

# Introduction to comparative analysis of cell-cell communication and spatial analysis

Ivan G. Costa, James Nagai, Mayra Ruiz, Barbara Di Camillo, Giacomo Baruzzo,  
Giulia Cesaro

Institute for Computational Genomics, Joint Research Centre for Computational Biomedicine, RWTH Aachen University, Germany

Department of Information Engineering, University of Padova, Padova, Italy



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# 1. Differential Cell Cell Communication Analysis



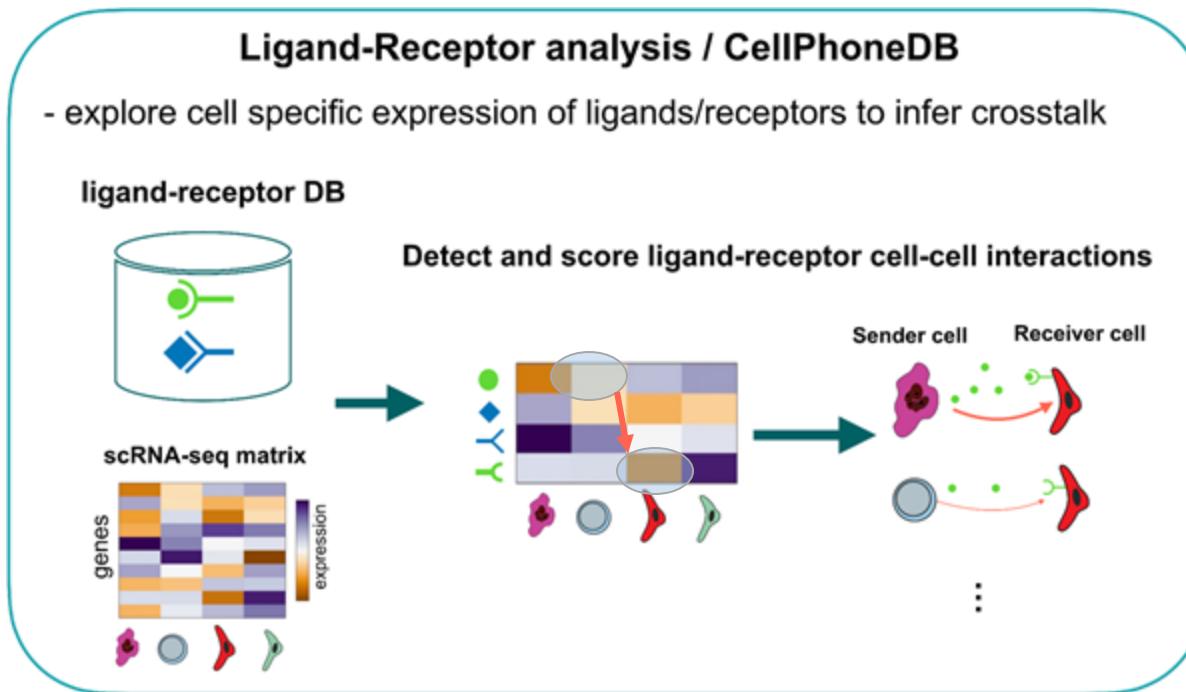
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# Computational Inference of Cell Cell Communication



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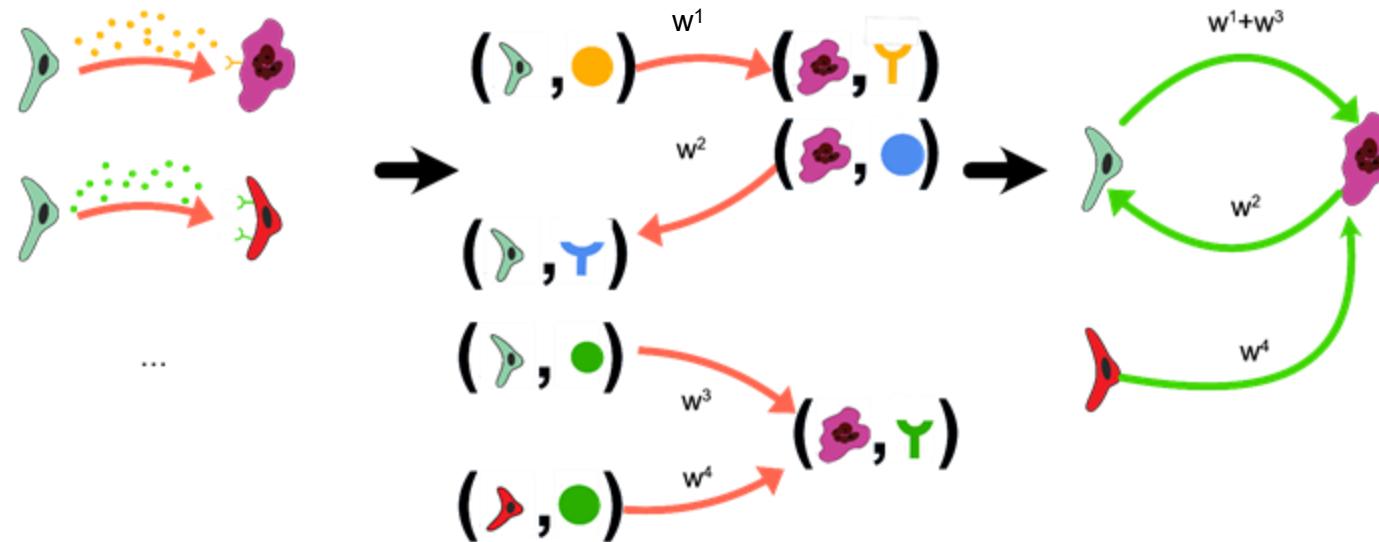
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# From ligand-receptor pairs to cell-cell networks

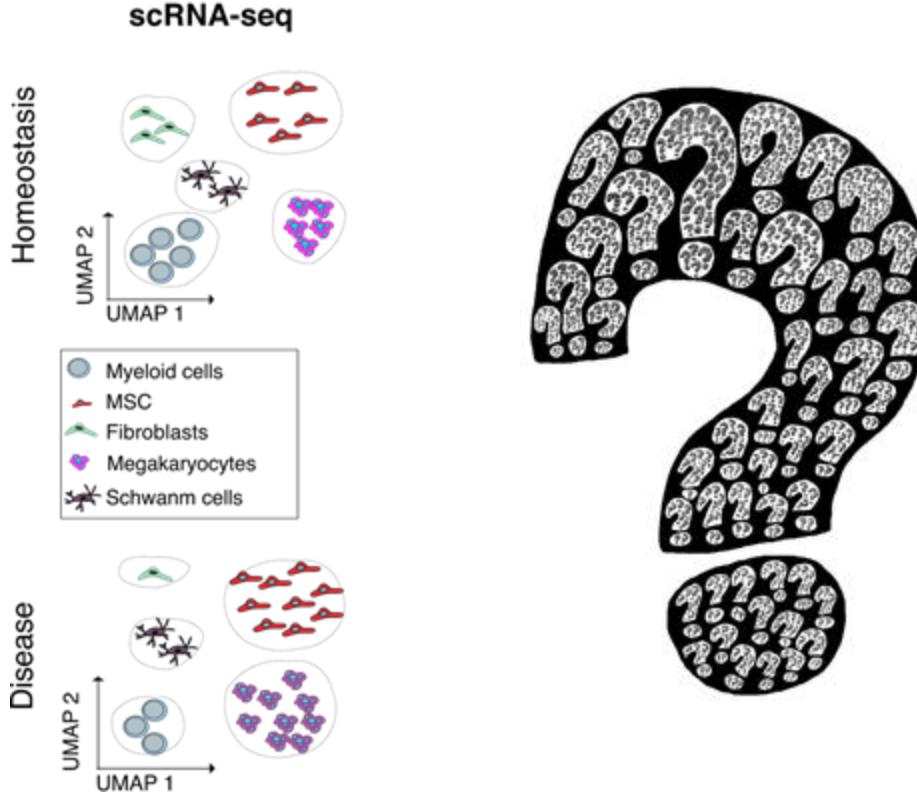
Cell-Ligand / Cell Receptor Network

Gene-Cell Network

Cell-Cell Network



# Differential Cell Cell Communication Analysis



Nagai, James S., et al. "CrossTalker: analysis and visualization of ligand–receptor networks." *Bioinformatics* 37.22 (2021): 4263-4265.



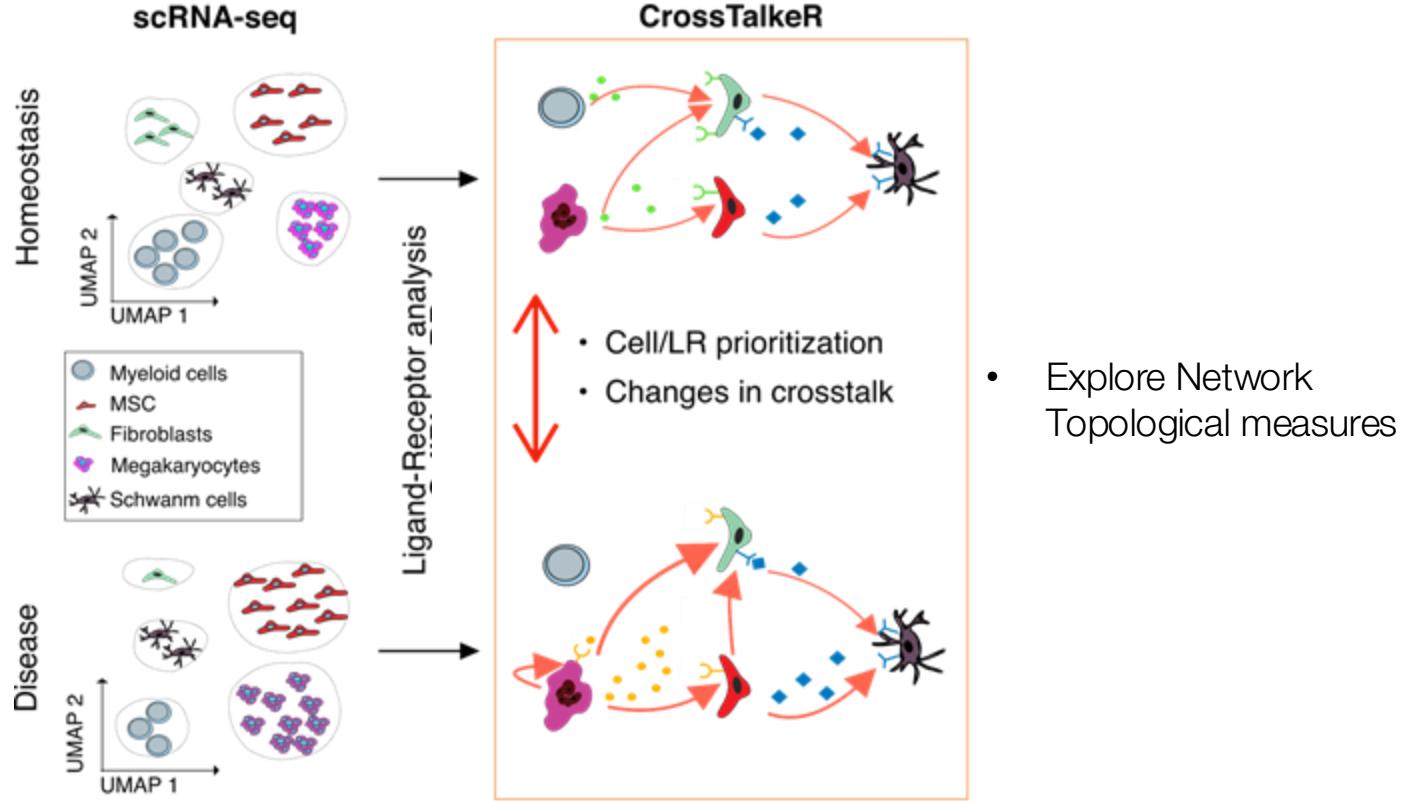
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# Differential Cell Cell Communication Analysis



Nagai, James S., et al. "CrossTalker: analysis and visualization of ligand–receptor networks." *Bioinformatics* 37.22 (2021): 4263-4265.

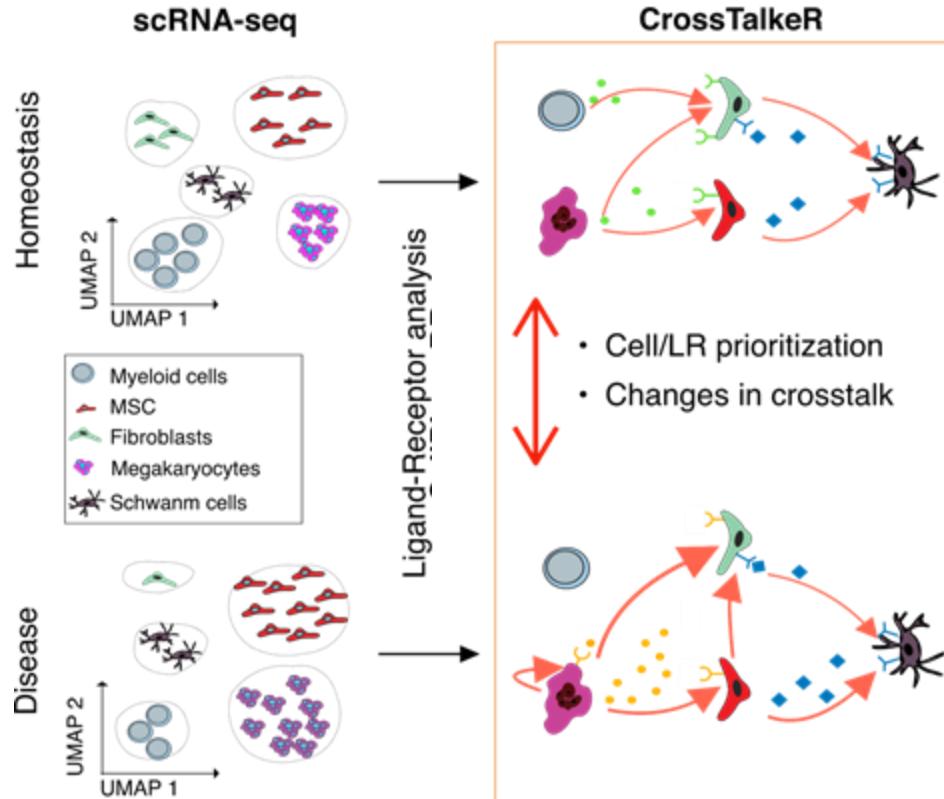


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# CrossTalker Local Measures



Nagai, James S., et al. "CrossTalker: analysis and visualization of ligand–receptor networks." *Bioinformatics* 37.22 (2021): 4263-4265.

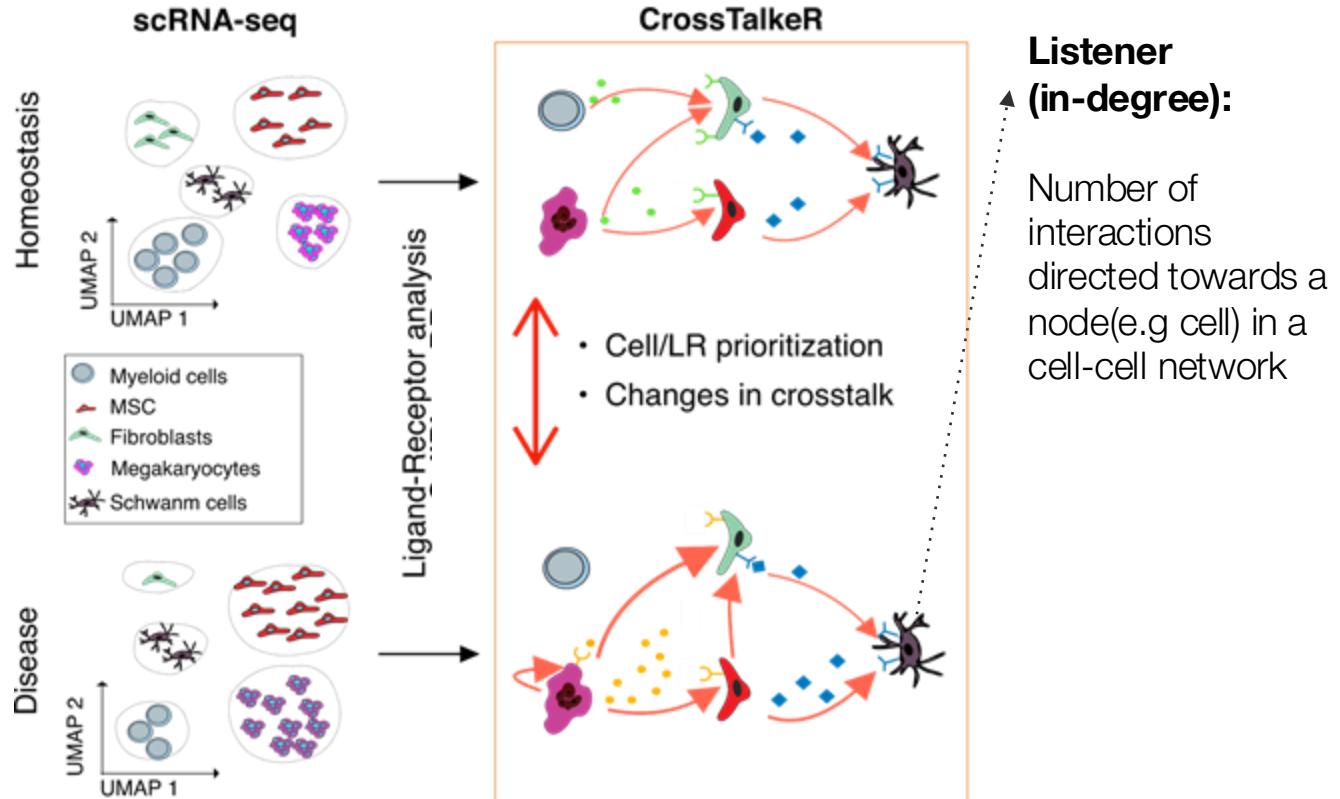


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# CrossTalker Local Measures



Nagai, James S., et al. "CrossTalker: analysis and visualization of ligand–receptor networks." *Bioinformatics* 37.22 (2021): 4263-4265.



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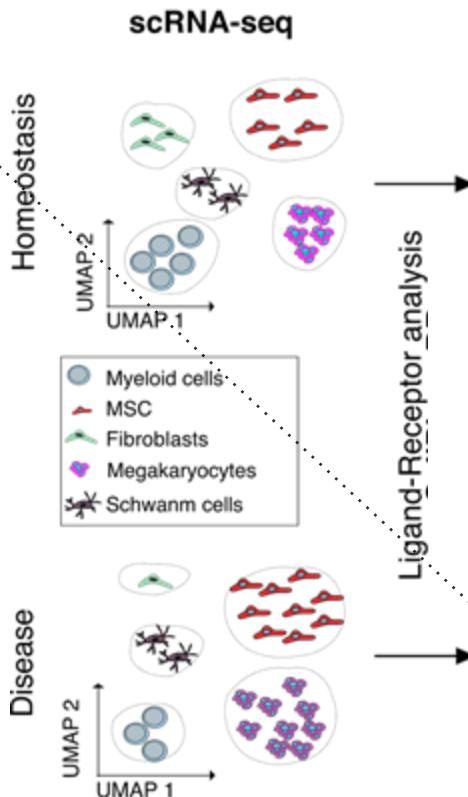
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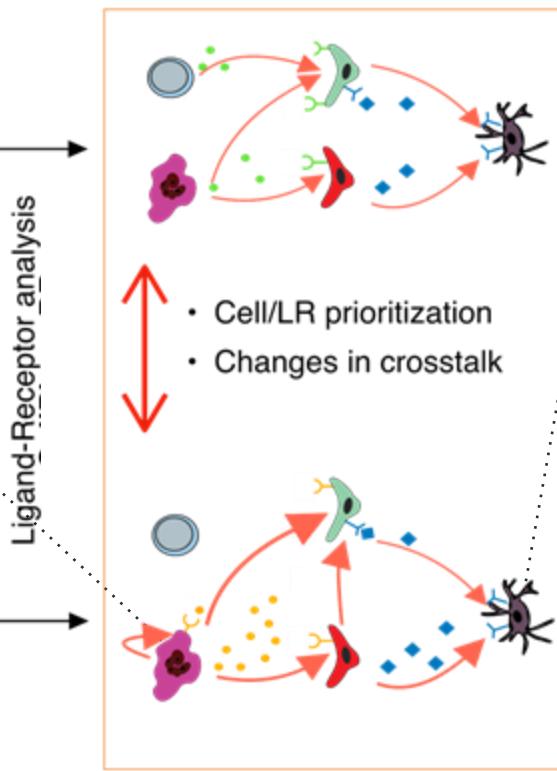
# CrossTalker Local Measures

## Influencer (out-degree):

Number of interactions directed out of a node (e.g. cell) in a cell-cell network



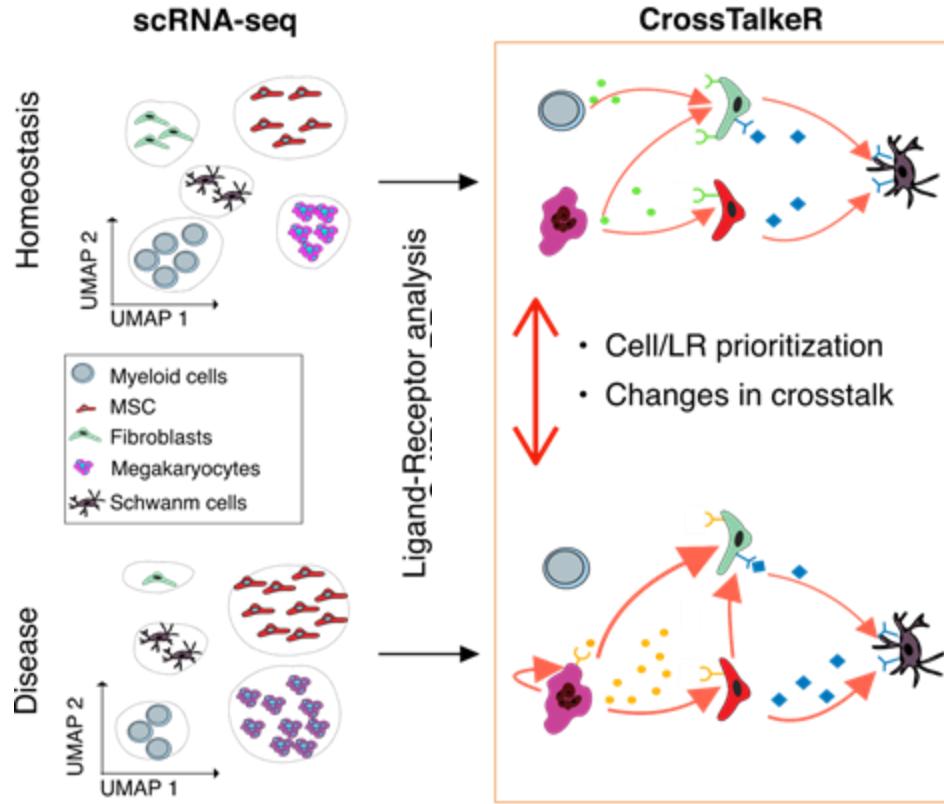
## CrossTalker



## Listener (in-degree):

Number of interactions directed towards a node(e.g cell) in a cell-cell network

# CrossTalker Global Measures



Nagai, James S., et al. "CrossTalker: analysis and visualization of ligand–receptor networks." *Bioinformatics* 37.22 (2021): 4263-4265.



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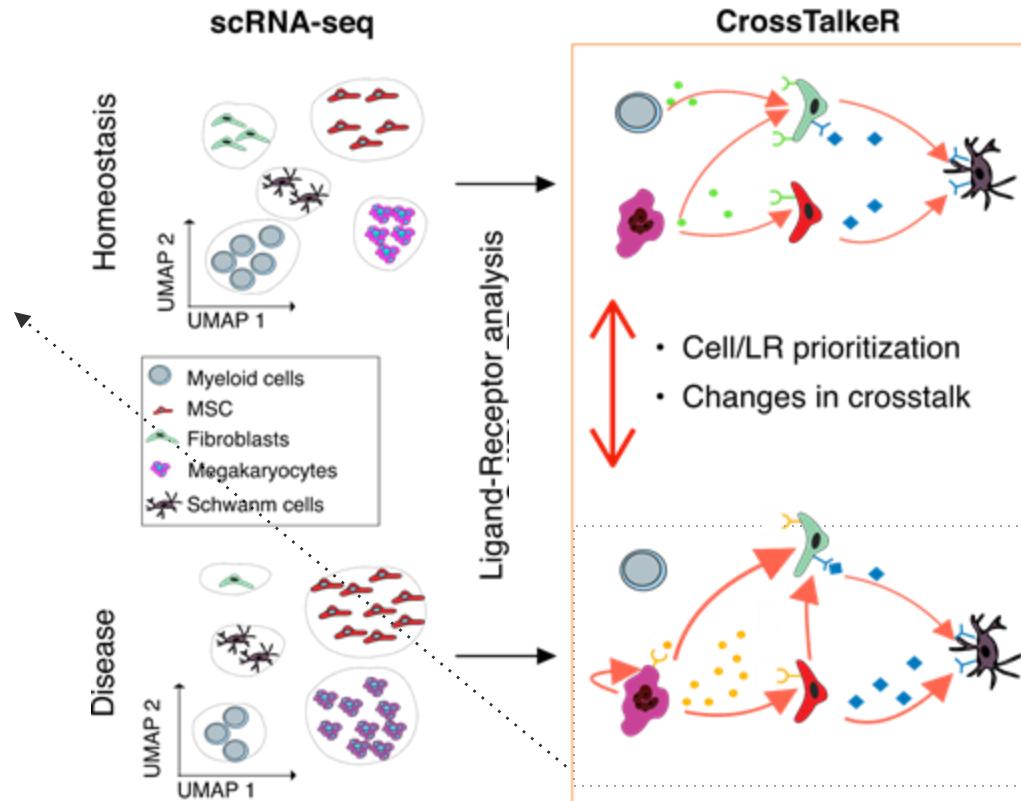
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# CrossTalker Global Measures

## Pagerank:

Algorithm used to rank the importance of web-pages. It is the probability of arriving at a cell after a large number of cell-cell interactions



Nagai, James S., et al. "CrossTalker: analysis and visualization of ligand–receptor networks." *Bioinformatics* 37.22 (2021): 4263-4265.



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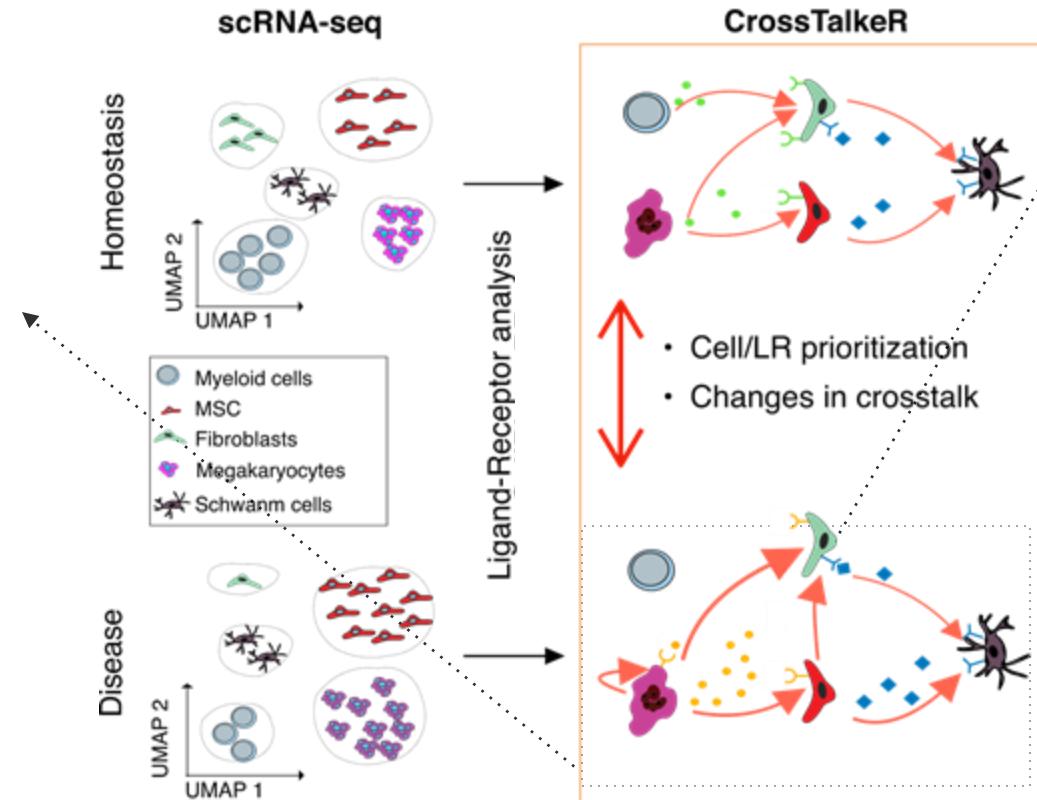
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# CrossTalker Global Measures

## Pagerank:

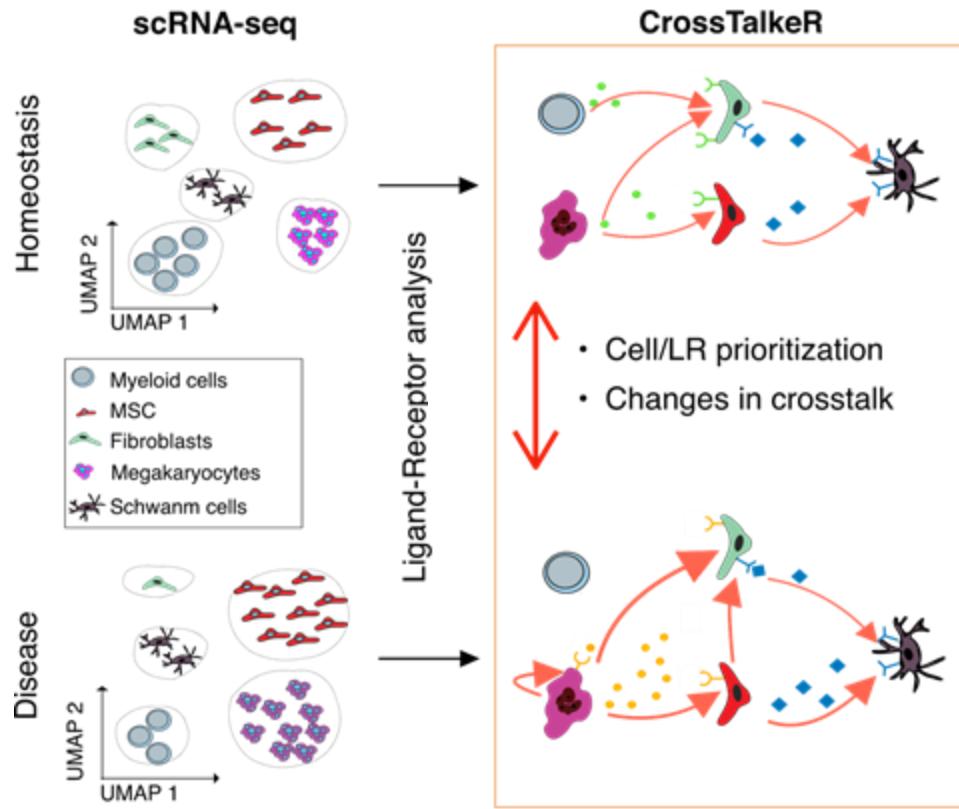
Algorithm used to rank the importance of web-pages. It is the probability of arriving at a cell after a large number of cell-cell interactions



## Mediator (Betweenness Centrality):

overall fraction of shortest path including a certain node

# CrossTalker Differential Analysis



## Differential Analysis ( $\Delta$ ):

- LogOdds (Pagerank (Dis.)/Pagerank(Hom.))
- Mediator (Dis.) - Mediator (Hom)

Nagai, James S., et al. "CrossTalker: analysis and visualization of ligand–receptor networks." *Bioinformatics* 37.22 (2021): 4263-4265.



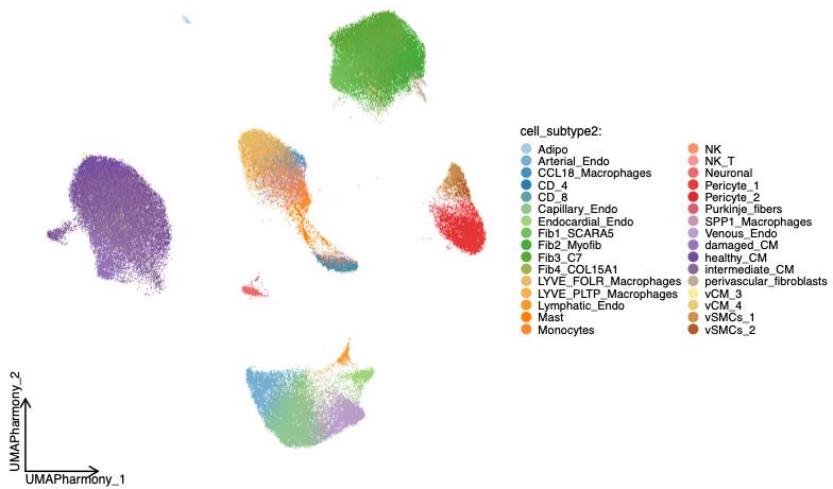
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# Example: Myocardial Infarction

## Single cell – Cell Types/States



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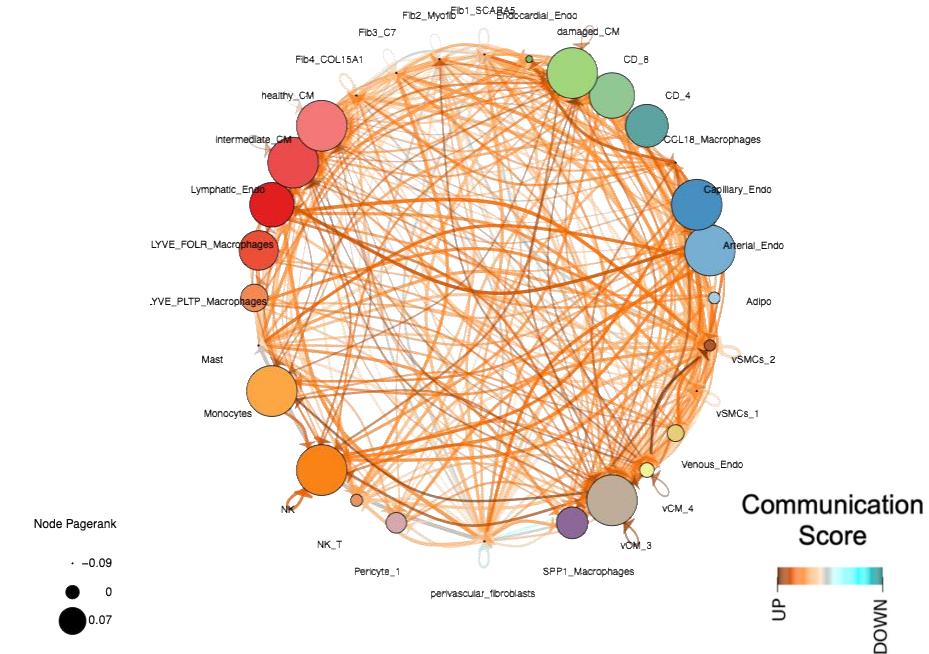
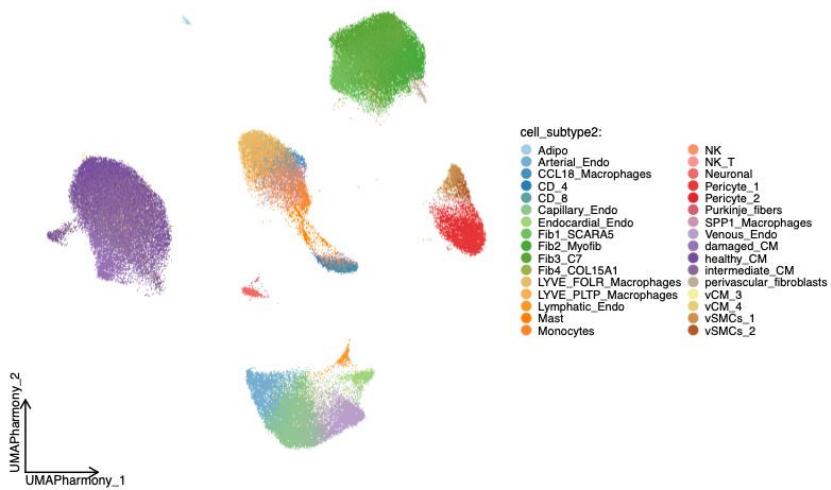
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# Example: Myocardial Infarction

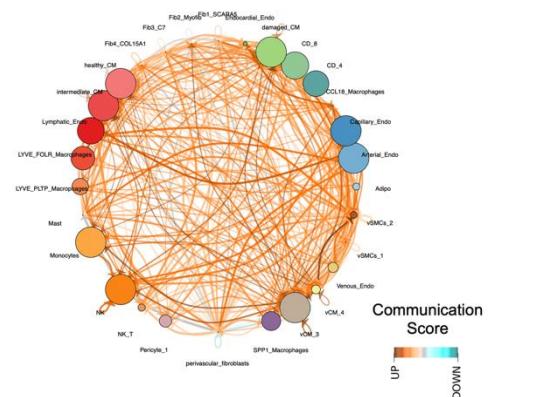
CCI Plot – Ischemic vs Myogenic

Single cell – Cell Types/States



# Example: MI – Network based Analysis

CCI Plot  
Ischemic vs Myogenic



Nagai, James S., et al. "CrossTalker: analysis and visualization of ligand–receptor networks." *Bioinformatics* 37.22 (2021): 4263-4265.



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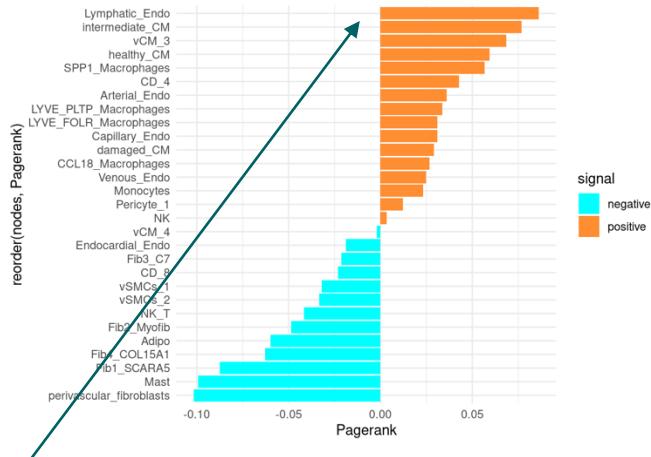
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# Example: MI – Network based Analysis

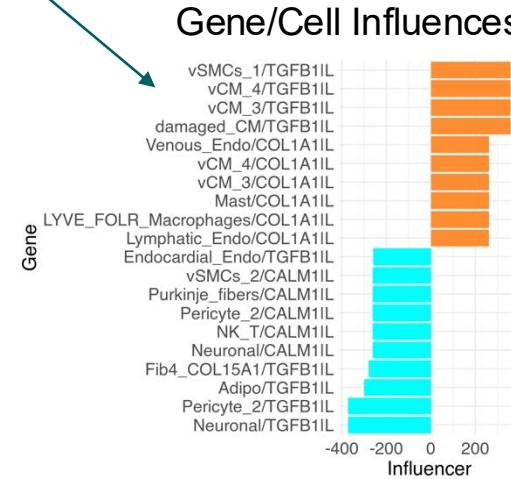
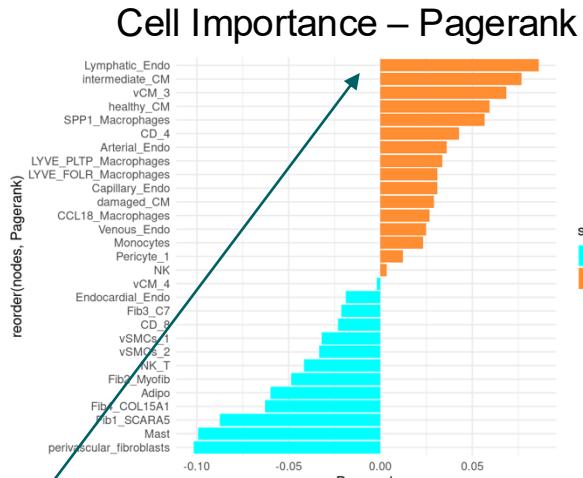
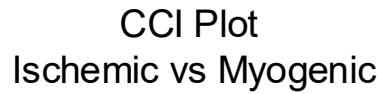
CCI Plot  
Ischemic vs Myogenic

Cell Importance – Pagerank



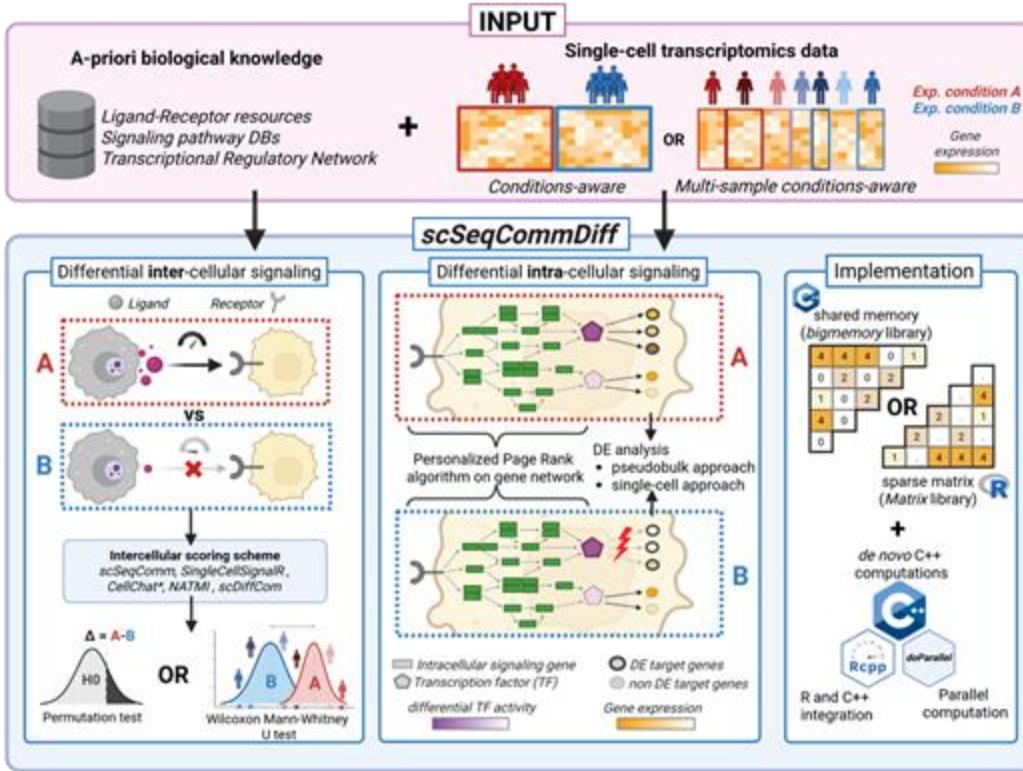
Lymphatic endothelial cell – responsible for immune cell invasion/recruitment  
CM\_intermediary – initial injured state of cardiomyocytes (CM)  
SPP\_macrophages – phagocytic cells performing tissue clearance

# Example: MI – Network based Analysis



Lymphatic endothelial cell – responsible for immune cell invasion/recruitment  
CM\_intermediary – initial injured state of cardiomyocytes (CM)  
SPP\_macrophages – phagocytic cells performing tissue clearance

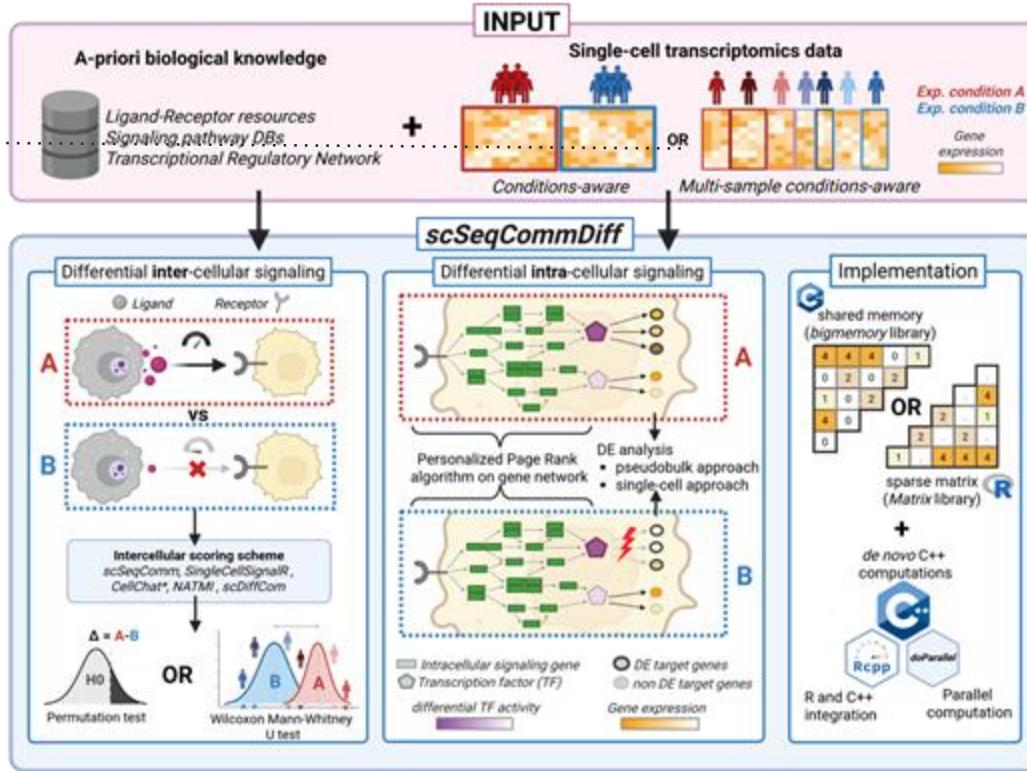
# scSeqCommDiff



# scSeqCommDiff

## Experimental design:

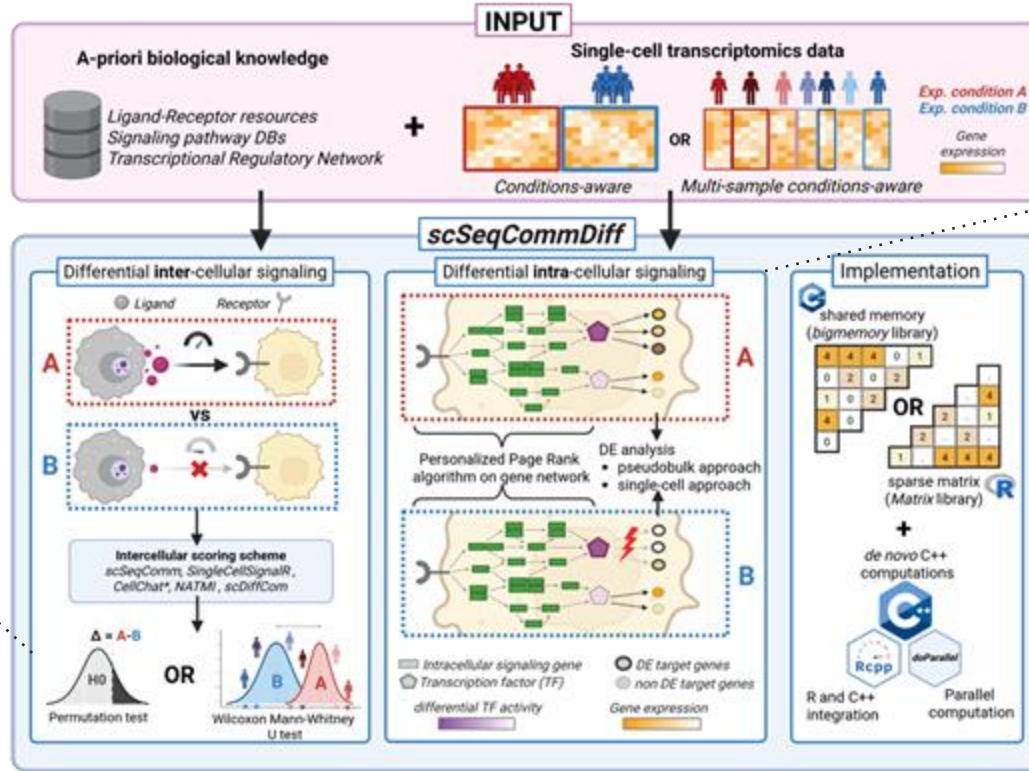
Identify condition-level differences (*conditions-aware*) or capturing within-condition heterogeneity (*multi-sample conditions-aware*)



# scSeqCommDiff

## Differential Inter-cellular signaling

non-parametric statistical test for assessing significant differential changes in intercellular scores between conditions



## Differential Intra-cellular signaling

Network-based and statistical-based approach to identify differential transcriptional regulation of regulome modules

## 2. Spatial transcriptomics analysis



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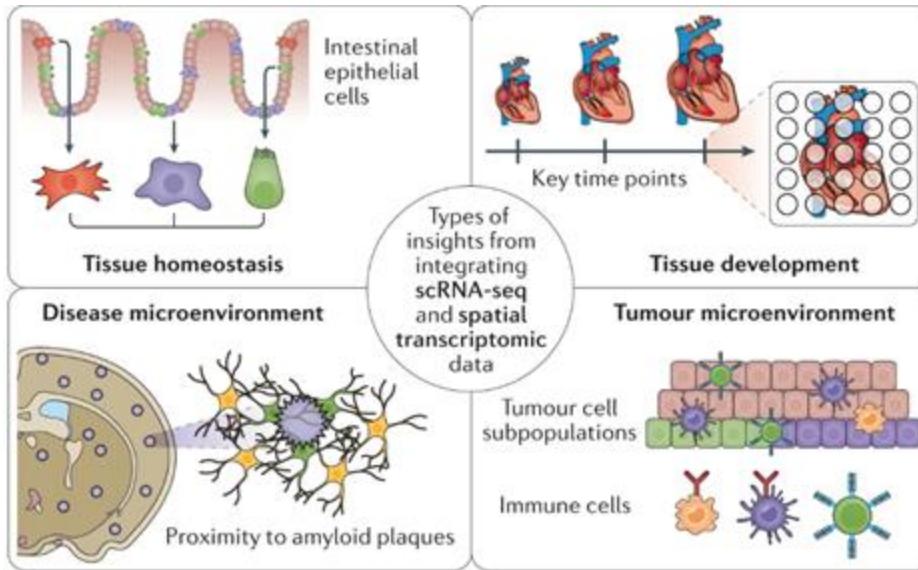
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# Spatial Transcriptomics

organization of cells is crucial to understand diseases

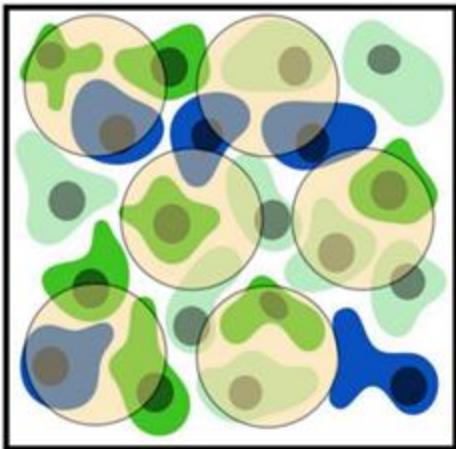


single cell dissociate tissue / spatial information is lost

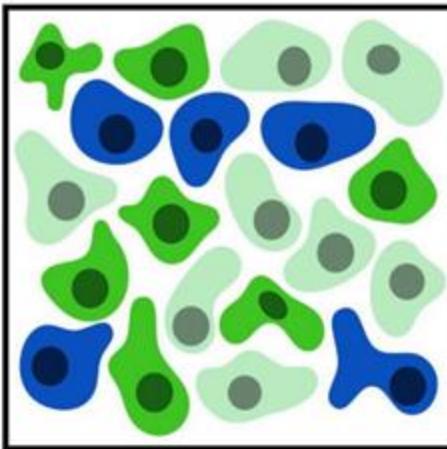
# Spatial Transcriptomics - Technologies

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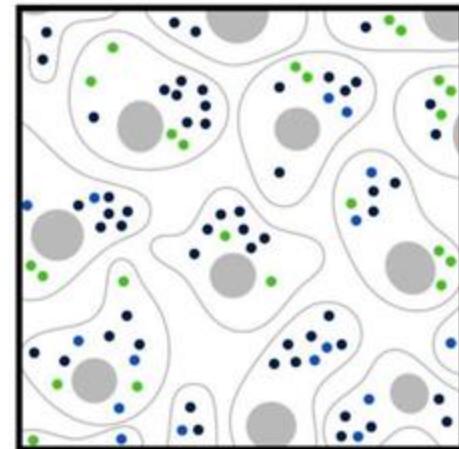
**Multi-cell resolution**



**Single-cell resolution**



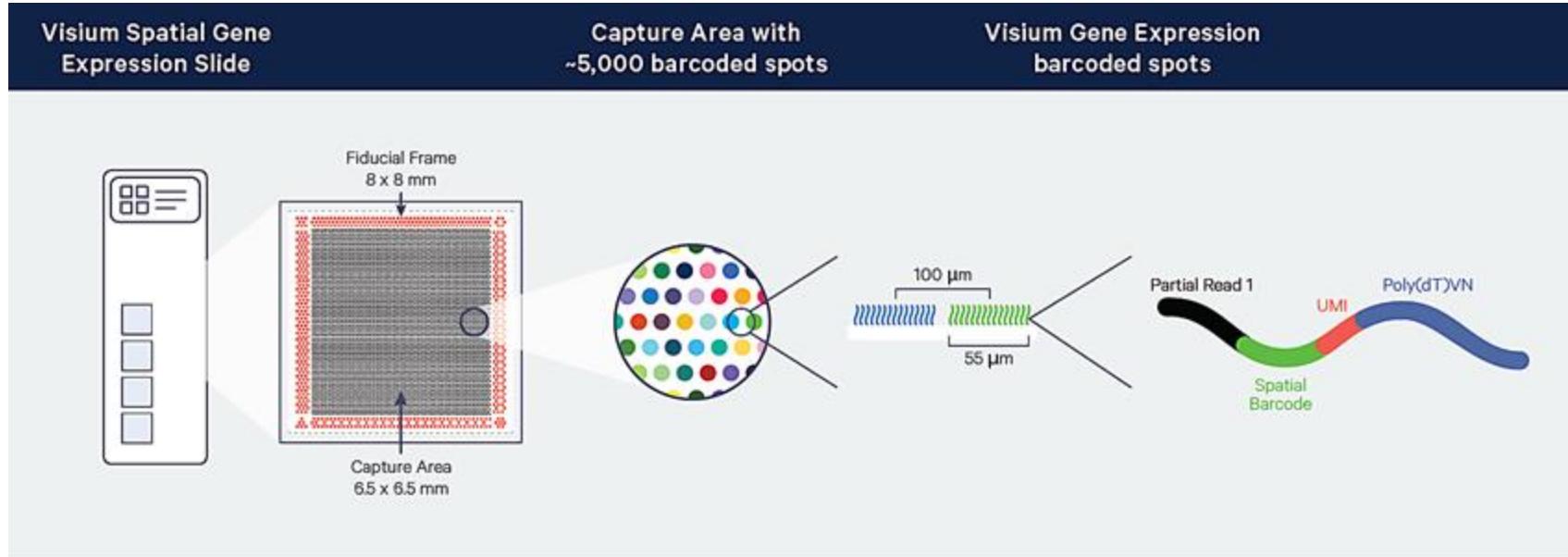
**Sub-cellular resolution**



- all genes
- cannot resolve single cells

- measure only some genes (100-5000)
- higher costs

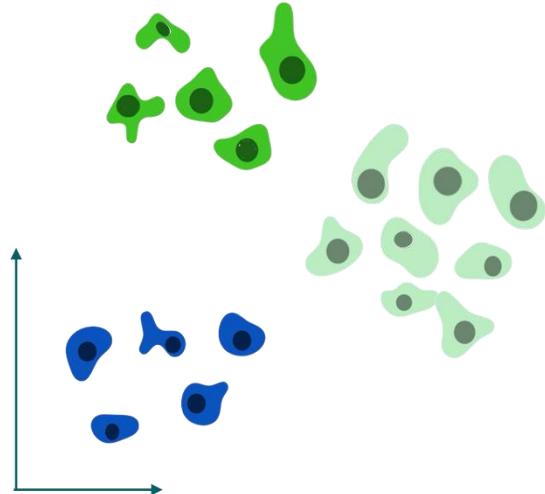
# Spatial Transcriptomics - Visium 10X



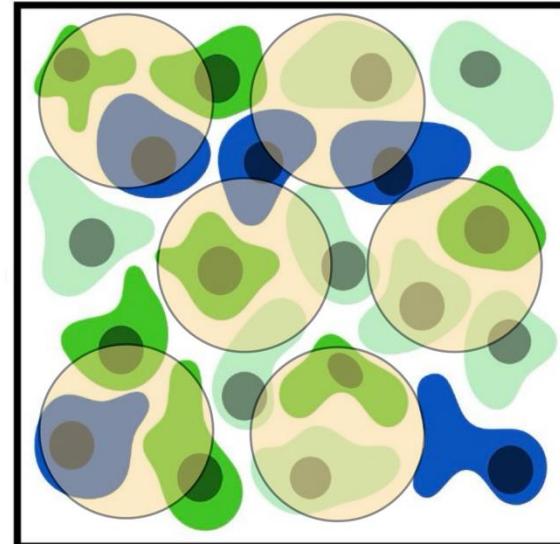
# Spatial Transcriptomics – Cell Deconvolution

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**Reference Single Cell**



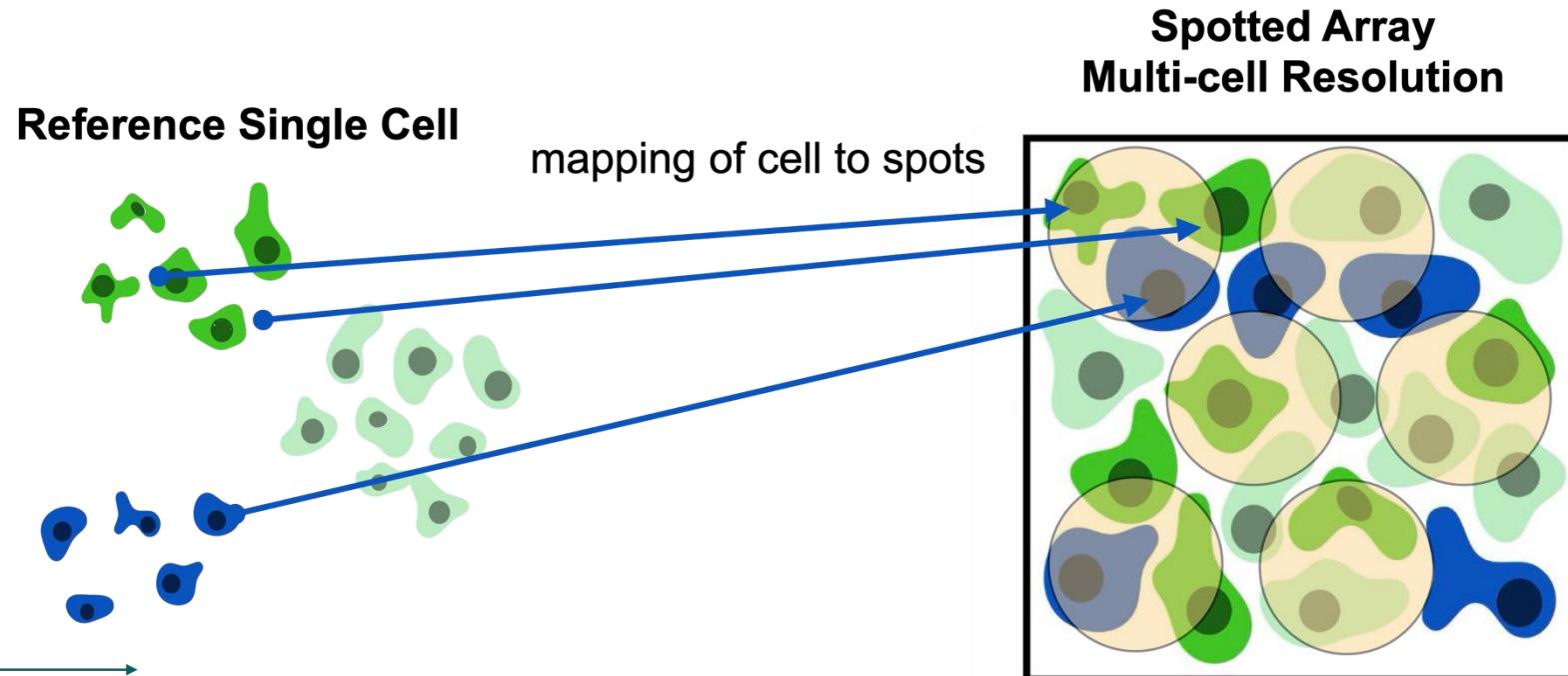
**Spotted Array  
Multi-cell Resolution**



How do we know what celltypes are in each spot??

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# Spatial Transcriptomics – Cell Deconvolution

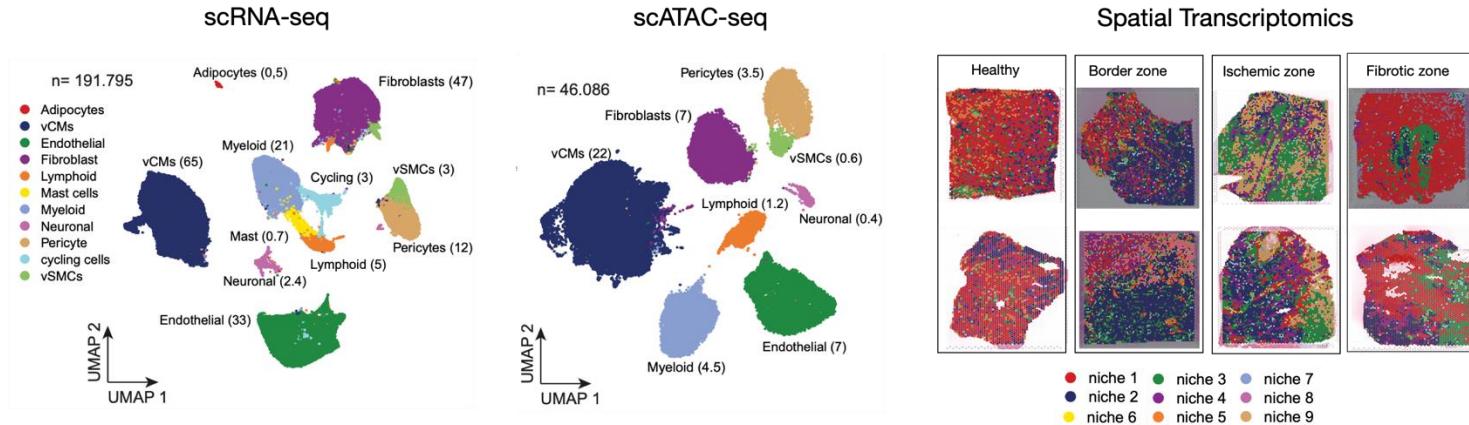
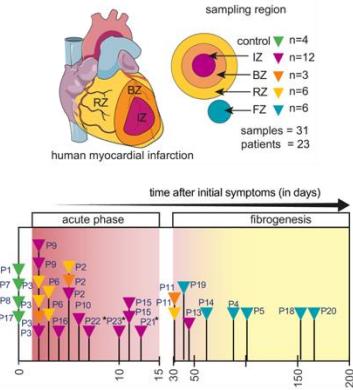


Solution: Optimal Transport (NovoSparc, MOSCOT);  
Cell Deconvolution (Cell2Location; STdeconvolve)

# Example – Myocardial Infarction

Multimodal data (single cell, spatial and histology) of 31 heart samples

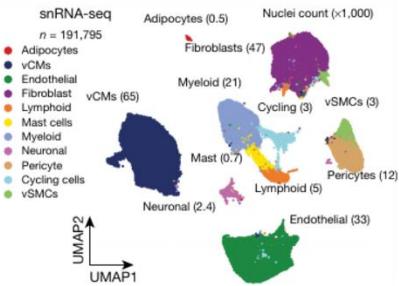
## study design



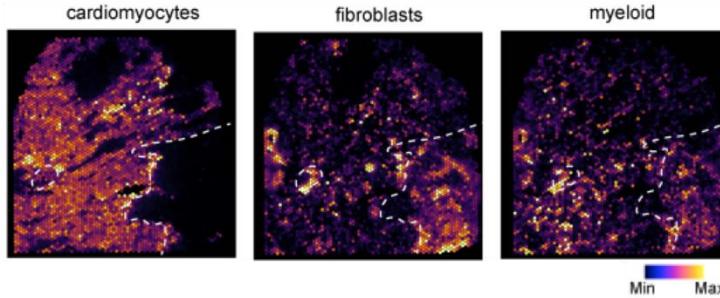
Samples were either from **Myogenic** (Control + Remote Zones), **Ischemic** (IZ) or **Fibrotic** (FB)

# Cell Deconvolution – Myocardial Infarction

## snRNA-seq

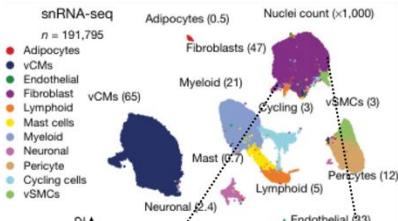


cell2location  
→

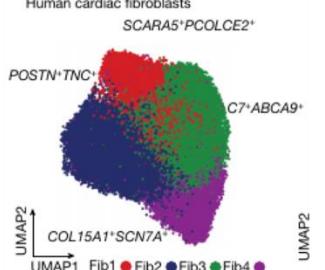
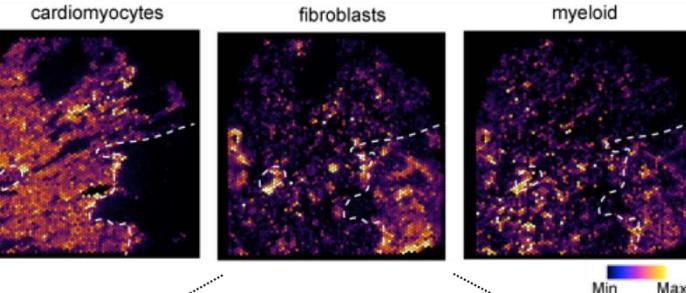


# Cell Deconvolution – Myocardial Infarction

## snRNA-seq

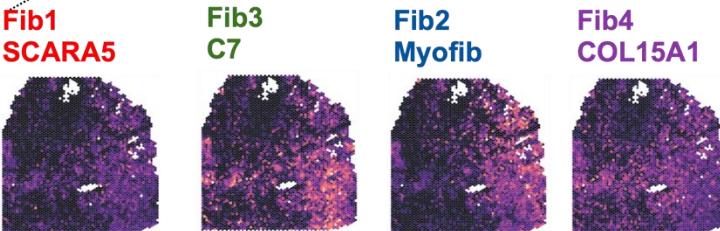


cell2location



Personalized OT  
(Moscot)

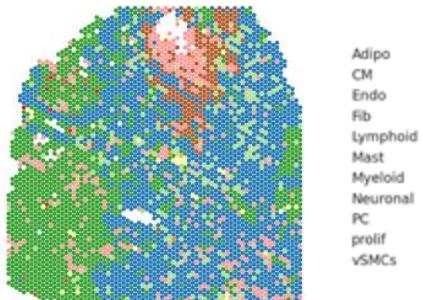
Sample specific  
scRNA-seq to ST mapping



## Fibroblast sub-clustering

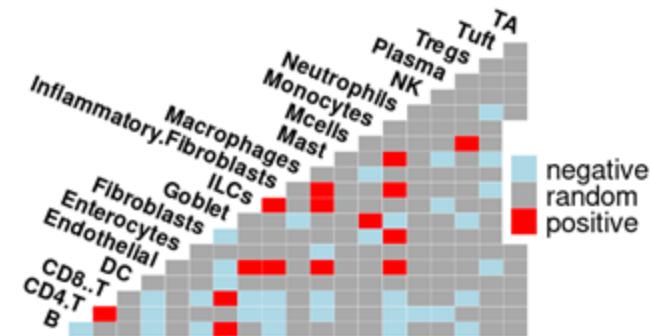
# Cell type co-localization analysis (ISCHIA)

Cell types in space



Which **cell type pairs** can be found **in the same spot** more often than expected by chance?

Cell type co-localization matrix

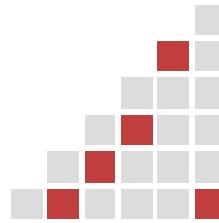


# Differential co-localization networks

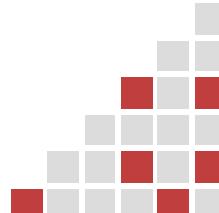
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## Cell type co-localization

Condition 1



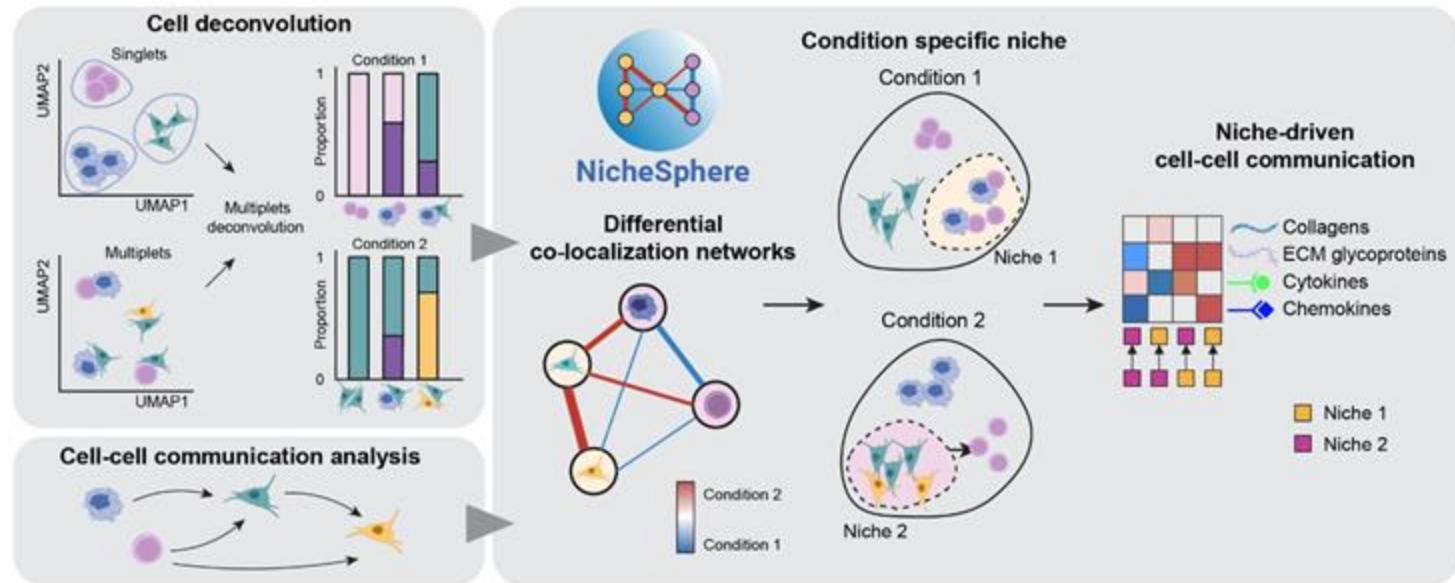
Condition 2



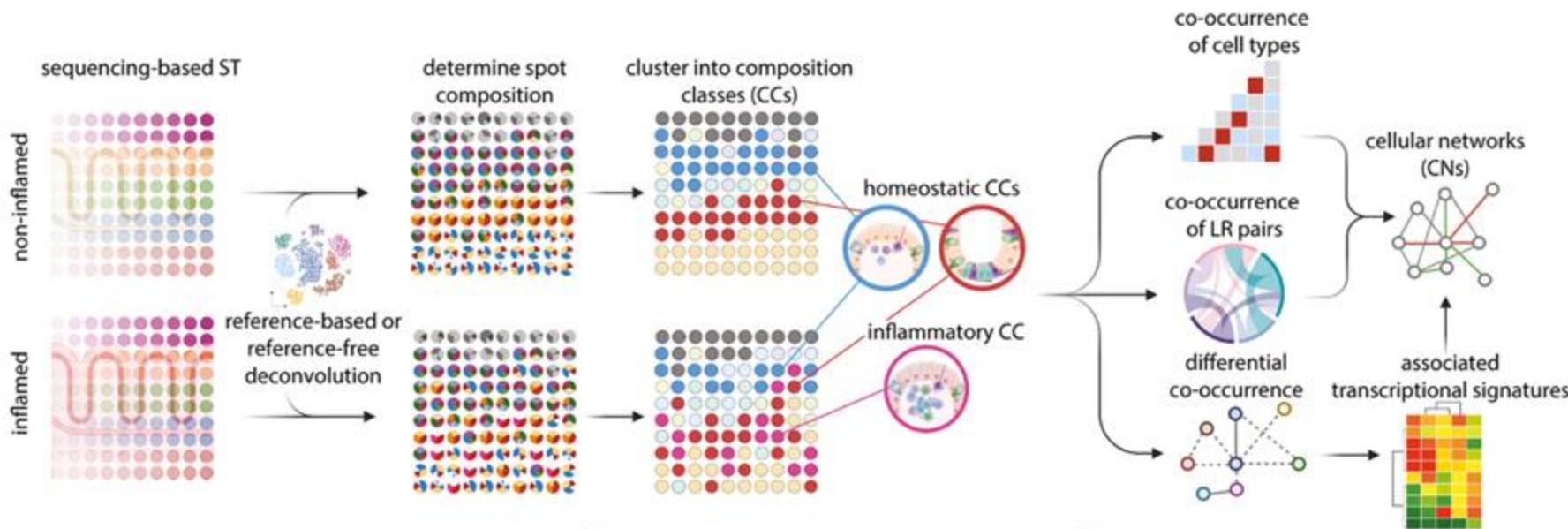
Which **cell types get together** in a specific **condition** (eg: a disease)?  
How do **Ligand - Receptor interactions** relate to these changes?

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# Spatially driven differential cell-cell communication (Nichesphere)



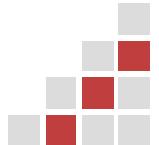
# Cell type co-localization analysis (ISCHIA)



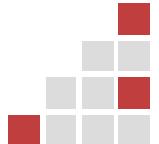
# Differential co-localization networks

Cell type co-localization

Condition 1

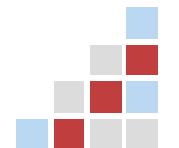


Condition 2



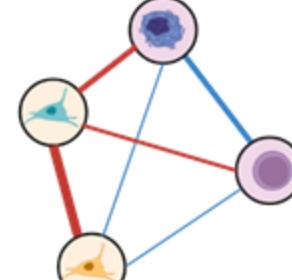
Differential co-localization test

Differential co-localization scores



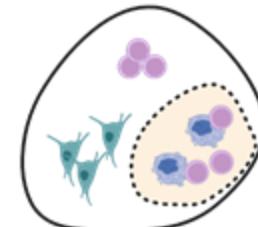
- Condition 2
- Condition 1

Network and community detection

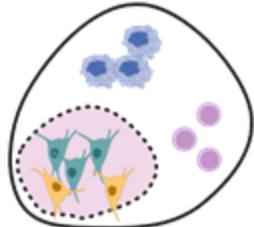


- Niche 1
- Niche 2

Condition 1



Condition 2



# Differential co-localization networks – Myocardial Infarction

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Cell State  
Spatial Mapping

Myogenic



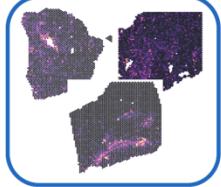
Ishemic



# Differential co-localization networks – Myocardial Infarction

## Cell State Spatial Mapping

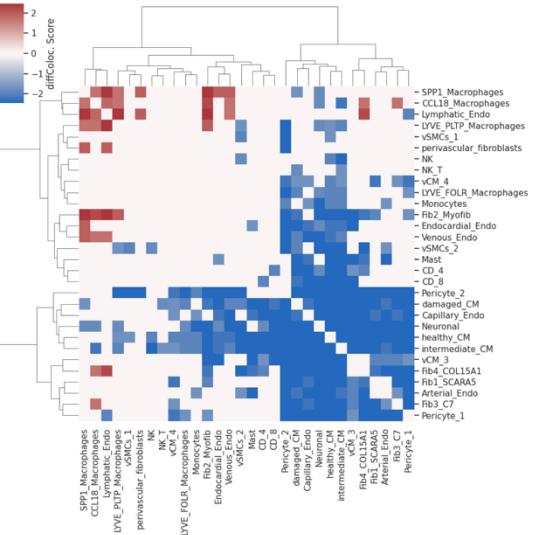
### Myogenic



statistical test

### Differential Co-localization

Disease  
Controls



# Differential co-localization networks – Myocardial Infarction

## Cell State Spatial Mapping

Myogenic

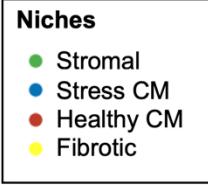
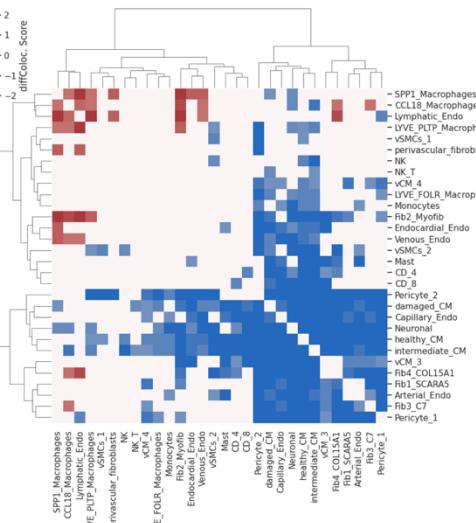


Ischemic

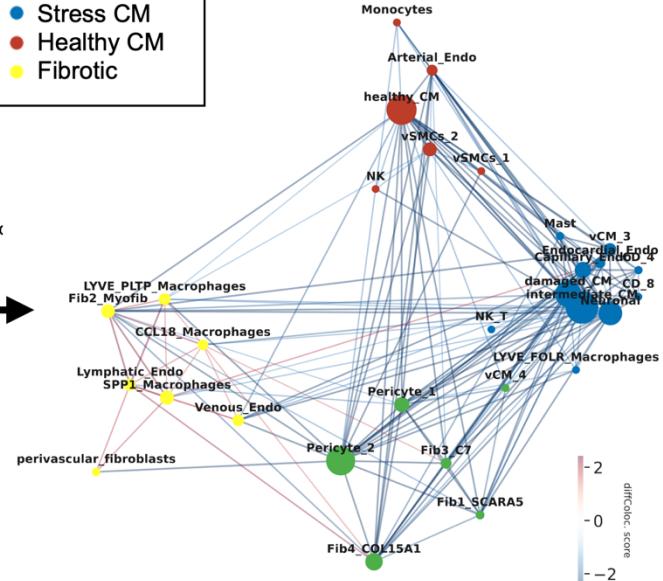


Disease  
Controls  
statistical test

## Differential Co-localization

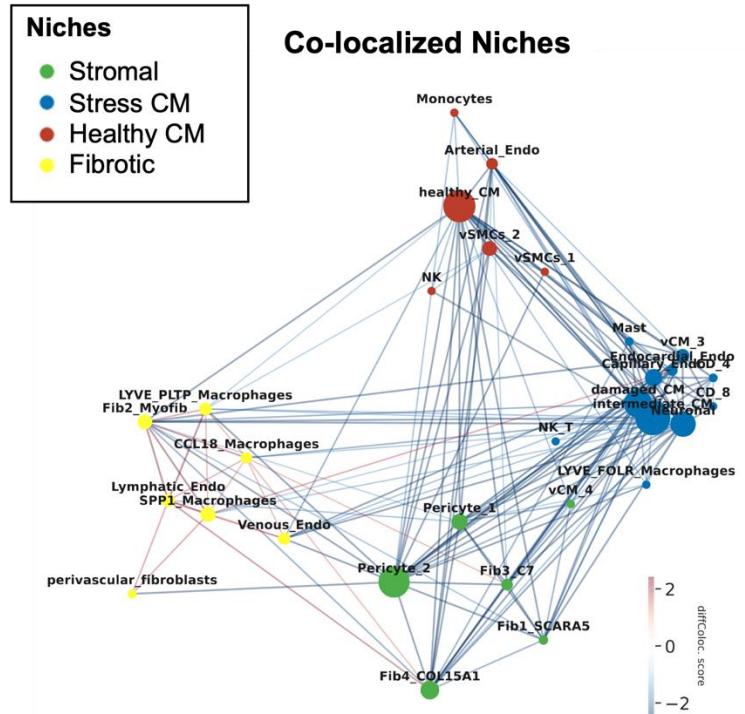


## Co-localized Niches

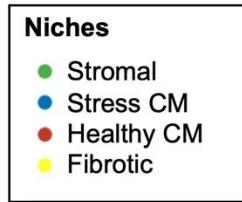


Graph representation  
+ Community  
Detection

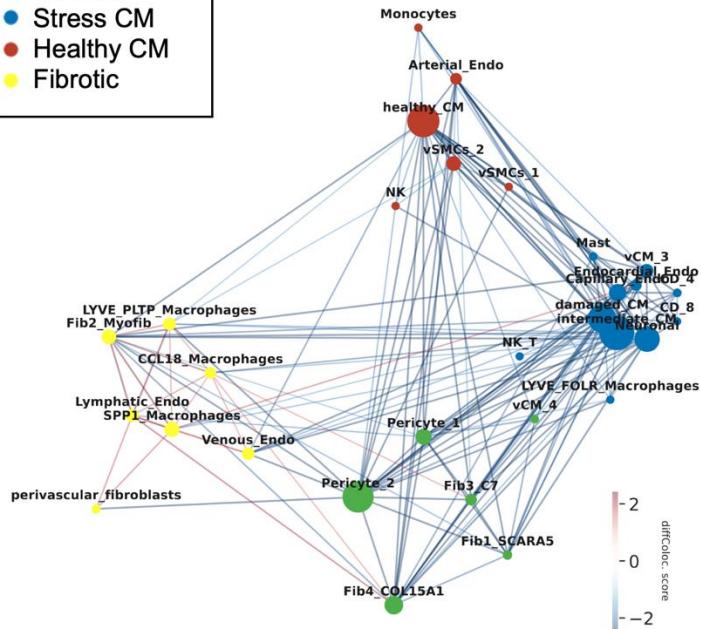
# Differential co-localization networks – Myocardial Infarction



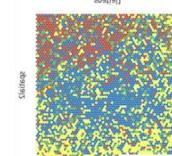
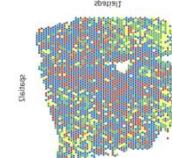
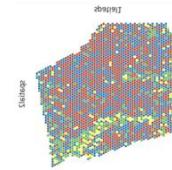
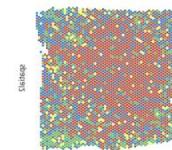
# Differential co-localization networks – Myocardial Infarction



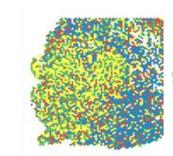
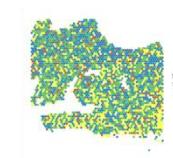
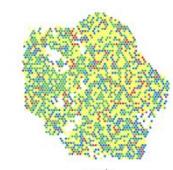
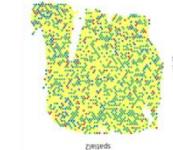
## Co-localized Niches



## Myogenic

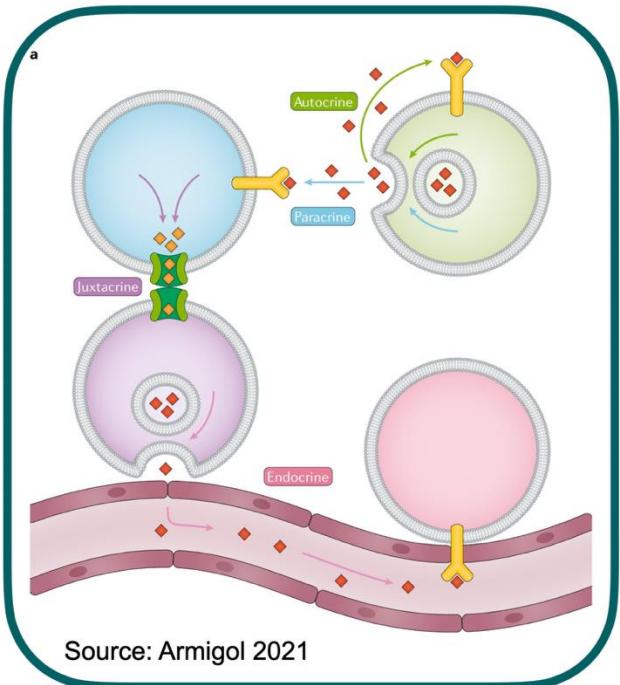


## Ischemic



# Process based niche driven cell - cell communication

## Ligand-Receptor Analysis



Focus on curated / fibrosis relevant LR mechanism

### Extracellular matrix (Matrissome)

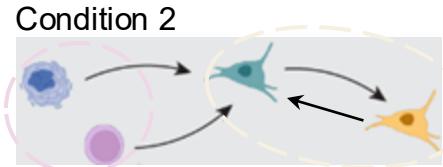
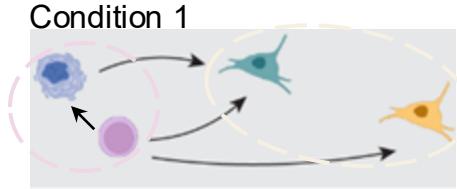
**Collagens** => Tissue structure  
**ECM regulators** => Modifiers of ECM  
**ECM glycoproteins & proteoglycans** => ECM assembly, signalling, lubrication  
**ECM affiliated** => Secreted factors that bind to solid phase complexes (example - SEMA3)

### Immune cell recruitment (CytoSig)

**Cytokines**-> immune signalling  
**Chemokines** => cell migration  
**Secreted and Growth factors** => Interaction with ECM proteins, signalling

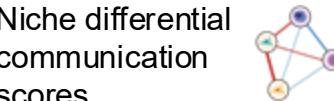
# Process based niche driven cell - cell communication

Communication through  
Glycoproteins  
(filtered by diff. co-localization)

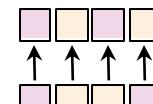


Niches differential  
communication test

Niche differential  
communication  
scores



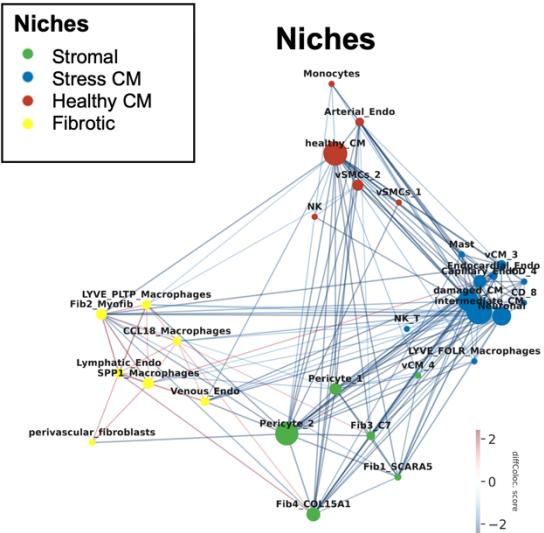
Condition 2  
Condition 1



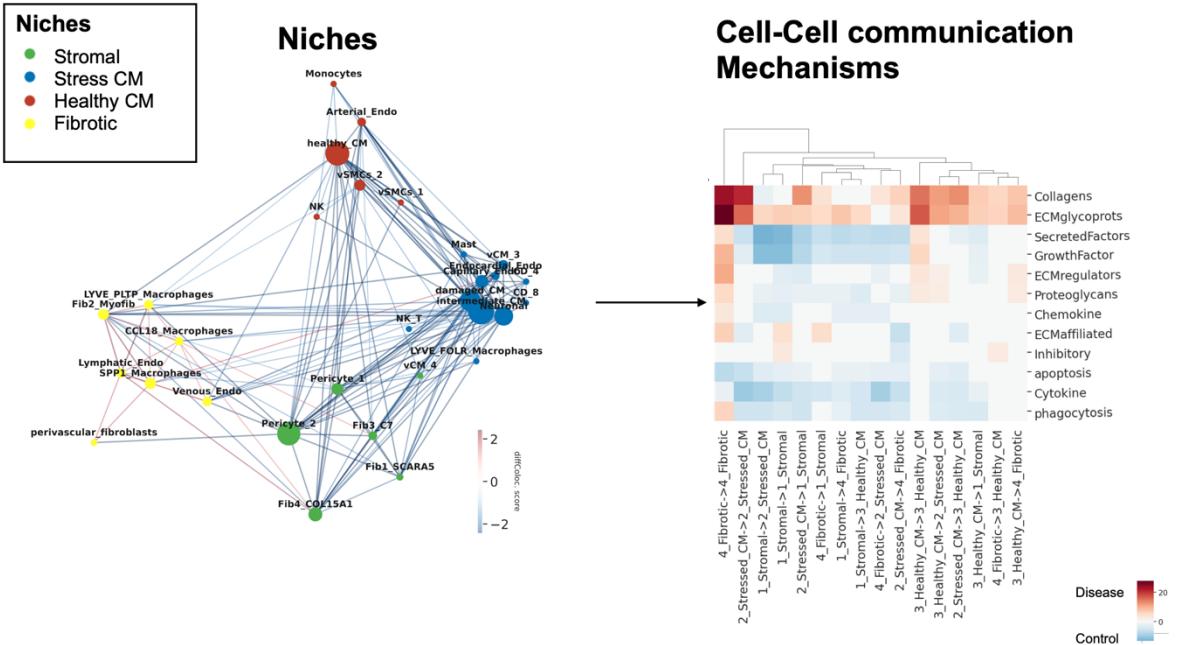
Niche 1  
Niche 2

# Process based niche driven cell - cell communication

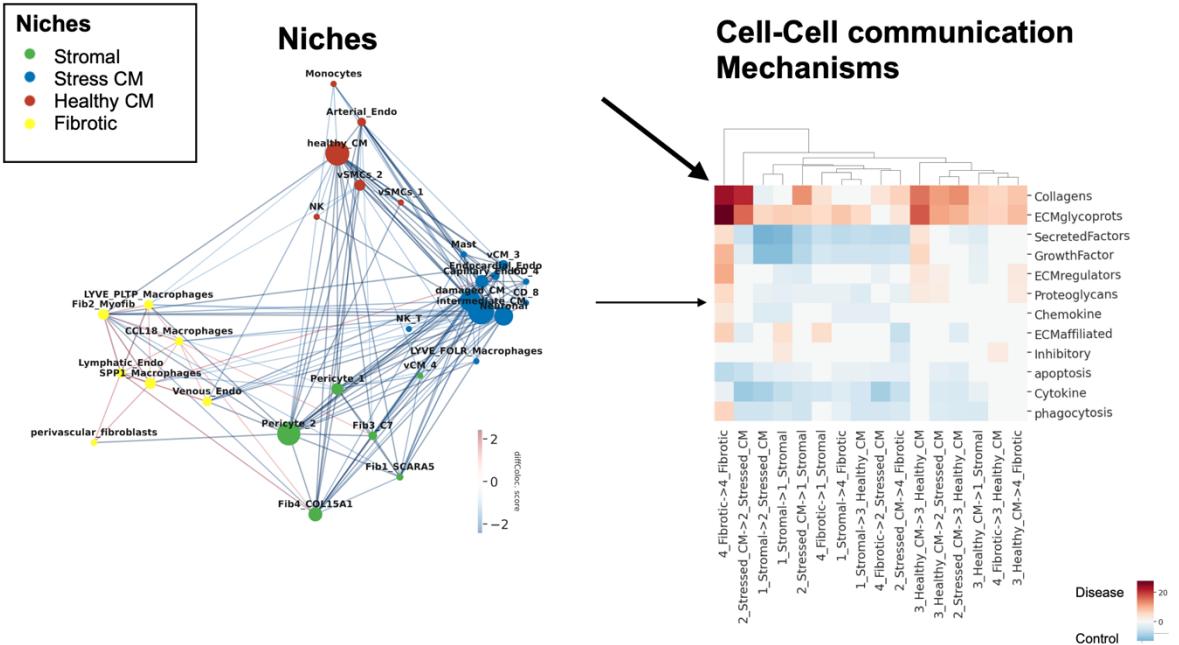
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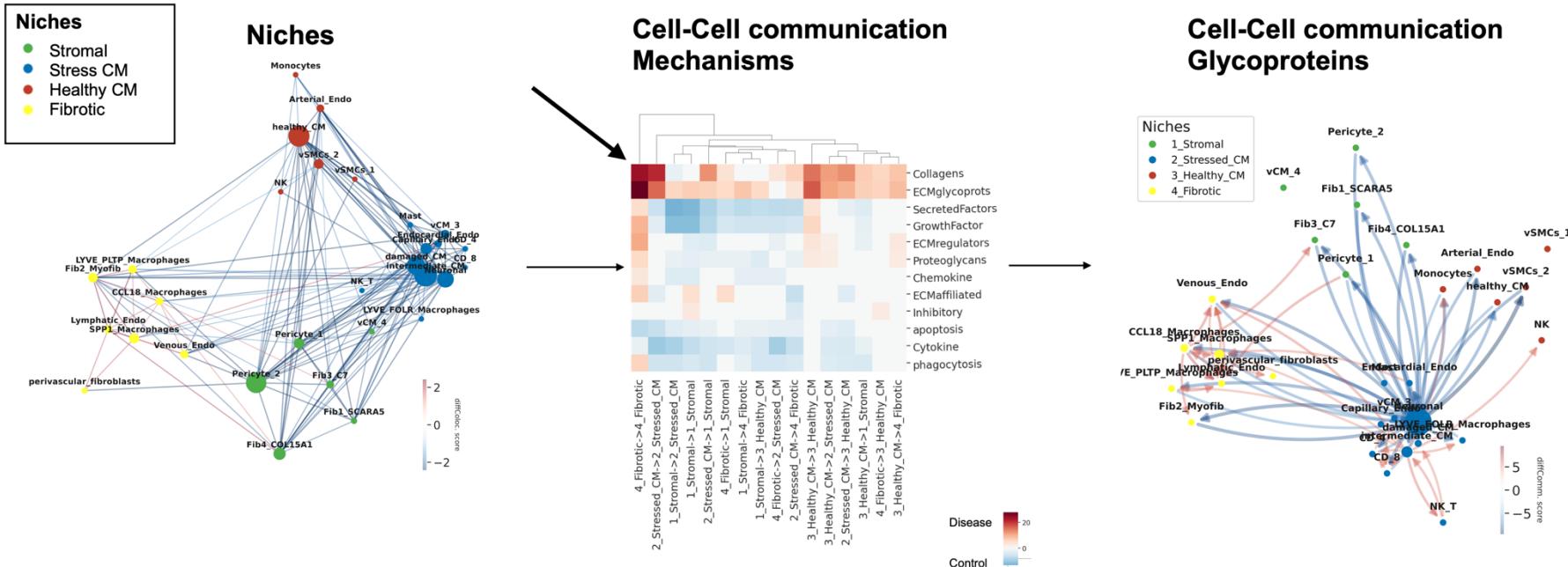
# Process based niche driven cell - cell communication



# Process based niche driven cell - cell communication

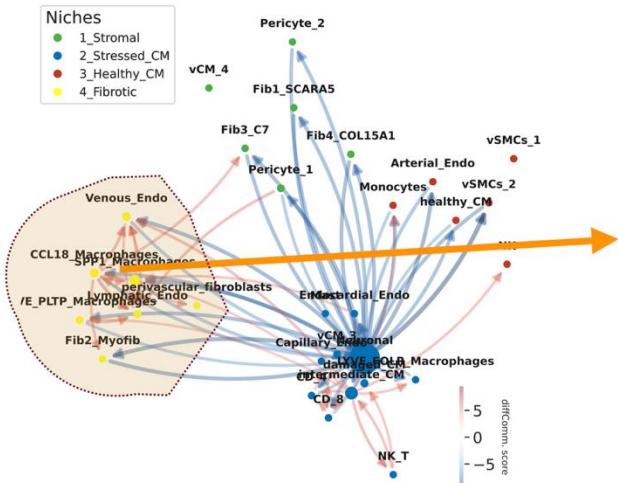


# Process based niche driven cell - cell communication

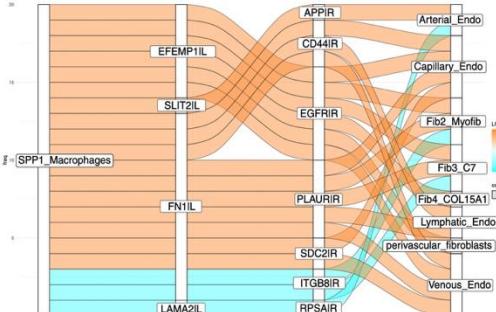


# Process based niche driven cell - cell communication

## Cell-Cell communication Glycoproteins

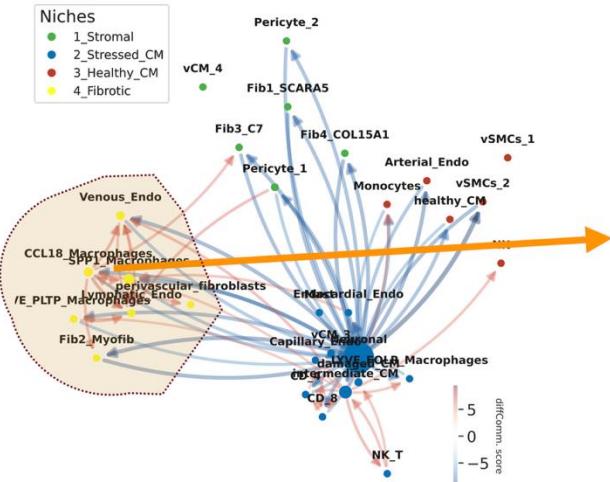


## Glycoprotein Signaling from SPP1+ macrophages

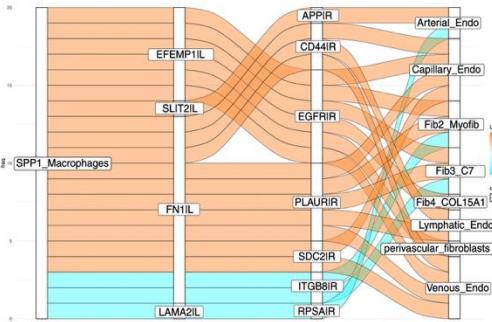


# Process based niche driven cell - cell communication

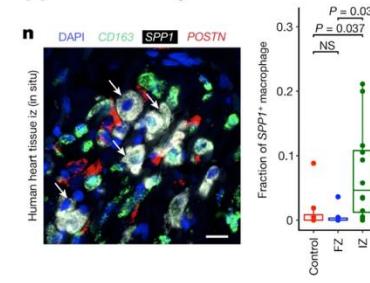
## Cell-Cell communication Glycoproteins



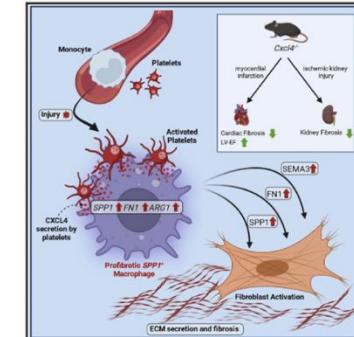
## Glycoprotein Signaling from SPP1+ macrophages



**SPP1 Macs + Myob. Co-localization**  
(Kuppe et al 2022)



**Fibrosis signalling from SPP1+ Mac. To myofibroblasts via FN1 in heart and kidney fibrosis models**



Hoeft, Schaefer et al. Cell Rep. 2023

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# **Hands on analysis of Myocardial Infarction ST and scRNA-seq**

*Nagai, Ruiz*



# Questions & Answers

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# Feedback survey

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Please take a moment to complete the end-of-tutorial questionnaire!  
Click [here](#) or scan the QR code.



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# Thank you for your attention!



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