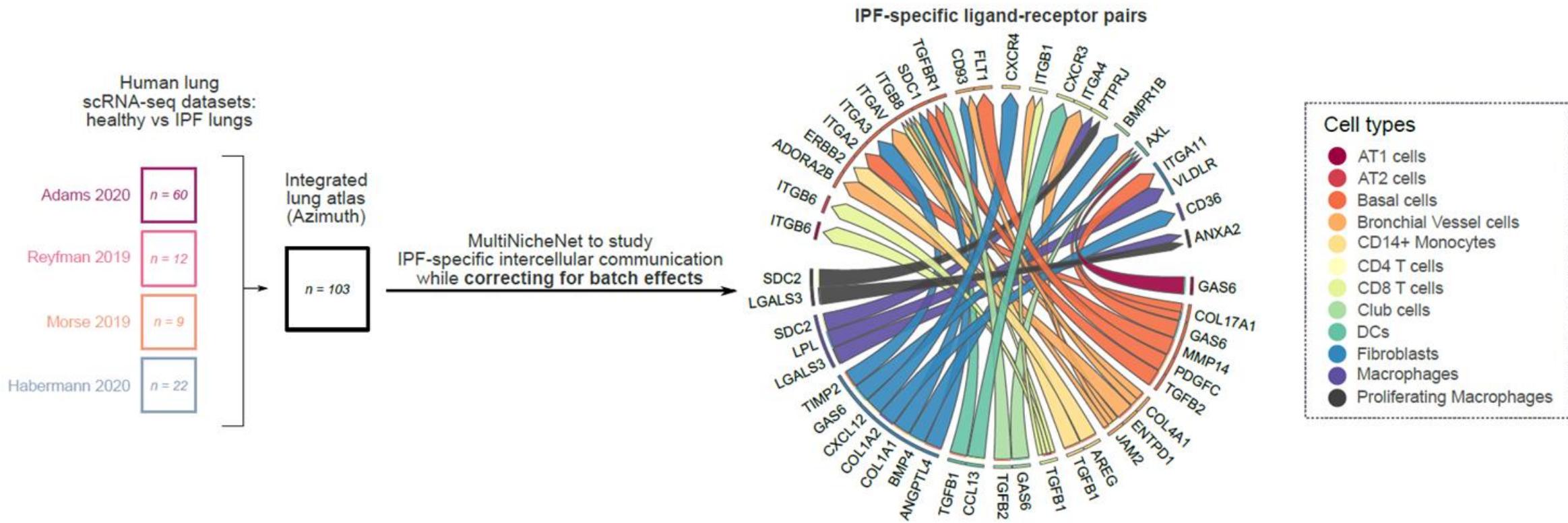
```
```{r}
contrasts_oi = c("M-(S+A)/2", "S-(M+A)/2", "A-(S+M)/2")
contrast_tbl = tibble(contrast =
 c("M-(S+A)/2", "S-(M+A)/2", "A-(S+M)/2"),
 group = c("M", "S", "A"))
```

```

Case-control: batch effect correction

Case-control: batch effect correction

Human lung atlas data: healthy vs idiopathic pulmonary fibrosis (IPF)



Case-control: batch effect correction

```
```{r}
covariates = NA
batches = "dataset_origin"
contrasts_oi = c("'idiopathic.pulmonary.fibrosis-normal','normal-idiopathic.pulmonary.fibrosis'")
contrast_tbl = tibble(contrast =
 c("idiopathic.pulmonary.fibrosis-normal", "normal-idiopathic.pulmonary.fibrosis"),
 group = c("idiopathic.pulmonary.fibrosis", "normal"))
...
```

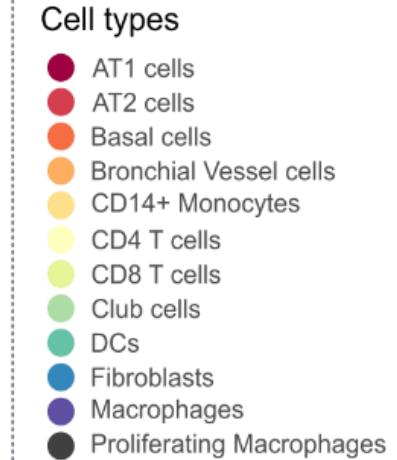
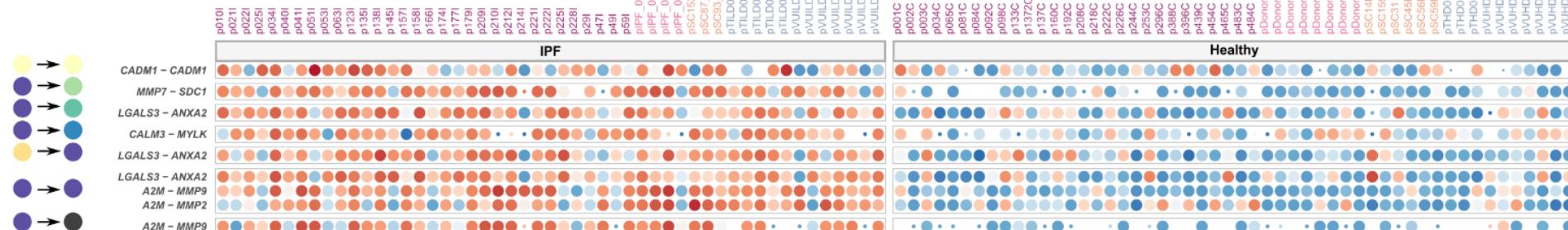
```

MultiNicheNet corrects for batch effects in integrated lung atlas data to reveal dysregulated cell-cell communication in idiopathic pulmonary fibrosis (IPF)

Interactions more highly ranked with batch effect correction - visualization with non-corrected pseudobulk expression values



Interactions more highly ranked with batch effect correction - visualization with corrected pseudobulk expression values



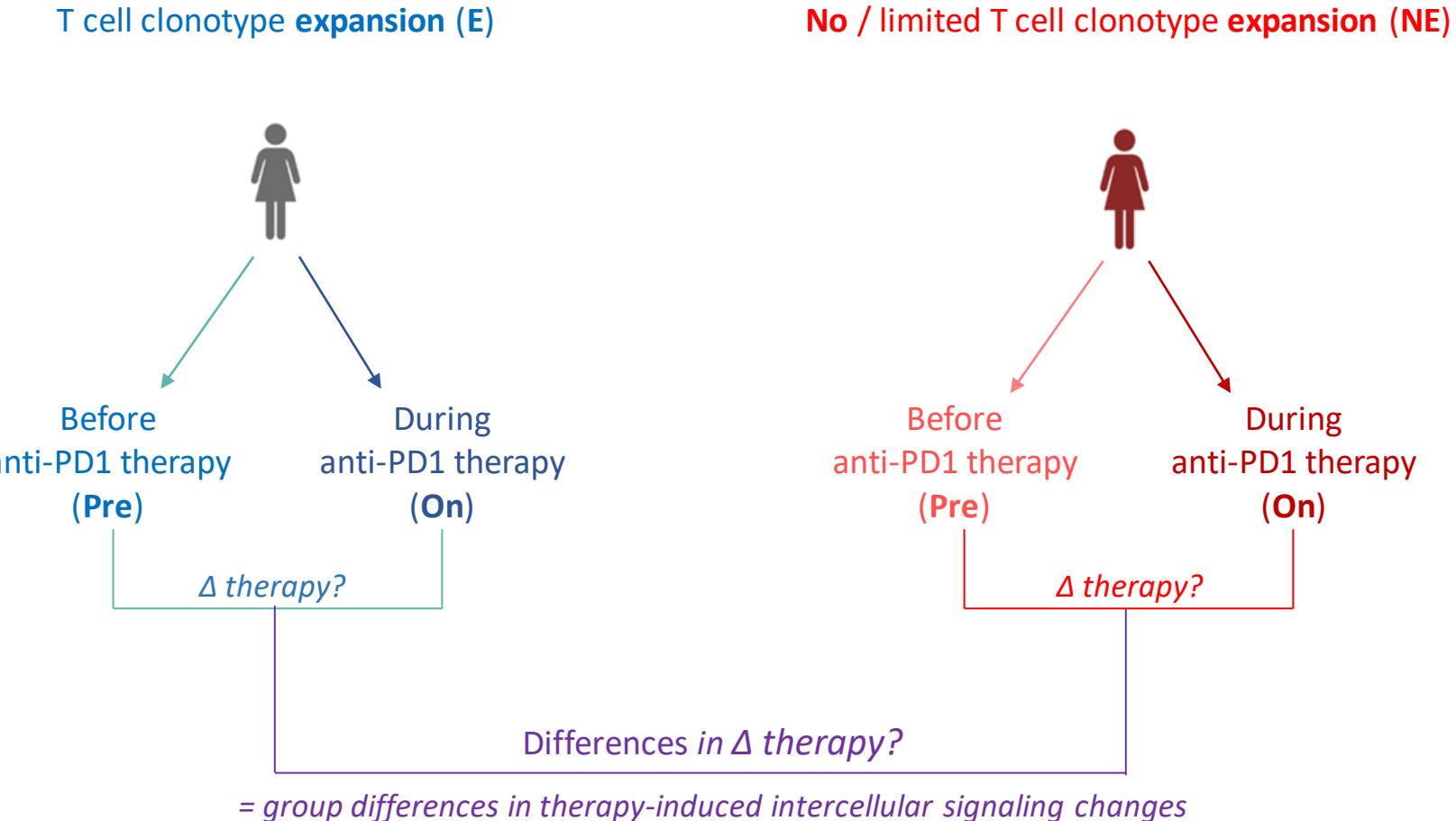
Condition differences in treatment effects

MultiNicheNet to tackle complex questions & compare cell-cell communication between multiple groups

Patient group:
Anti-PD1
therapy response

scRNAseq data:
Point of sampling

contrast:
 $(OnE - PreE) - (OnNE - PreNE)$



Condition differences in treatment effects

Treatment effect
group 1

```
```{r}
contrasts_oi = c("G1.T2-G1.T1", "G1.T1-G1.T2")
contrast_tbl = tibble(
 contrast = c("G1.T2-G1.T1", "G1.T1-G1.T2"),
 group = c("G1.T2", "G1.T1")
)
````
```

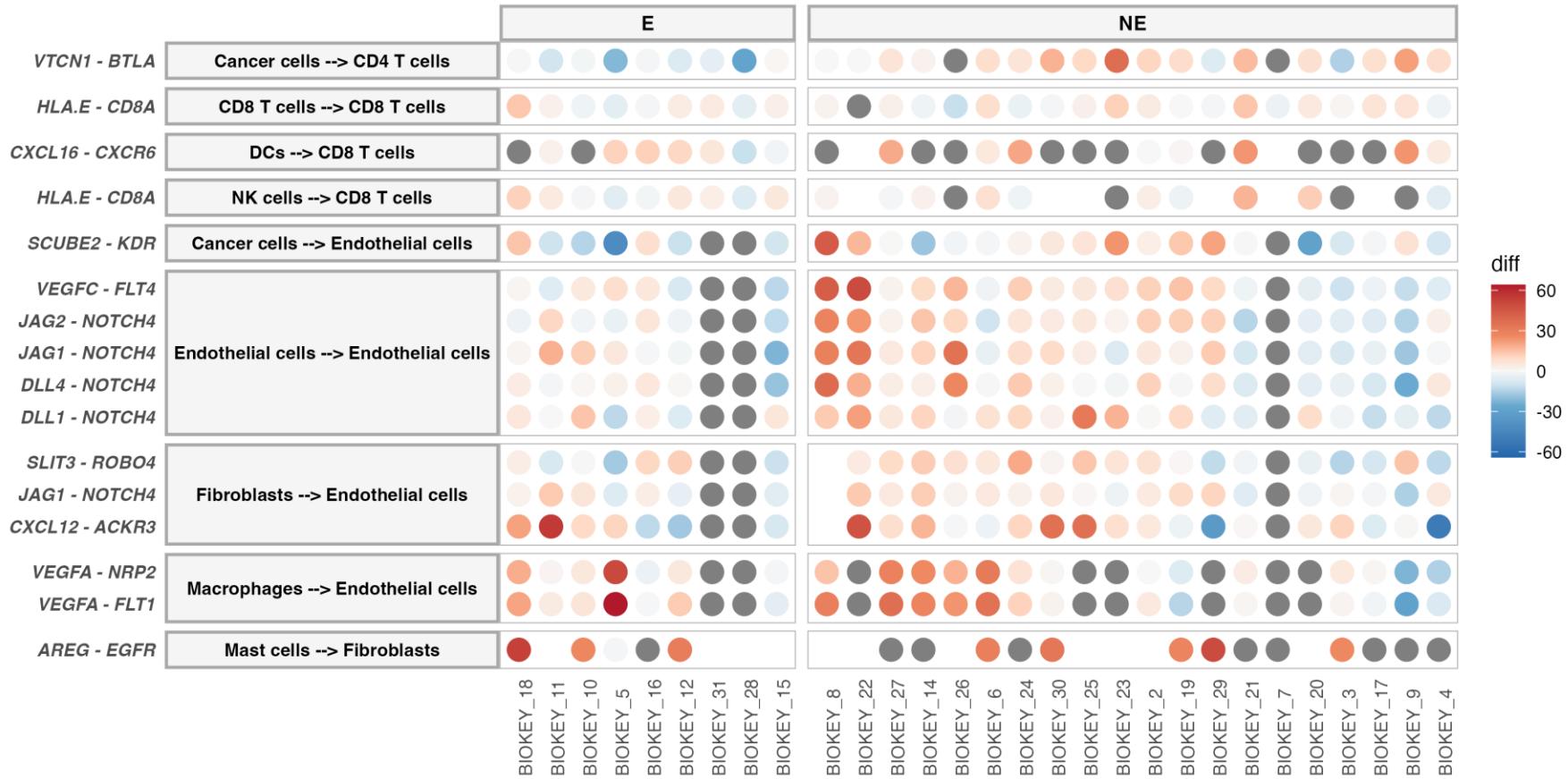
Treatment effect
group 2

```
```{r}
contrasts_oi = c("G2.T2-G2.T1", "G2.T1-G2.T2")
contrast_tbl = tibble(
 contrast = c("G2.T2-G2.T1", "G2.T1-G2.T2"),
 group = c("G2.T2", "G2.T1")
)
````
```

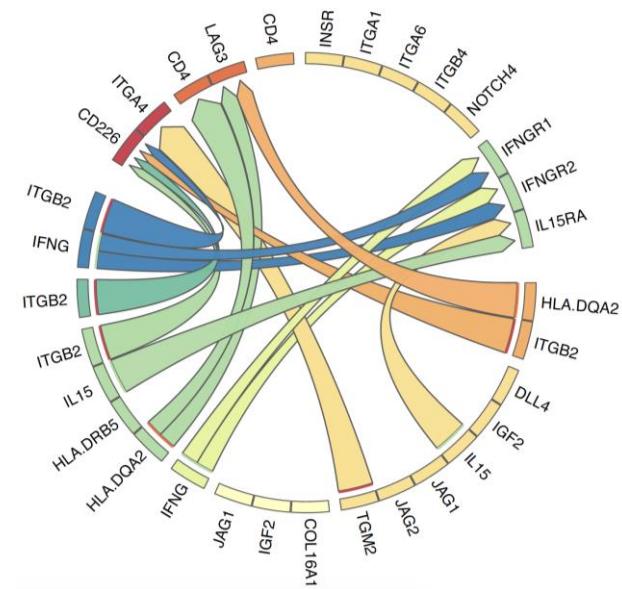
Group difference in
treatment effect

```
```{r}
contrasts_oi = c("(G1.T2-G1.T1)-(G2.T2-G2.T1)", "(G2.T2-G2.T1)-(G1.T2-G1.T1)", "(G1.T1-G1.T2)-(G2.T1-G2.T2)", "(G2.T1-G2.T2)-(G1.T1-G1.T2)")
contrast_tbl = tibble(contrast =
 c("(G1.T2-G1.T1)-(G2.T2-G2.T1)",
 "(G2.T2-G2.T1)-(G1.T2-G1.T1)",
 "(G1.T1-G1.T2)-(G2.T1-G2.T2)",
 "(G2.T1-G2.T2)-(G1.T1-G1.T2)"),
 group = c("G1.T2", "G2.T2", "G1.T1", "G2.T1"))
````
```

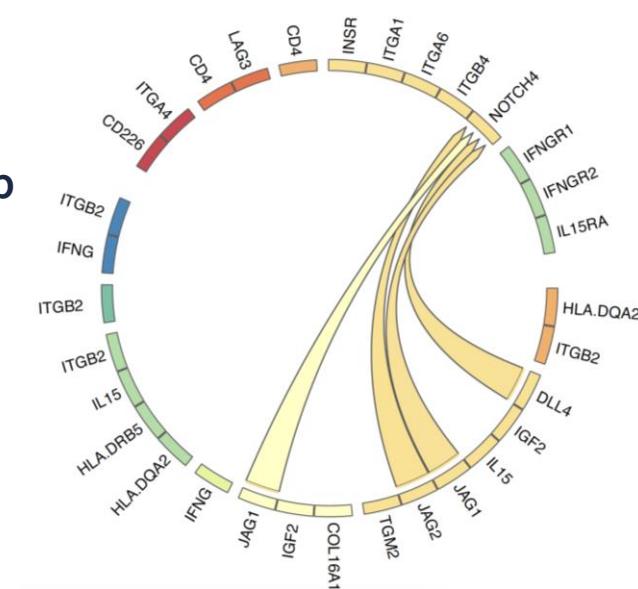
Specific increase in NE-group after therapy
 --> visualizations show within-group heterogeneity



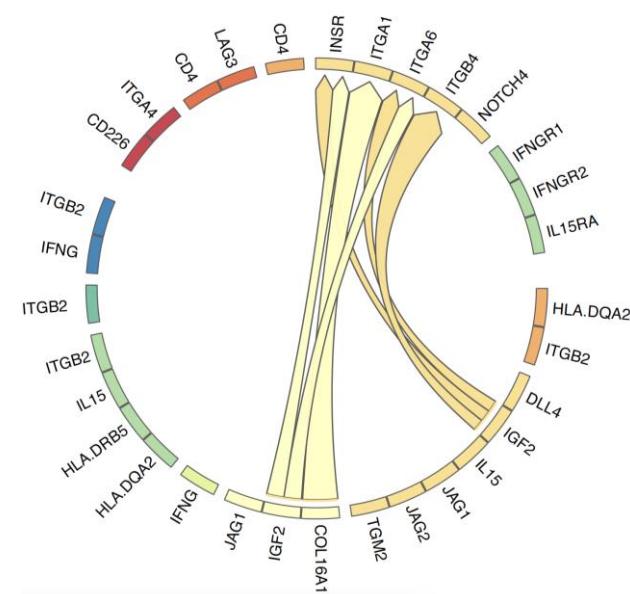
Specific increase in E-group after therapy



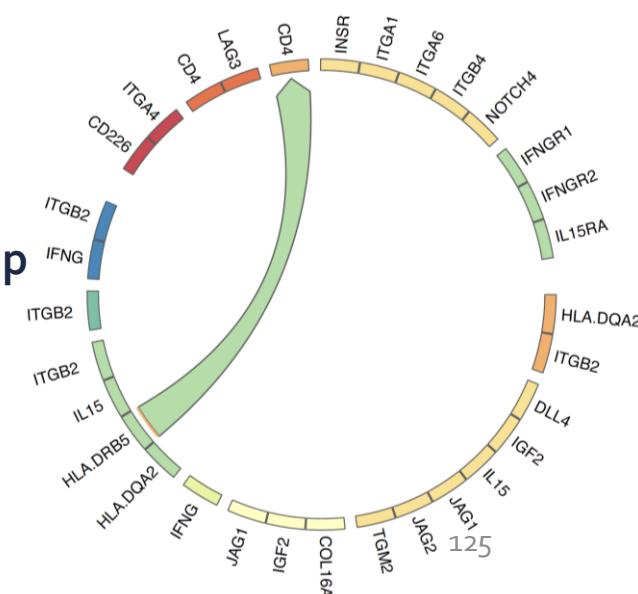
Specific increase in NE-group after therapy



Specific decrease in E-group after therapy



Specific decrease in **N**-group after therapy

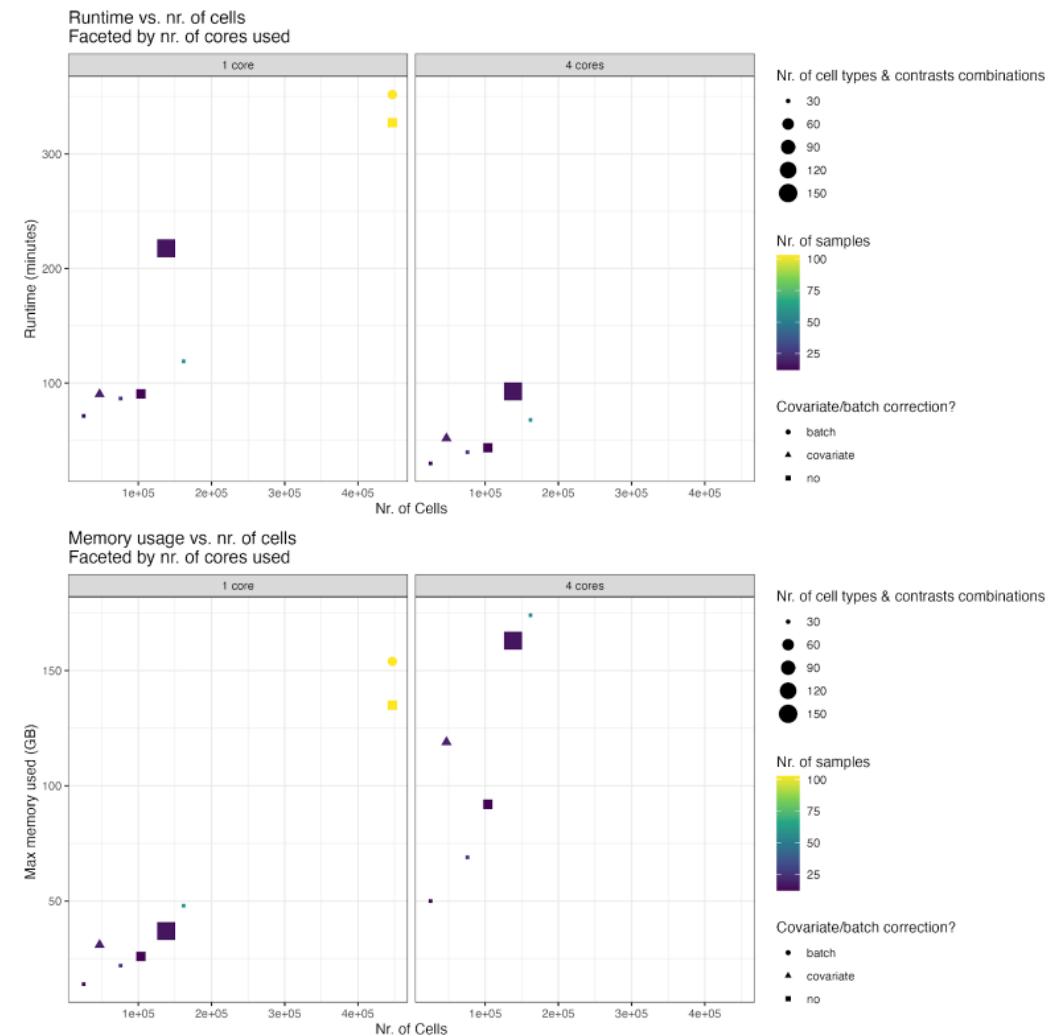


Running MultiNicheNet in practice

Quite some computational power required for big datasets

Suggested to:

1. Run the analysis on HPC infrastructure
2. Save the output object
3. Explore and interpret the output locally
 1. Focus on ligand-receptor interactions
 2. Zoom in on ligand-target interactions



What if I want to perform differential CCC analysis but I don't have (many) replicates?

Possible, but...

Realize that the analysis is based on a limited number of samples, and it will be impossible to draw strong conclusions. This may sometimes be the best you can get out of your data, but it is not a practice we recommend.

https://github.com/saeyslab/multinichener/blob/main/vignettes/basic_analysis_steps_MISC_SACL.knit.md

What else can I find on the github repository?

- <https://github.com/saeyslab/multinichener>

Guidelines for parameter changes and interpretation of output figures

To help users in interpreting parameter values and output figures, we provide the following two files:

- [Parameter interpretation](#): provides an explanation of different parameter choices - can help users in deciding when the default parameter values would not be optimal for their own dataset
- [Output figure interpretation](#): provides an explanation of different output figures - can help users in drawing hypotheses based on the output figures

MultiNicheNet parameter interpretation guidelines

| Parameter | Consequences of being more lenient | Consequences of being more stringent | Notes and recommendations |
|--|--|--|--|
| <p>Sample filtering:
 <i>min_cells = 10</i></p> <p>For each cell type, the considered samples are those samples with nr. of cells \geq <i>min_cells</i> parameter</p> | <p>You can be more lenient by decreasing this value.</p> <p>This will keep more samples per cell type in the analysis, and thus possibly more cell types as well.</p> <p>However, DE analysis-based results and prioritization criteria may be less trustworthy if based on several samples with a low nr of cells.</p> <p>Based on the mock analysis, the potential disadvantage of being lenient is likely to be limited (if not too extreme).</p> | <p>You can be more stringent by increasing this value.</p> <p>This can lead to a loss of cell types relevant to the condition of interest. For example, cell types that are more abundant in the condition of interest can be left out from analysis if not sufficiently present in the steady-state condition.</p> <p>Based on the mock analysis, being more stringent will probably not lead to improved prioritization of interactions between abundant cell types.</p> | <p>For datasets with several lowly abundant cell types of interest, we recommend using <i>min_cells</i> = 5.</p> <p>We explicitly recommend against using <i>min_cells</i> < 5 and <i>min_cells</i> > 50.</p> |
| <p>Gene filtering - sample proportion:
 <i>min_sample_prop = 0.50</i></p> <p>For each cell type, we consider genes expressed if they are expressed in at least <i>min_sample_prop</i> fraction of samples in the smallest condition.</p> | <p>You can be more lenient by decreasing this value.</p> <p>Based on the mock analysis, the influence of decreasing this parameter is limited.</p> | <p>You can be more stringent by increasing this value.</p> <p>Based on the mock analysis, the influence of increasing this parameter is limited.</p> | <p>We recommend using the default value.</p> |

MultiNicheNet output interpretation guidelines

Interaction validation guidelines

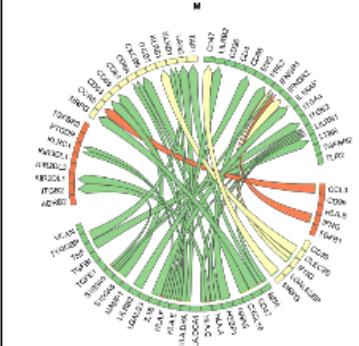
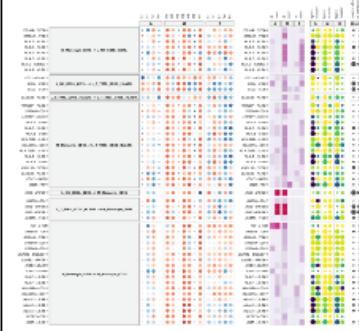
What are the properties of interactions that we recommend for follow-up experimental validation? In which downstream visualizations can MultiNicheNet users assess these properties?

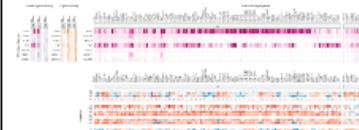
Ligand-receptor validation guidelines

| Properties of ideal interactions for follow-up experimental validation | Downstream visualizations | | | |
|--|----------------------------------|---|---|---|
| | <i>Interpretable bubble plot</i> | <i>Ligand activity - target gene combination plot</i> | <i>Intercellular regulatory network</i> | <i>Ligand-receptor single-cell expression violin plot</i> |
| Ligand and receptor are upregulated in the condition of interest | ✓ | | | ✓ |
| The ligand has a strong scaled "upregulatory" ligand activity in the condition of interest | ✓ | | | |
| The predicted ligand activity seems to be the result of an enrichment of multiple and specific target genes in the condition of interest | | ✓ | | |
| The ligand and receptor are cell-type specifically expressed | ✓ | | | |
| The ligand and receptor are sufficiently expressed in the majority of samples in the group of interest | ✓ | | | |
| The ligand-receptor interaction is a trustworthy protein-protein interaction with downstream signaling potential as supported by several databases | ✓ | | | |
| The ligand and receptor are target genes regulated by another prioritized ligand-receptor interaction. | | | ✓ | |
| | | | | |
| <u>The prioritized interaction is concordant with additional data on the research question.</u> | Not applicable | | | |

Visualization interpretation guidelines

The following table documents the goals and limitations of each downstream visualization of MultiNicheNet's output:

| Visualization type | Aims of the visualization | Limitations of the visualization |
|---|---|--|
| ChordDiagram circos plot
 | Summary of the top prioritized senderLigand-receiverReceptor interactions per condition (between all cell types or between cell type pairs of interest). | Does not visualize data underlying the prioritization of these interactions. |
| Interpretable bubble plot
 | Interpret the prioritization: help users decide which interactions may be most interesting for follow-up experimental validation.

This visualization shows differential expression, ligand activity, cell-type specific expression, fraction of expression, and Omnipath database metrics for a selected subset of senderLigand-receiverReceptor interactions. | Does not visualize the specific target genes downstream of the prioritized interactions. Hereby, the user cannot assess whether high activity values may be due to a reasonable number of specific target genes. |
| Ligand activity - target gene combination plot
 | Inspect the predicted target genes behind ligand activity predictions. The genes shown are the top target genes of the ligand that have contributed to the ligand activity prediction of that interaction. Users can inspect the regulatory potential scores of each ligand-target link and the expression of each target gene in each sample. | Shows how well ligand-target links are supported by general prior knowledge, but not whether they are likely to be active in the system under study. |

Conclusions

MultiNicheNet

= comprehensive tool for differential cell-cell communication analysis
from multi-sample scRNA-seq data with complex designs

Running MultiNicheNet is straightforward. The most important aspect is taking time to explore all its output to understand what is going on in your data.

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Ligand-Receptor interaction inference with CellChat



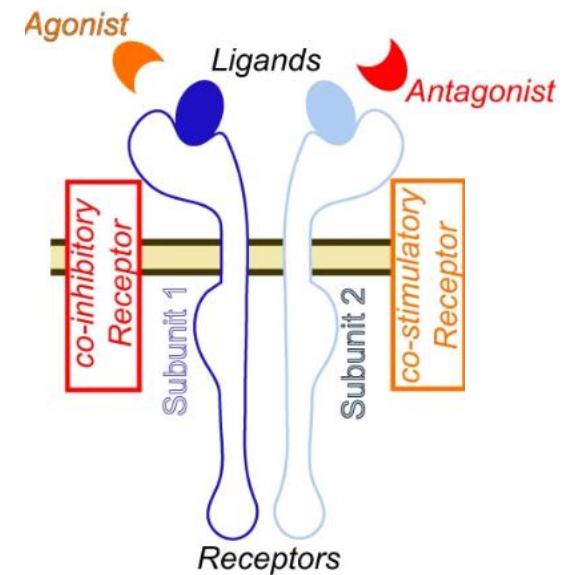
CellChat: main concepts (I)

- Modelling of LR pairs with complex architecture (*multi-subunit*)
- Conservative modelling: multi-subunit complexes are required to show expression of all components (*geometric mean*)

$$L_i = \sqrt[m_1]{L_{i,1} \cdots L_{i,m_1}} \quad R_j = \sqrt[m_2]{R_{j,1} \cdots R_{j,m_2}} \cdot \frac{1 + RA_j}{1 + RI_j}.$$

i : sender cell type *j : receiver cell type*

- Complex estimation of LR interaction activity (*agonist/antagonist and co-inhibitory/stimulatory subunits*)



CellChat: main concepts (II)

- LR interaction activity estimated via Hill functions leveraging for the presence of agonists and antagonists

$$P_{i,j}^k = \frac{L_i R_j}{K_h + L_i R_j} \times \left(1 + \frac{AG_i}{K_h + AG_i}\right) \cdot \left(1 + \frac{AG_j}{K_h + AG_j}\right) \\ \times \frac{K_h}{K_h + AN_i} \cdot \frac{K_h}{K_h + AN_j}$$

*k : ligand – receptor pair AG_{i/j} : agonist
i : sender cell type AN_{i/j} : antagonist
j : receiver cell type*

- Significance of each $P_{i,j}^k$ is evaluated by a permutation test via label switching

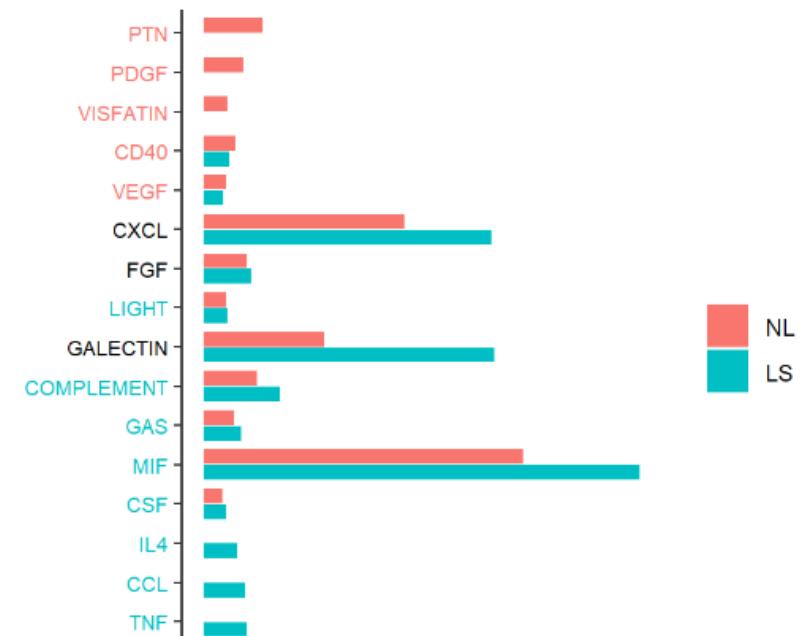
CellChat output: one LRI output for each condition

| source
<chr> | target
<chr> | ligand
<chr> | receptor
<chr> | prob
<dbl> | pval
<dbl> | ▶ |
|--------------------------|----------------------|-----------------|-------------------|---------------|---------------|---|
| Platelet | L_T_TIM3_CD38_HLADR. | PF4 | CXCR3 | 0.0139852121 | 0.00 | |
| Platelet | L_T_Proliferating | PF4 | CXCR3 | 0.0329946222 | 0.00 | |
| Platelet | L_Plasmablast | PF4 | CXCR3 | 0.0023576219 | 0.00 | |
| Platelet | M_DC_pDC | PF4 | CXCR3 | 0.0540138624 | 0.00 | |
| M_Monocyte_CD16 | L_T_TIM3_CD38_HLADR. | CXCL16 | CXCR6 | 0.0069209789 | 0.00 | |
| M_Monocyte_CD16 | L_T_Proliferating | CXCL16 | CXCR6 | 0.0032953796 | 0.00 | |
| L_T_CD4_Naive | L_T_TIM3_CD38_HLADR. | MIF | CD74_CXCR4 | 0.1107371599 | 0.00 | |
| L_T_TIM3_CD38_HLADR. | L_T_TIM3_CD38_HLADR. | MIF | CD74_CXCR4 | 0.0977901009 | 0.00 | |
| L_T_CD8_Naive | L_T_TIM3_CD38_HLADR. | MIF | CD74_CXCR4 | 0.1117472762 | 0.00 | |
| L_T_Memory_CCR6_enriched | L_T_TIM3_CD38_HLADR. | MIF | CD74_CXCR4 | 0.1187984406 | 0.00 | |

CellChat downstream analysis and comparison of multiple conditions

Possibility to combine different cell chat objects and perform systems-based analysis:

- LRIs pathway analysis (no single LRI result resolution)
- Identify signaling roles (e.g., dominant senders, receivers)
- Identify signals contributing to outgoing or incoming signaling of certain cell groups



CellChat recap

CellChat: main points

- Accurate Ligand-Receptor Interaction (LRI) modelling
- Complex LRI scoring functions (taking into account agonists, ...)
- Systems-based data exploration
- Conservative approach, relies on RNA levels as a proxy for LR components protein expression

CellChat & NicheNet as complementary tools to study CCC events:

- LRIs can be selected based on the orchestration of LR components (CellChat)
- Selected ligands can be ranked for their capacity to explain the differential expression induced by the active CCC event (NicheNet)