



Canadian Bioinformatics Workshops

www.bioinformatics.ca

bioinformaticsdotca.github.io



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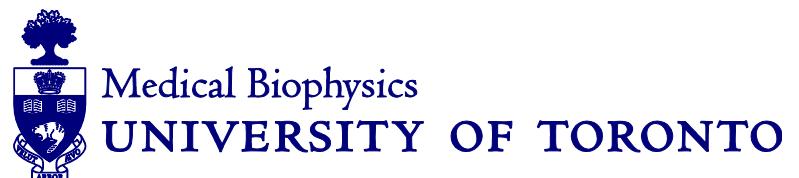
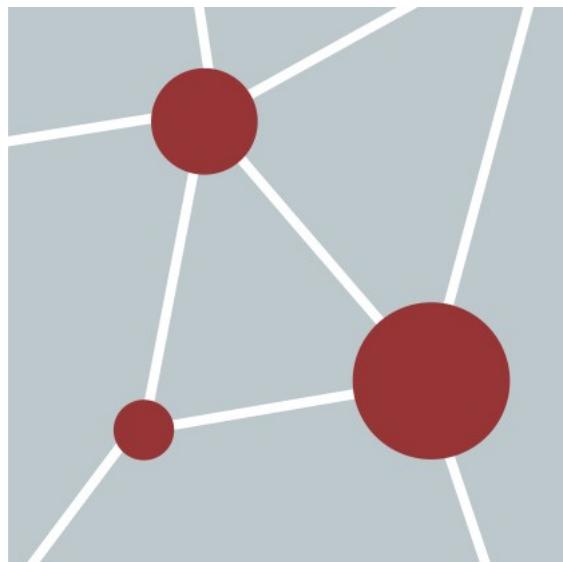
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Module #6: Cell-Cell Communication



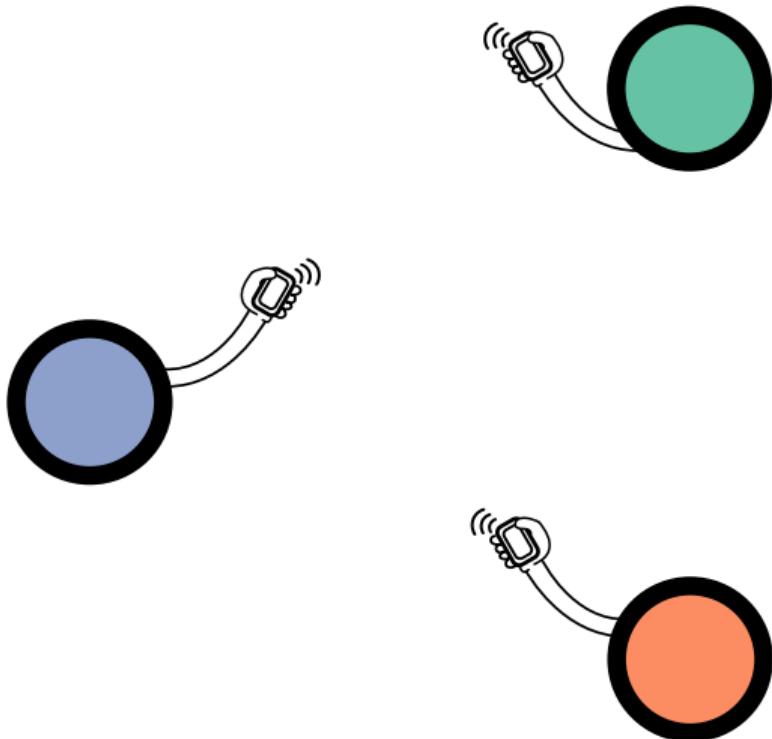
Gregory Schwartz
Pathway and Network Analysis
June 26-28, 2024



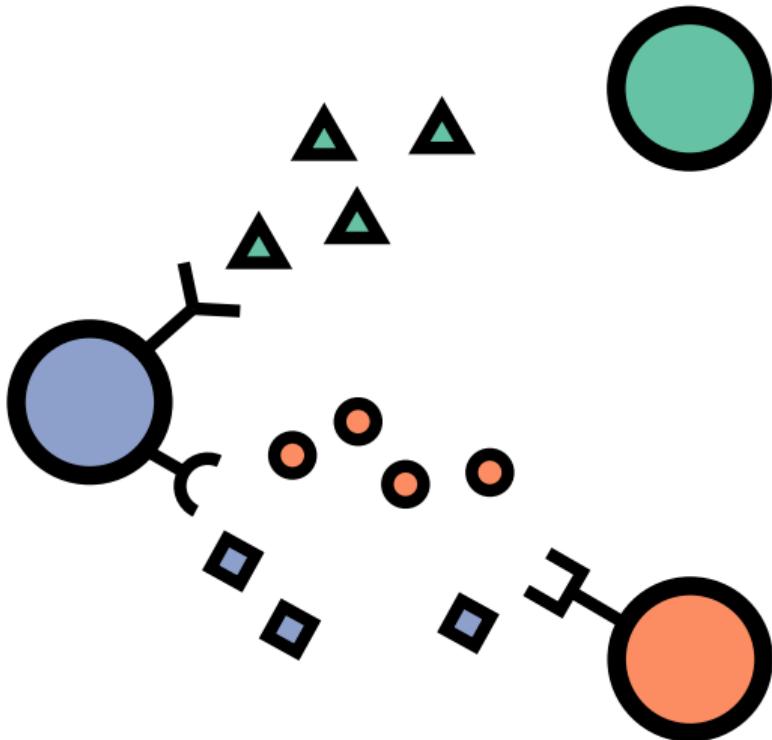
What you will learn

- The role and context of cell-cell communication
- Single-cell RNA sequencing for cellular transcriptomes
- General principles of cell-cell communication detection
- Inner workings of select single-cell RNA sequencing methods
- Spatial transcriptomics for communication detection
- Spatially-resolved cell-cell communication method

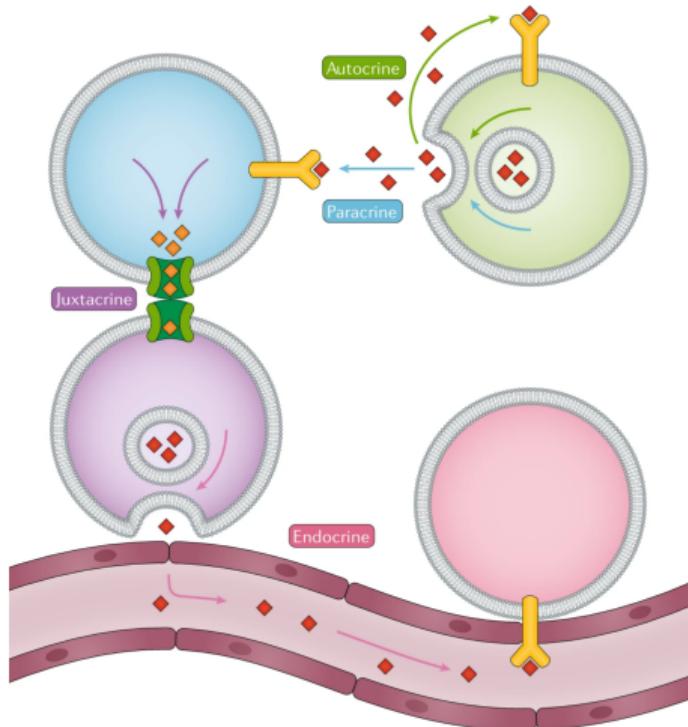
Cells communicate through intercellular signaling mechanisms



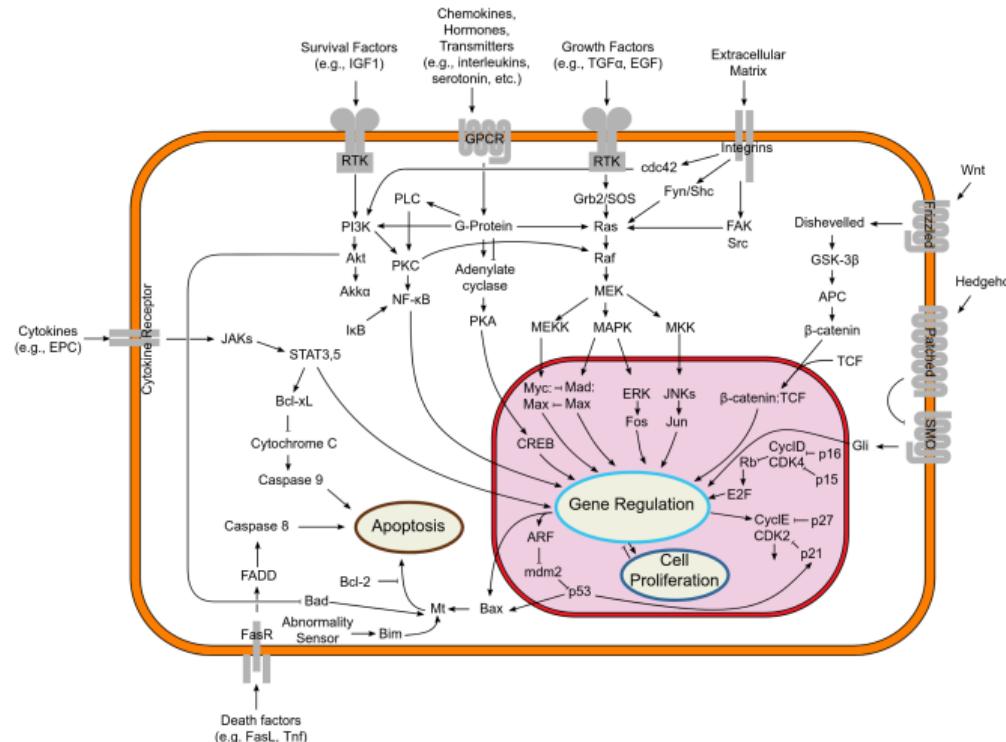
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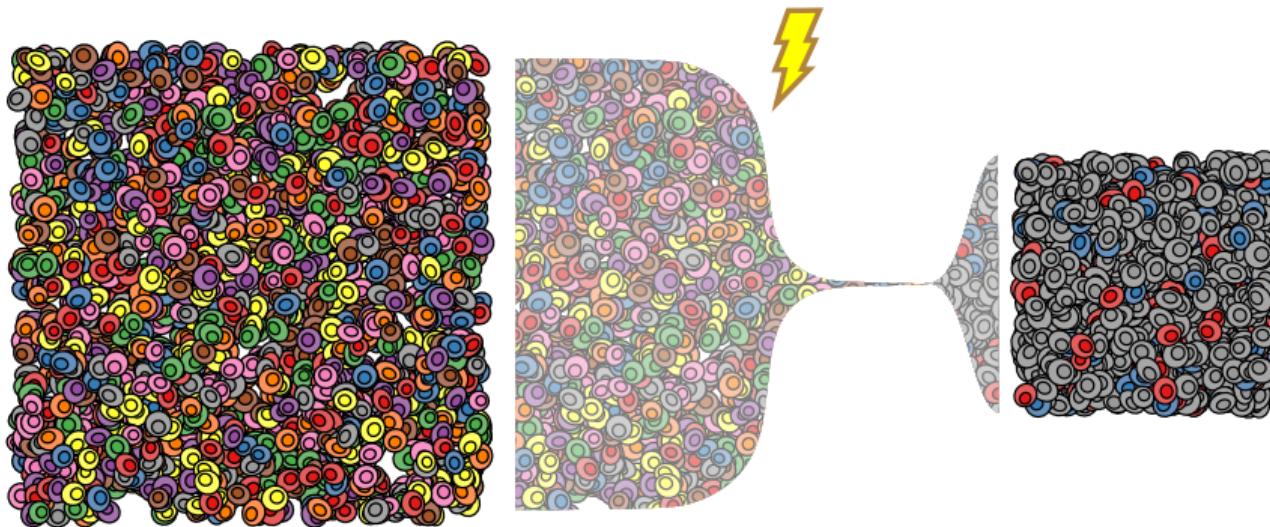
Ligands initiate different cell-cell communication mechanisms



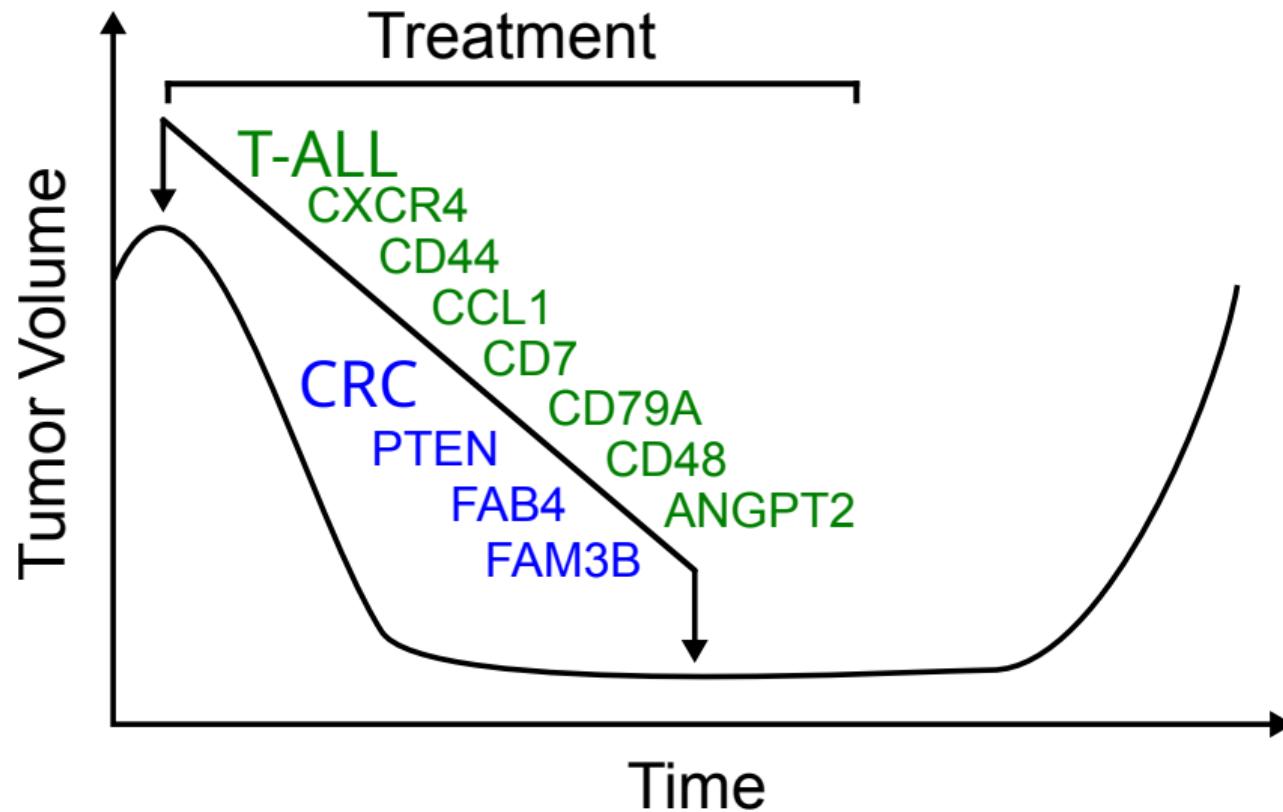
Communication triggers downstream cellular functionality



Cancer heterogeneity can reduce the efficacy of therapy



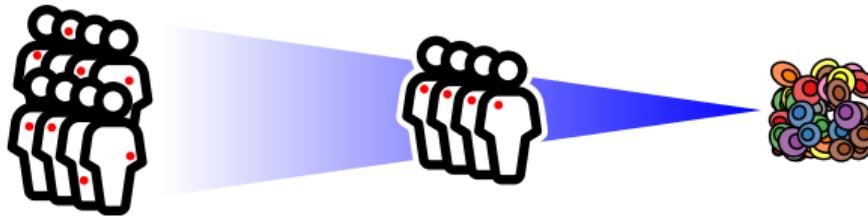
Genes involved in cell-cell communication are differentially expressed



inteGREAT

HeatITup

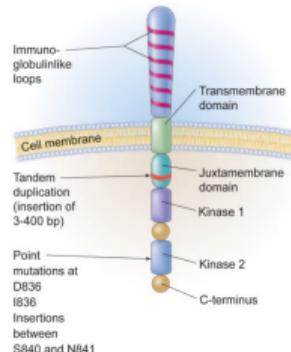
TooManyCells



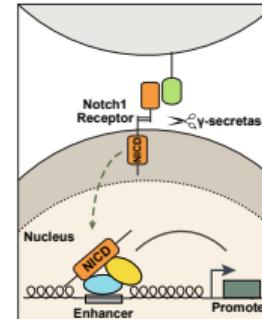
inteGREAT

HeatITup

TooManyCells



FLT3



Notch1

Hypothesis

Cell-cell communication contributes to progression and poor therapy response

Single-cell sequencing enables cell resolution analysis



Single-cell sequencing enables cell resolution analysis



Bulk Sequencing



Single-cell sequencing enables cell resolution analysis



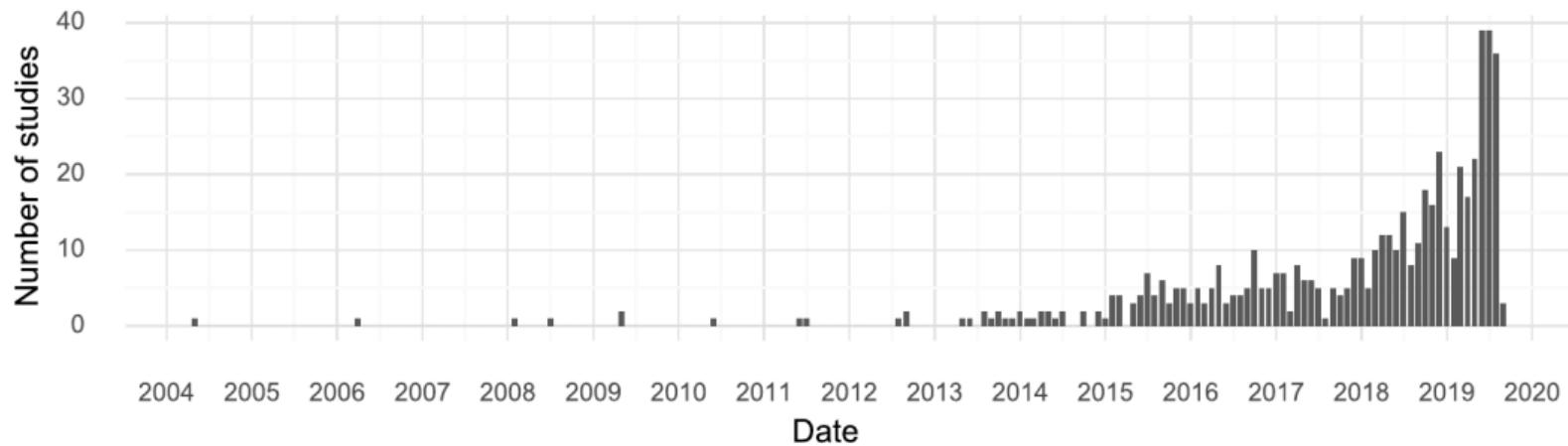
Bulk Sequencing



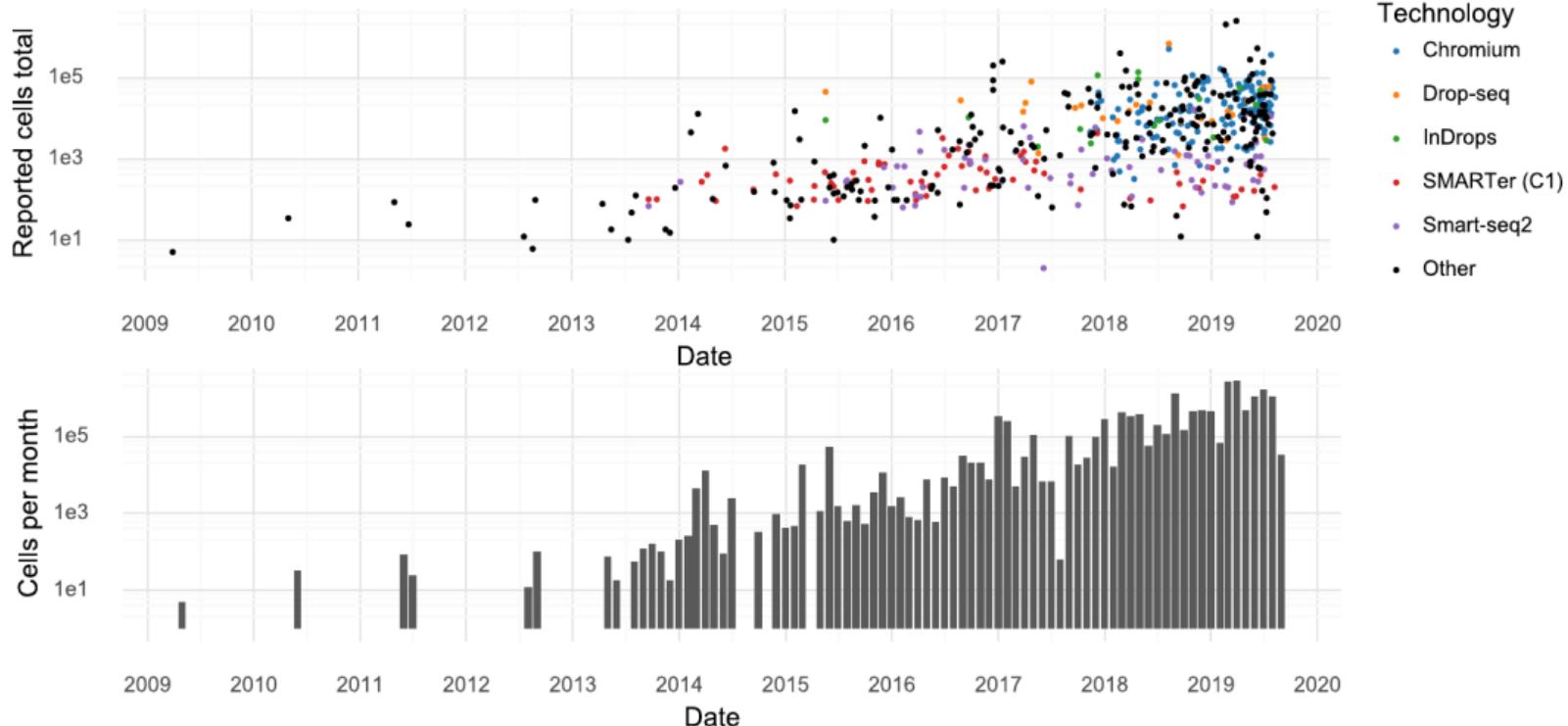
Single-cell Sequencing



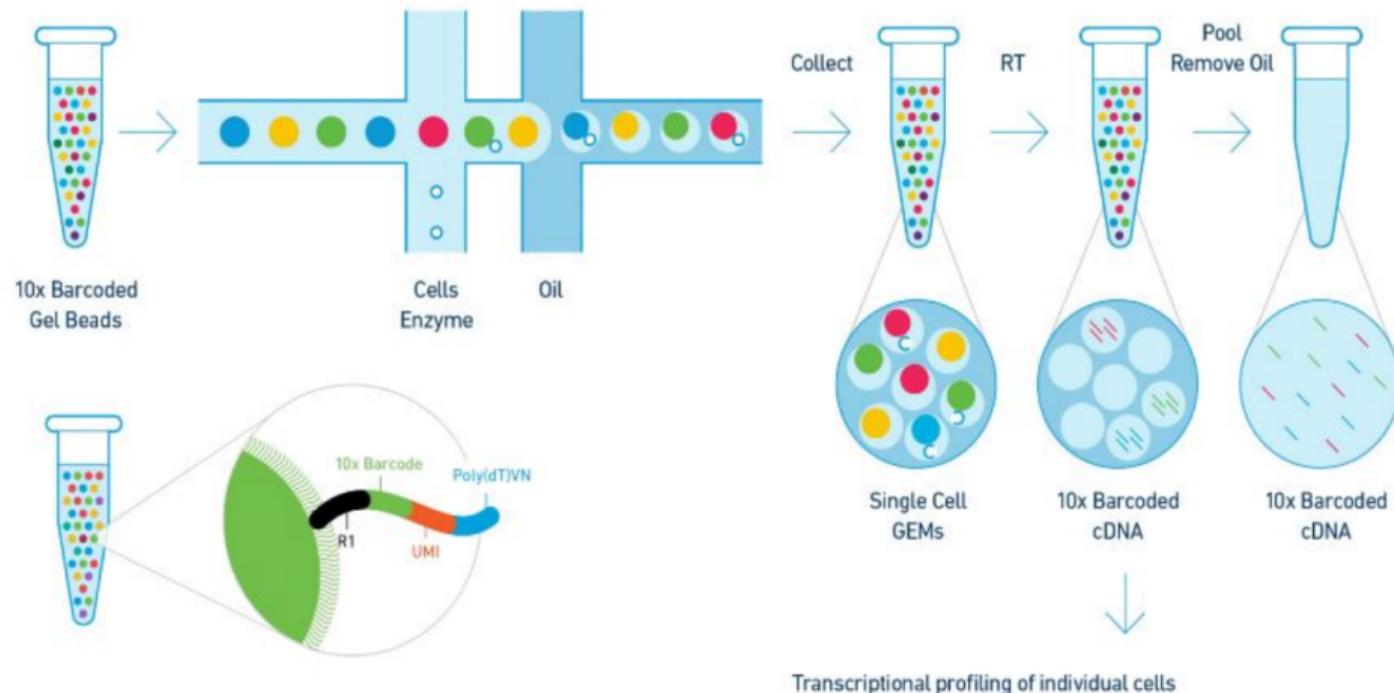
Single-cell transcriptomics is exponentially increasing



Single-cell transcriptomics is exponentially increasing



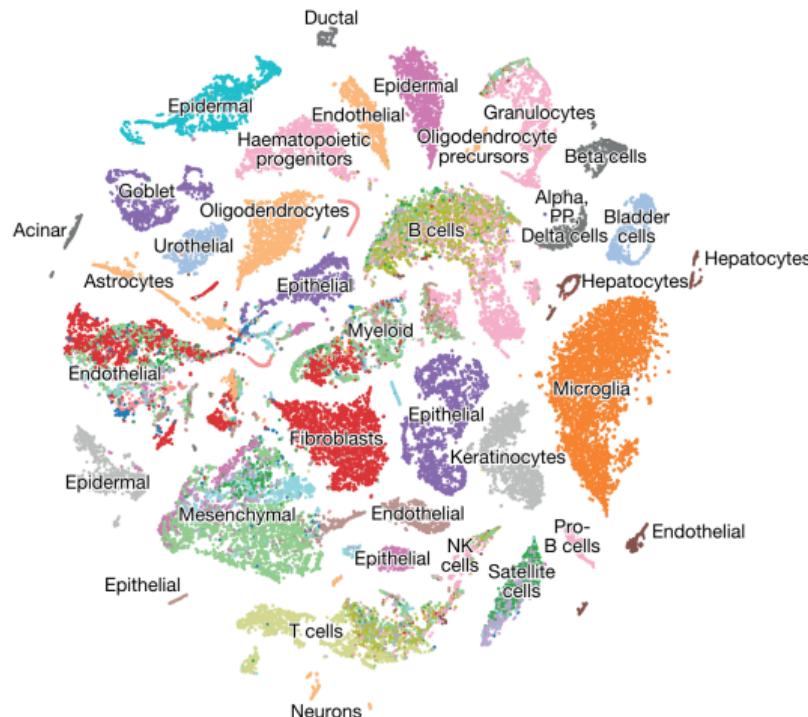
Microfluidics has high accuracy and throughput



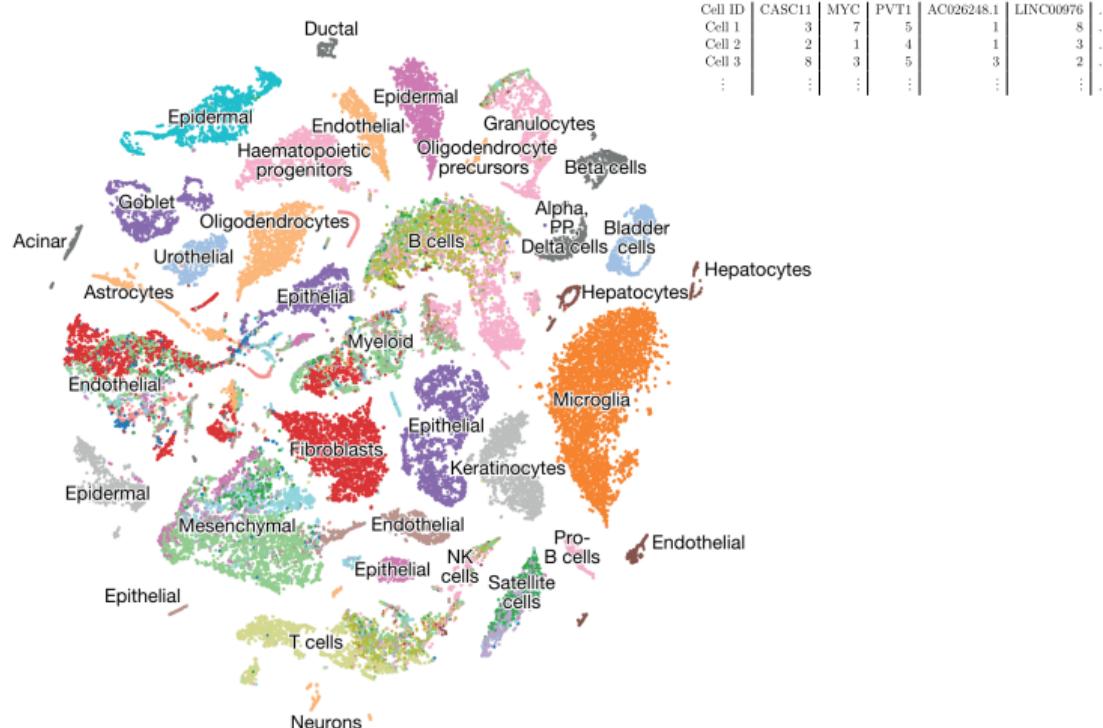
Microfluidics has high accuracy and throughput

Drop-seq (HyDrop)

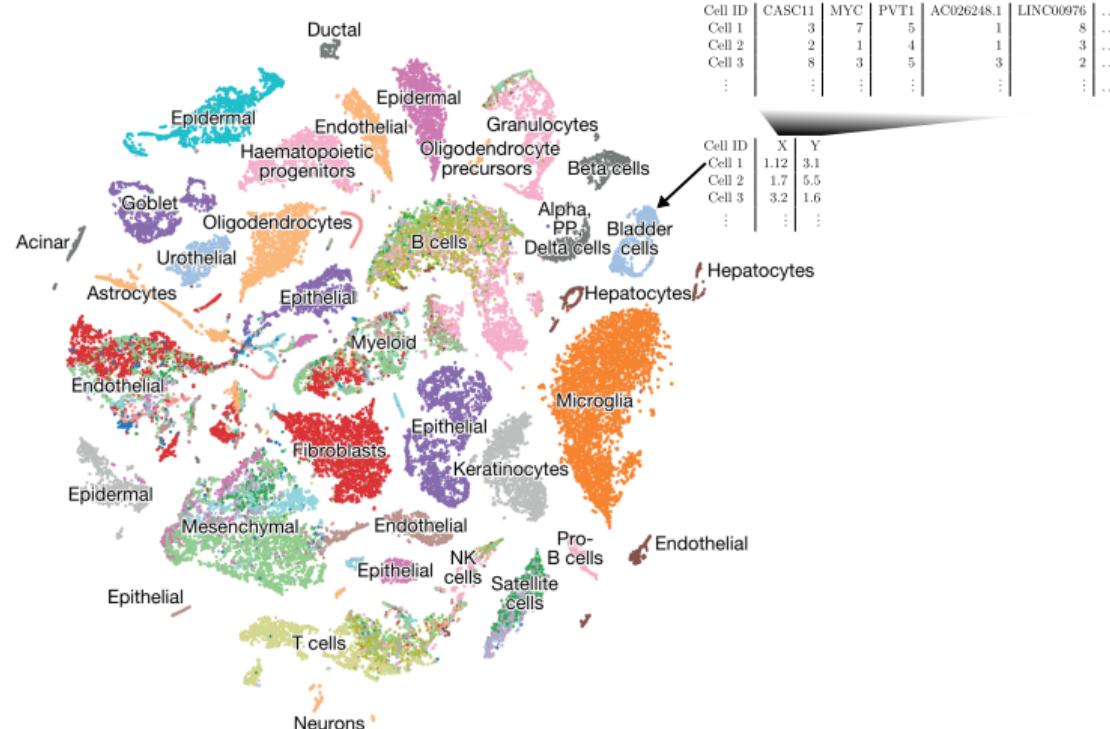
Typical workflow collapses many dimensions to only two



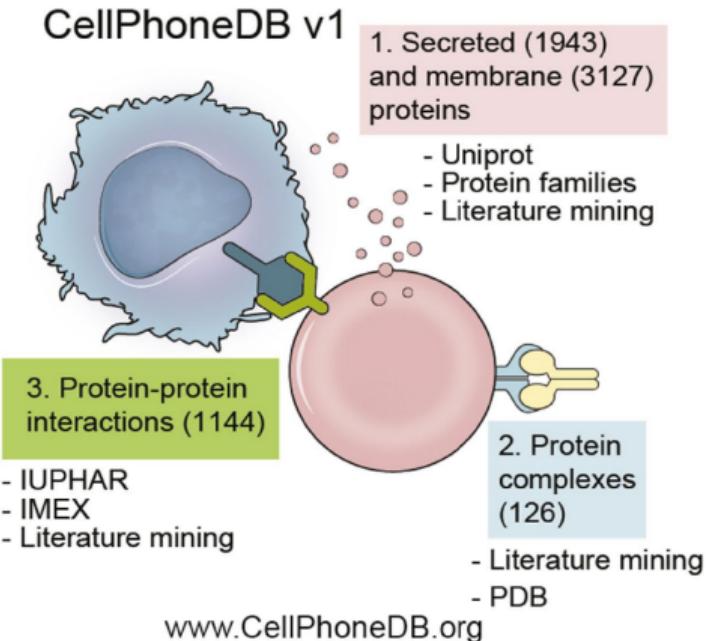
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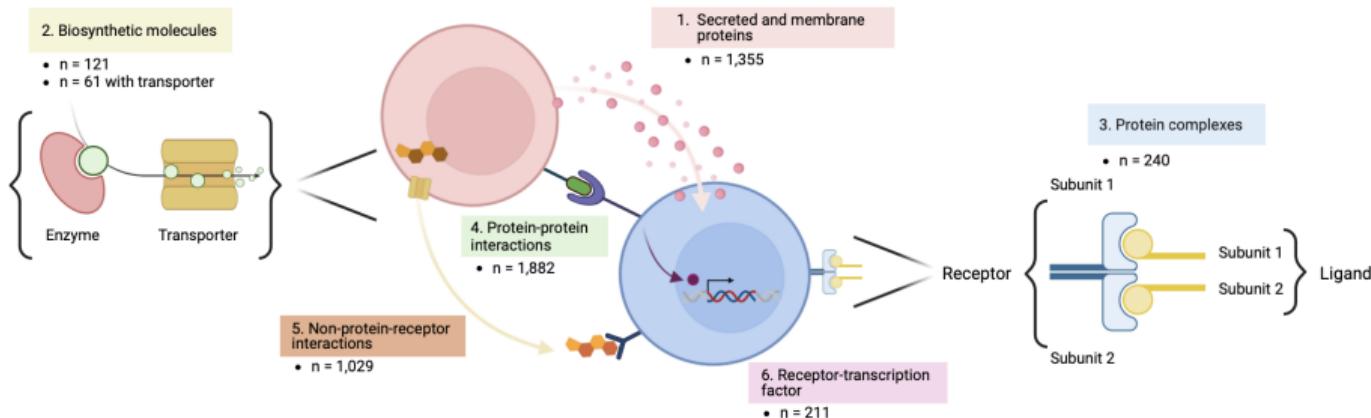
Typical workflow collapses many dimensions to only two



CellPhoneDB introduced a well-curated data set of signals



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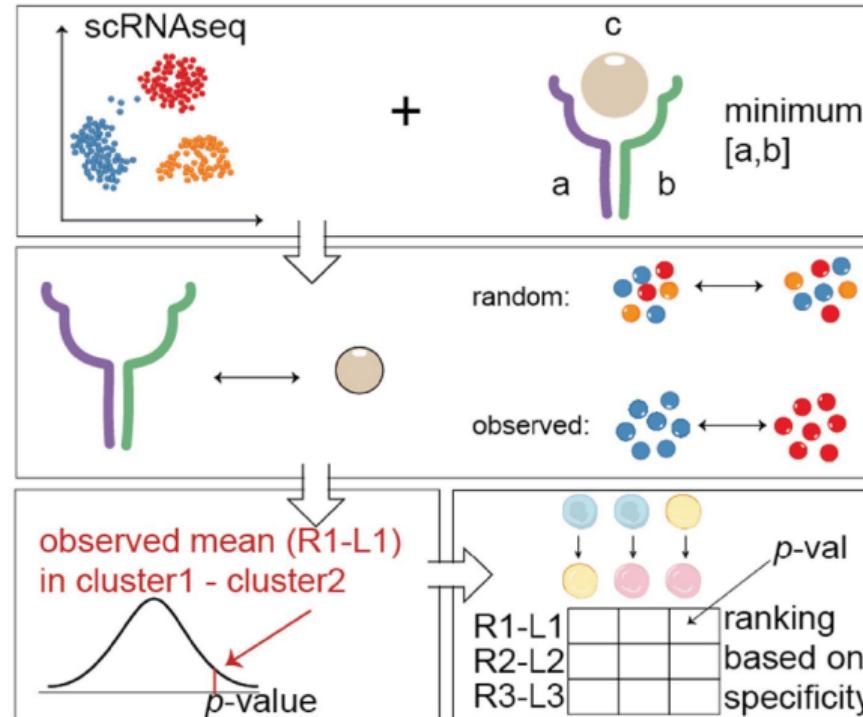
Flat table structure holds molecular interactions

id_interaction	id_cp_interaction	multidata_1_id	multidata_2_id	source	annotation_strategy	is_ppi	curator	directionality	classification
0	CPI-SC0A2DB962D	329	1507	PMID:12392763	curated	True	RVentoTormo	Adhesion-Adhesion	Adhesion by Cadherin
1	CPI-SC0B5CEA47D	716	1507	uniprot	curated	True	RVentoTormo	Adhesion-Adhesion	Adhesion by Collagen/Integrin
2	CPI-SC0C8B7BCBB	322	1507	uniprot	curated	True	RVentoTormo	Adhesion-Adhesion	Adhesion by Collagen/Integrin
3	CPI-SC0D3C12C3F	343	1507	uniprot	curated	True	RVentoTormo	Adhesion-Adhesion	Adhesion by Collagen/Integrin
4	CPI-SC0B86B7CED	930	1507	uniprot	curated	True	RVentoTormo	Adhesion-Adhesion	Adhesion by Collagen/Integrin
5	CPI-SC0FA343CEF	810	1507	uniprot	curated	True	RVentoTormo	Adhesion-Adhesion	Adhesion by Collagen/Integrin
6	CPI-SC0CCC9A7F	720	1507	uniprot	curated	True	RVentoTormo	Adhesion-Adhesion	Adhesion by Collagen/Integrin
7	CPI-SC0E85E1FB0	1347	1507	uniprot	curated	True	RVentoTormo	Adhesion-Adhesion	Adhesion by Collagen/Integrin

Flat table structure holds molecular interactions

id_multidata	name	receptor	receptor_desc	other	other_desc	secreted_highlight	secreted_desc	transmembrane	secreted	peripheral	integrin	is_complex
0	P03372	True		False		False		False	False	True	False	False
1	Q92753	True		False		False		False	False	False	False	False
2	O95477	False		False		False		True	False	False	False	False
3	Q13133	True		False		False		False	False	False	False	False
4	P04150	True		False		False		False	False	False	False	False
5	Q7Z5A7	False		False		True		False	True	False	False	False
6	Q9UNN8	True		False		False		False	False	False	False	False
7	Q96LR4	False		False		True		False	True	False	False	False
8	O60883	True		False		False		True	False	False	False	False
9	Q96RI1	True		False		False		False	False	False	False	False
10	P30533	False		False		False		False	False	False	False	False

CellPhoneDB compares high expression of each cell cluster



Pre-set thresholds control for noise

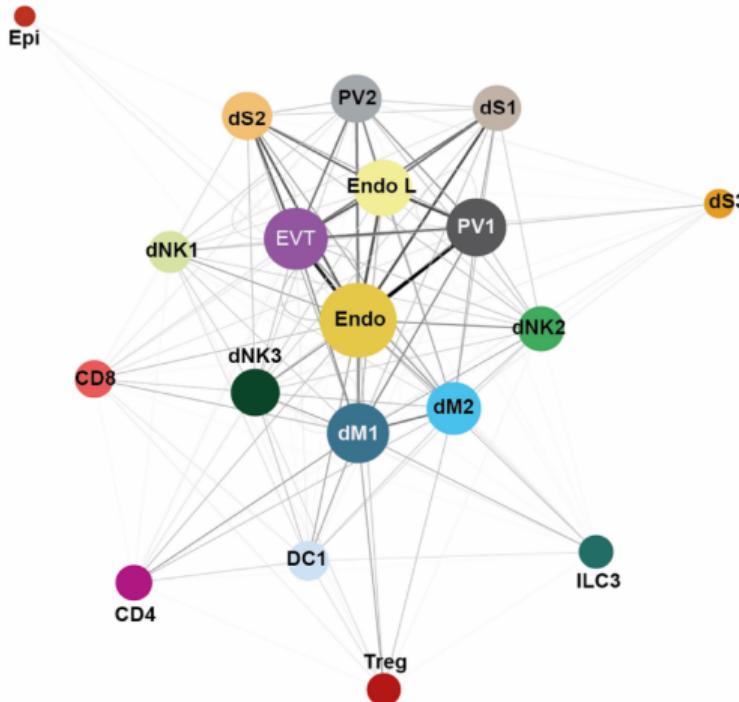
Only ligands and receptors in >10% of cells considered

Randomly permute cluster labels 1000 times

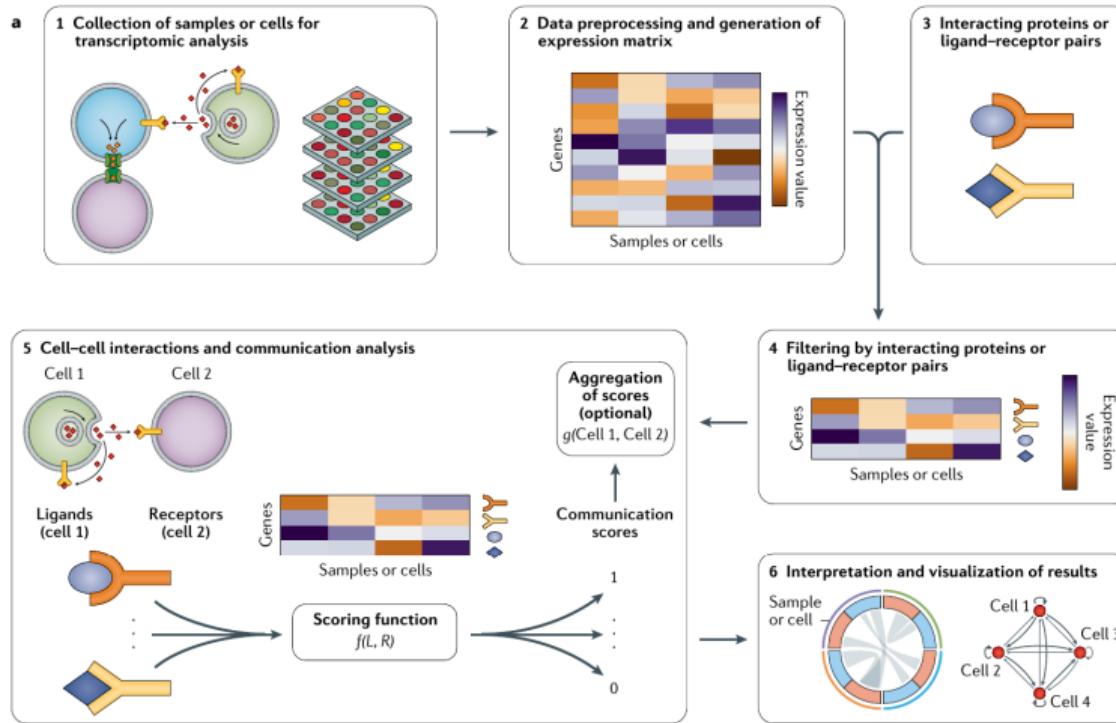
Calculate proportion of means “more extreme” than observed

The minimum of protein dimers are considered

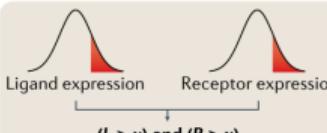
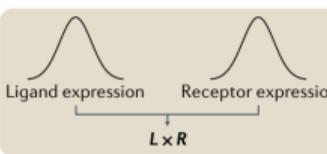
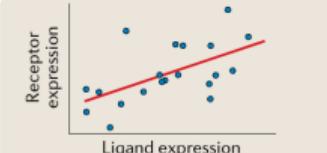
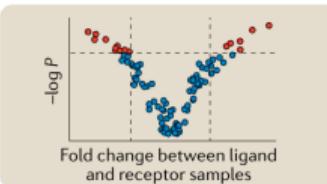
CellPhoneDB compares high expression of each cell cluster



Cell-cell communication algorithms decipher ligand-receptor pairs

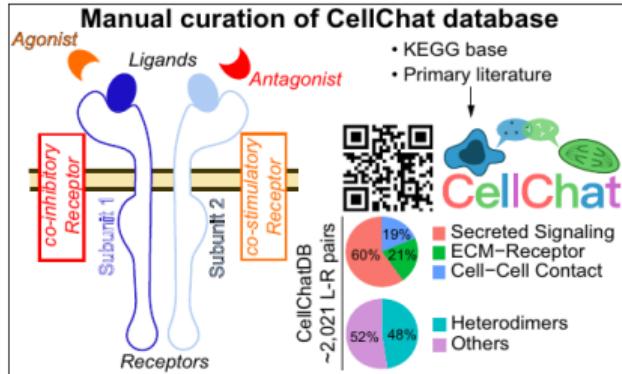


Cell-cell communication algorithms decipher ligand-receptor pairs

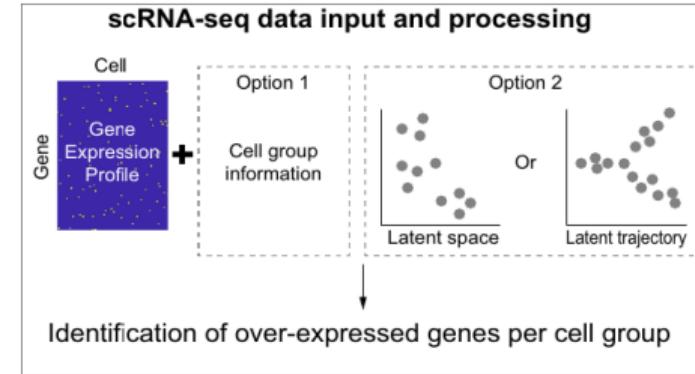
		Recommended data	Communication score
Expression thresholding		Bulk, single cell	Binary
Expression product		Single cell	Continuous
Expression correlation		Bulk, single cell	Continuous
Differential combinations		Bulk, single cell	Binary

CellChat alternatively uses law of mass action

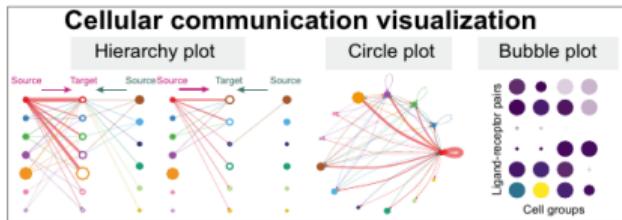
a



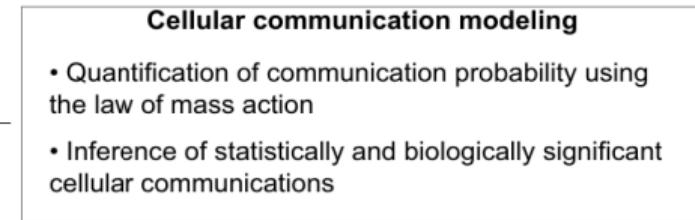
b



d



c



CellChat's estimation is based on Hill equations

$$\theta = \frac{[L]^n}{K_d + [L]^n} \quad (1)$$

θ = Fraction of receptor protein bound by ligand

$[L]$ = Ligand concentration

K_d = Dissociation constant from law of mass action (half binding)

n = Hill coefficient

CellChat's estimation is based on Hill equations

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

$$\times \frac{K_h}{K_h + AN_i} \cdot \frac{K_h}{K_h + AN_j} \times \frac{n_i n_j}{n^2},$$

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

CellChat's estimation is based on Hill equations

Ligand/receptor law of mass action

$$P_{i,j}^k = \frac{L_i R_j}{K_h + L_i R_j} \times \left(1 + \frac{AG_i}{K_h + AG_i}\right) \cdot \left(1 + \frac{AG_j}{K_h + AG_j}\right) \\ \times \frac{K_h}{K_h + AN_i} \cdot \frac{K_h}{K_h + AN_j} \times \frac{n_i n_j}{n^2},$$
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Geoetric mean of m_1 subunits Geoetric mean of m_2 subunits

CellChat's estimation is based on Hill equations

Ligand/receptor law of mass action

$$P_{i,j}^k = \frac{L_i R_j}{K_h + L_i R_j} \times \left(1 + \frac{AG_i}{K_h + AG_i}\right) \cdot \left(1 + \frac{AG_j}{K_h + AG_j}\right)$$
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Geoetric mean of m_1 subunits Geoetric mean of m_2 subunits

Averages of co-stimulatory / co-inhibitory receptor modeling

CellChat's estimation is based on Hill equations

$$P_{i,j}^k = \frac{L_i R_j}{K_h + L_i R_j} \times \left(1 + \frac{AG_i}{K_h + AG_i} \right) \cdot \left(1 + \frac{AG_j}{K_h + AG_j} \right) \times \frac{K_h}{K_h + AN_i} \cdot \frac{K_h}{K_h + AN_j} \times \frac{n_i n_j}{n^2},$$

Ligand/receptor law of mass action

Modulate with agonists

Averages of co-stimulatory / co-inhibitory receptor modeling

Geoetric mean of m_1 subunits

Geoetric mean of m_2 subunits

CellChat's estimation is based on Hill equations

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

Ligand/receptor law of mass action

Modulate with agonists

Modulate with antagonists

$$\times \frac{K_h}{K_h + AN_i} \cdot \frac{K_h}{K_h + AN_j} \times \frac{n_i n_j}{n^2},$$

Geoetric mean of m_1 subunits

Geoetric mean of m_2 subunits

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

Averages of co-stimulatory / co-inhibitory receptor modeling

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Ligand/receptor law of mass action

Modulate with agonists

Modulate with antagonists

Weigh by cell numbers

Averages of co-stimulatory / co-inhibitory receptor modeling

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

Geoetric mean of m_1 subunits

Geoetric mean of m_2 subunits

Many alternative methods exist

CellPhoneDB

CellChat

Connectome

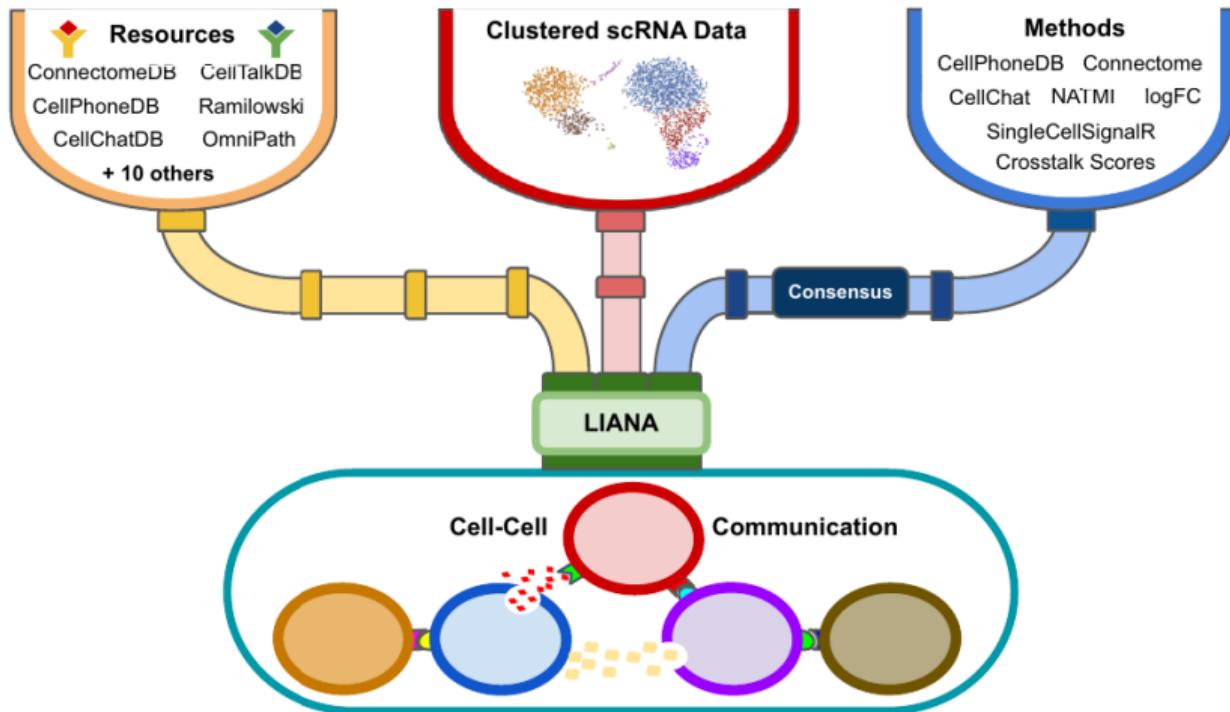
SingleCellSignalR

Scriabin

NATMI

...

LIANA aggregates many resources and methods



What to do next?

Traditional differential expression analysis

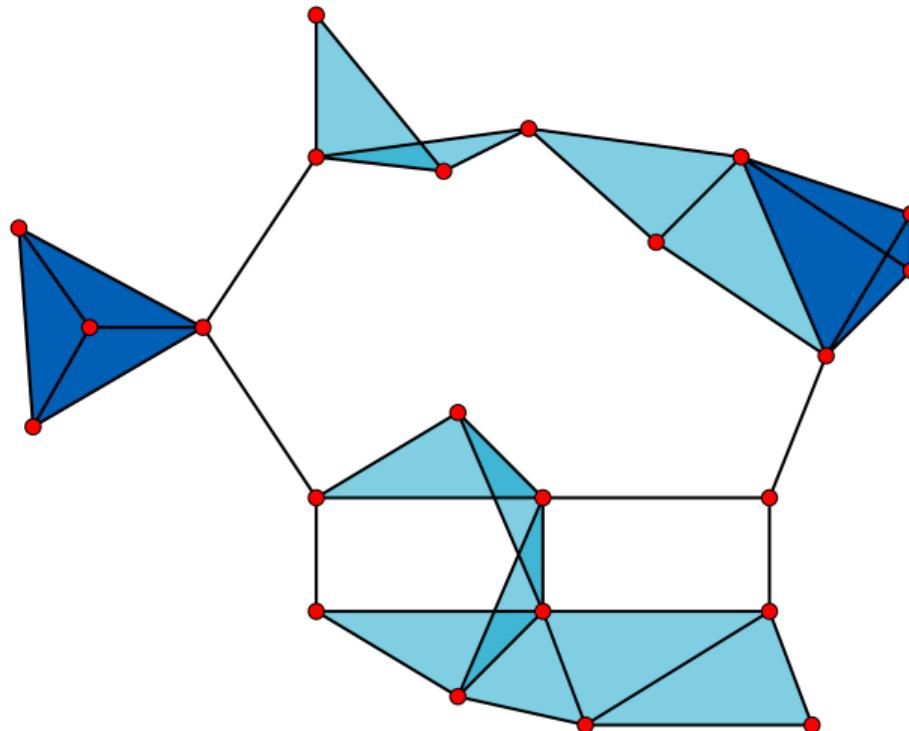
Traditional pathway analysis

Cliques

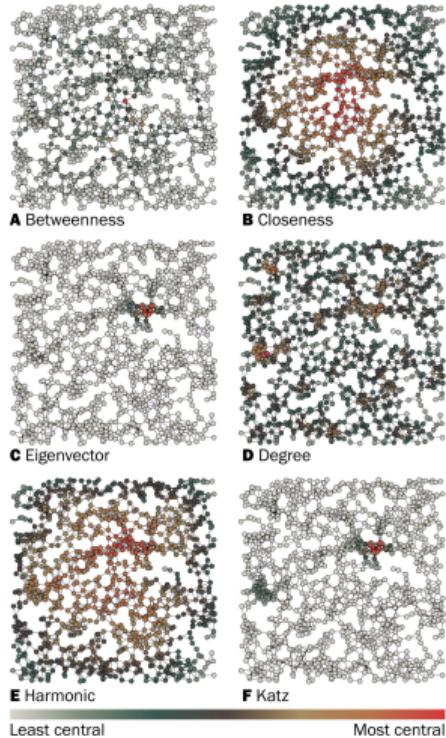
Graph centrality

Vertex hubs

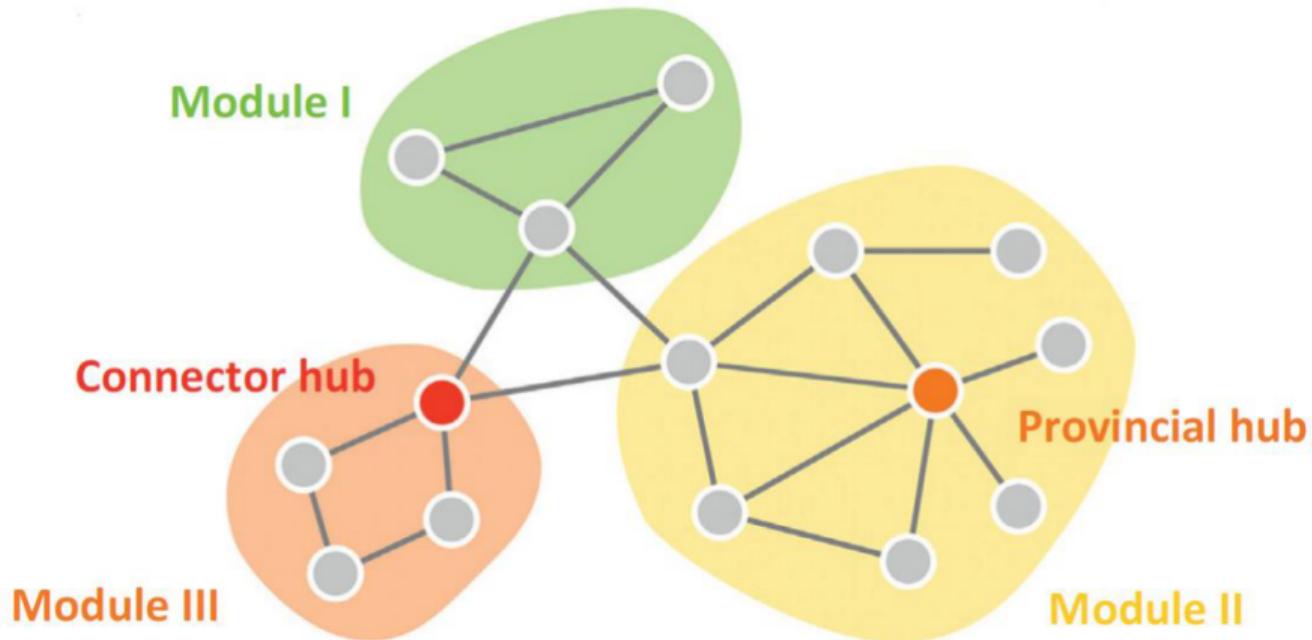
Cliques show highly-connected communities



Graph centrality identifies vertices with easy access to most of the network



Hubs reveal highly visited vertices



Hypothesis

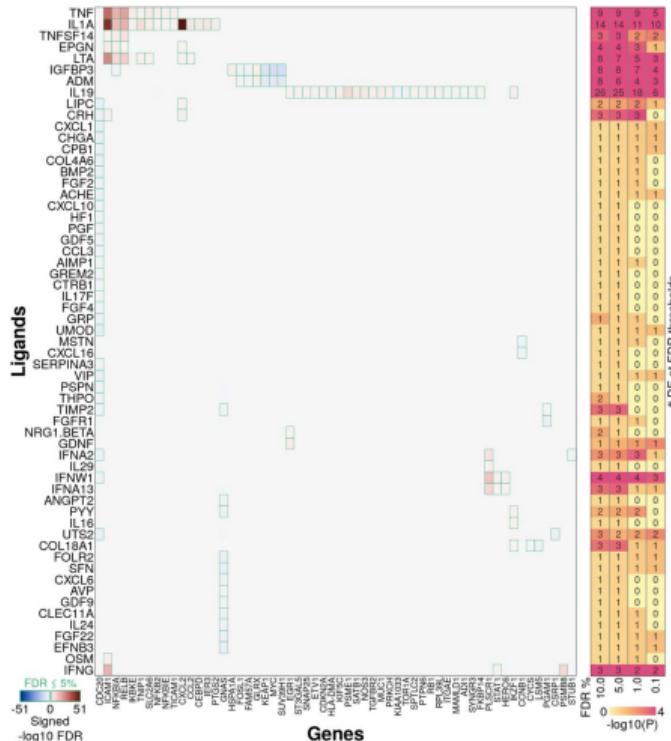
Cell-cell communication contributes to progression and poor therapy response

Hypothesis

Cell-cell communication contributes to progression and poor therapy response

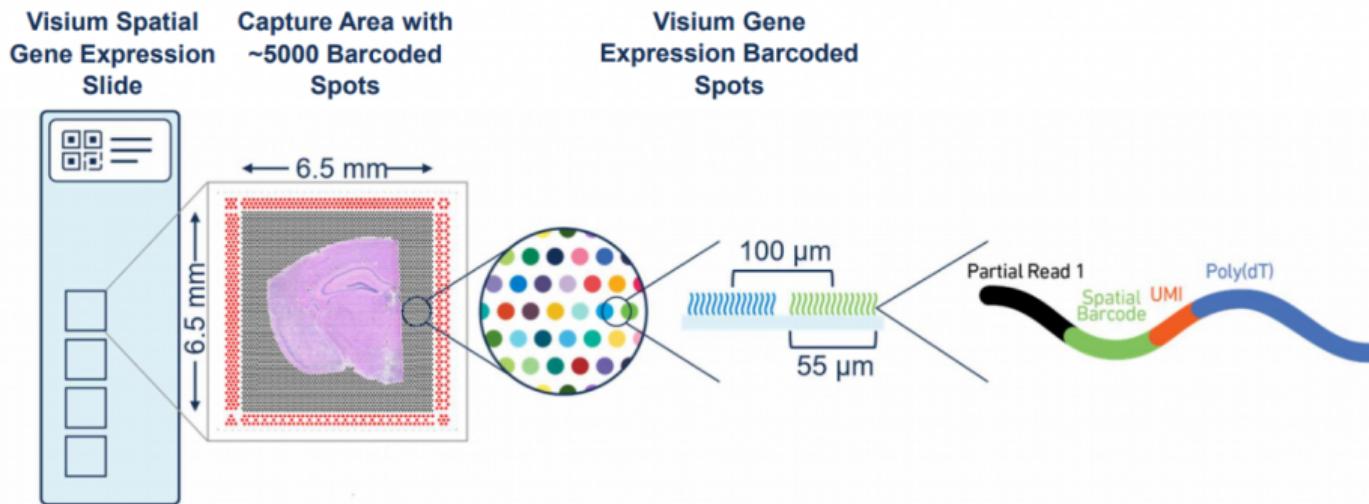
A single transcriptomic layer is **insufficient** to test this hypothesis

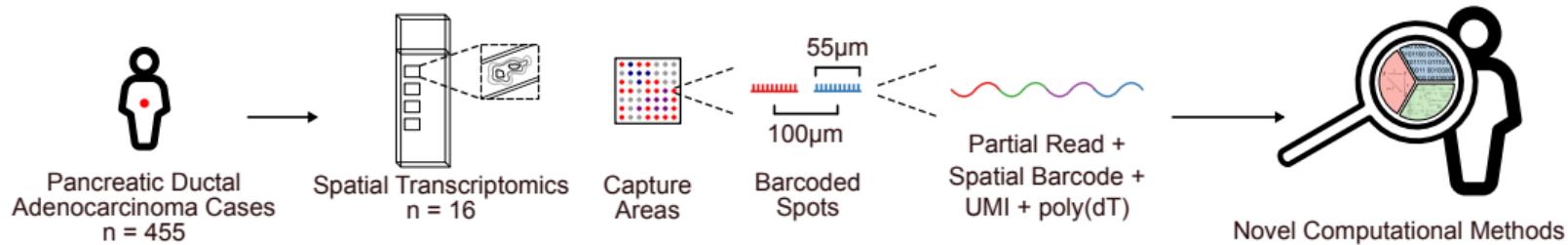
Cell lines exhibit minimal change in expression when treated with ligands



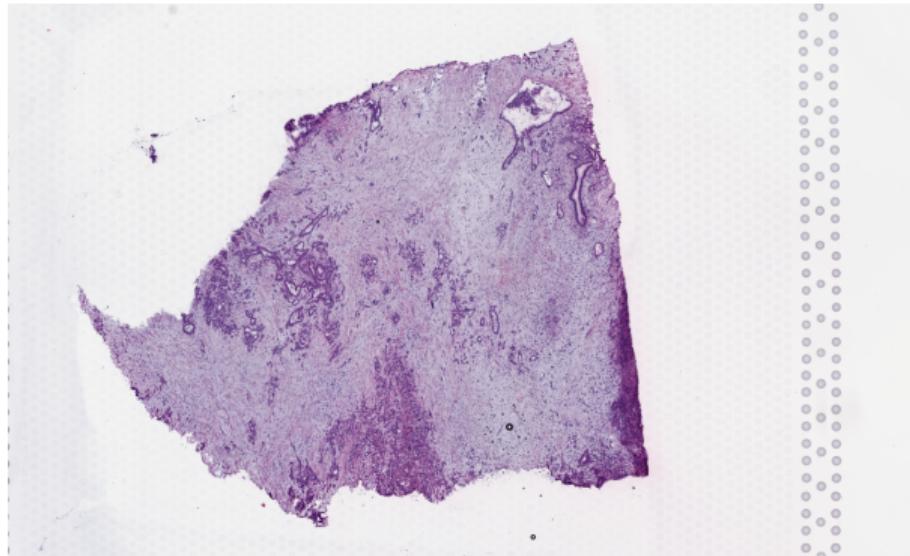
What about spatial transcriptomics data?

Spatial transcriptomics spatially resolves gene expression

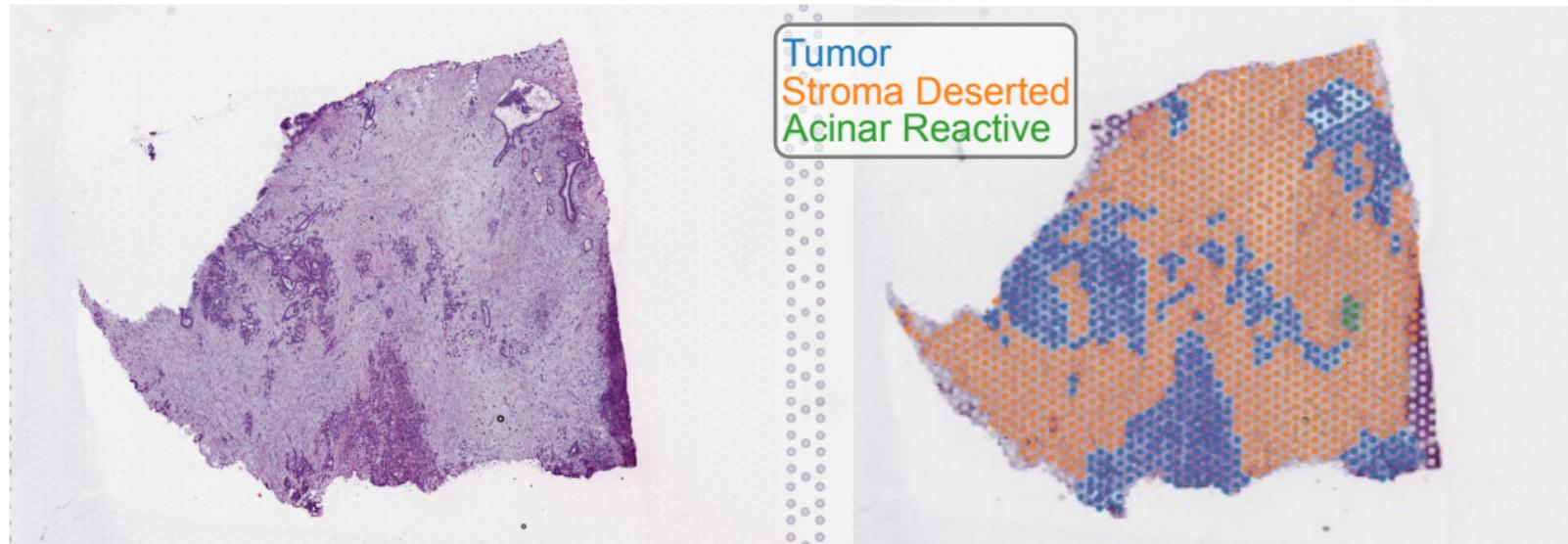




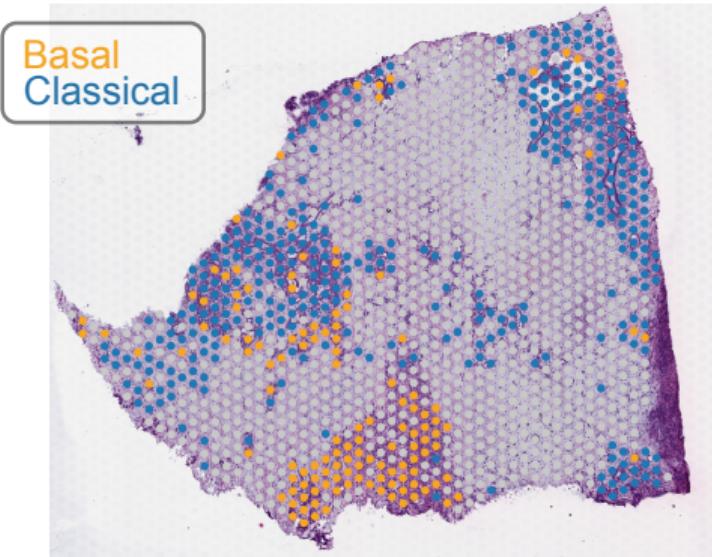
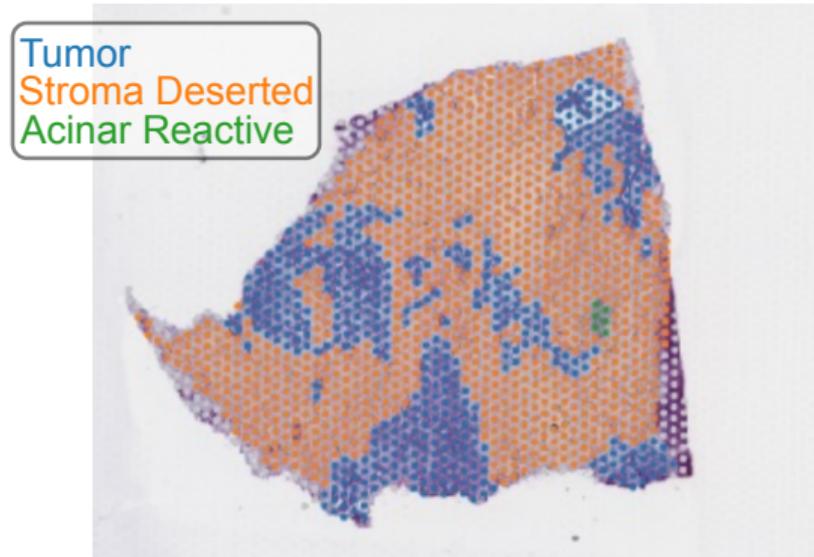
Spatial information paired with molecular profiles is a powerful tool



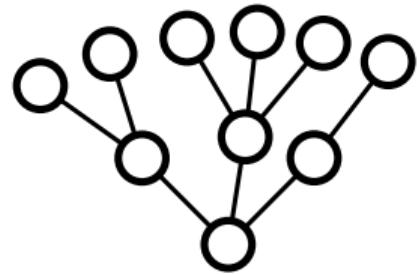
Spatial information paired with molecular profiles is a powerful tool



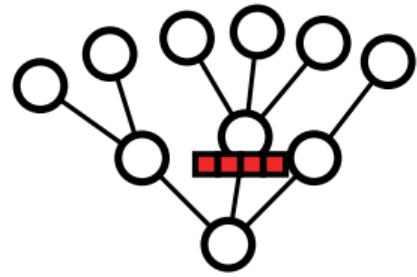
Spatial transcriptomics reveals pancreatic ductal adenocarcinoma signatures



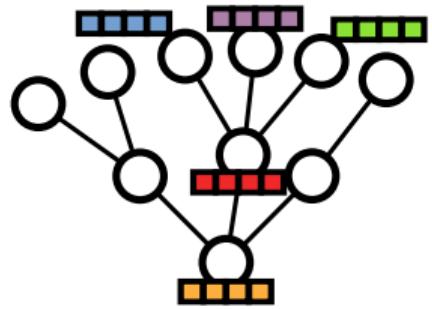
Graph convolutional networks learn node embeddings for downstream analysis



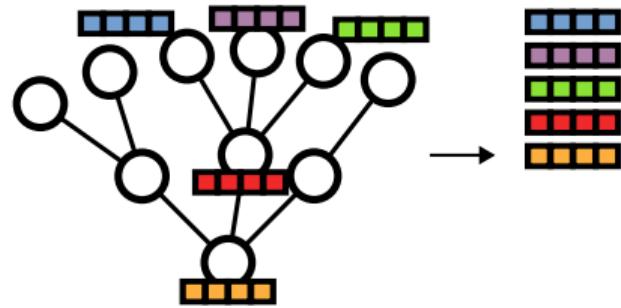
Graph convolutional networks learn node embeddings for downstream analysis



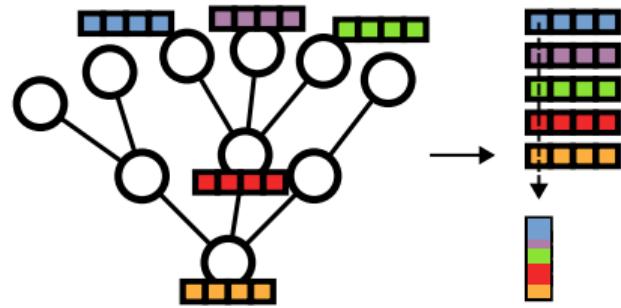
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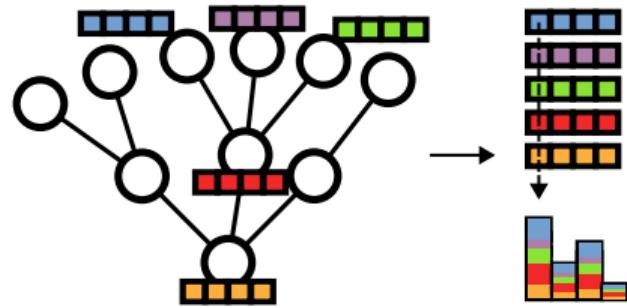
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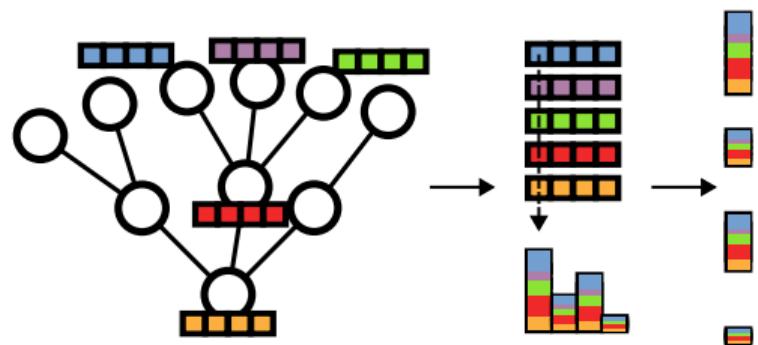
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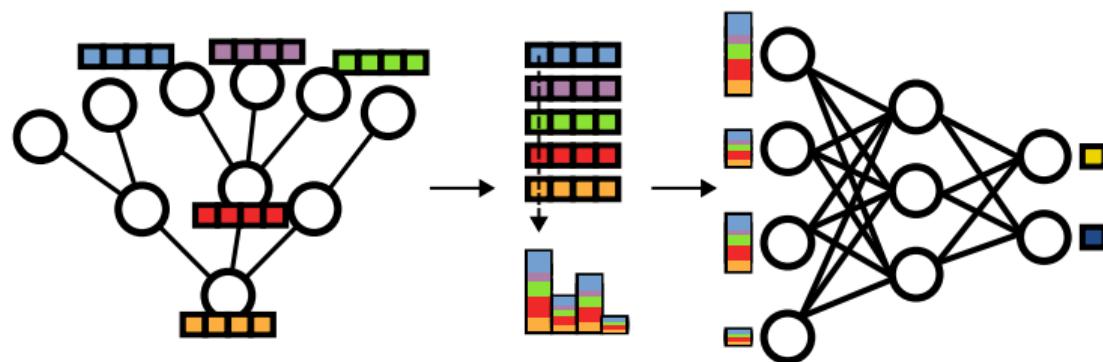
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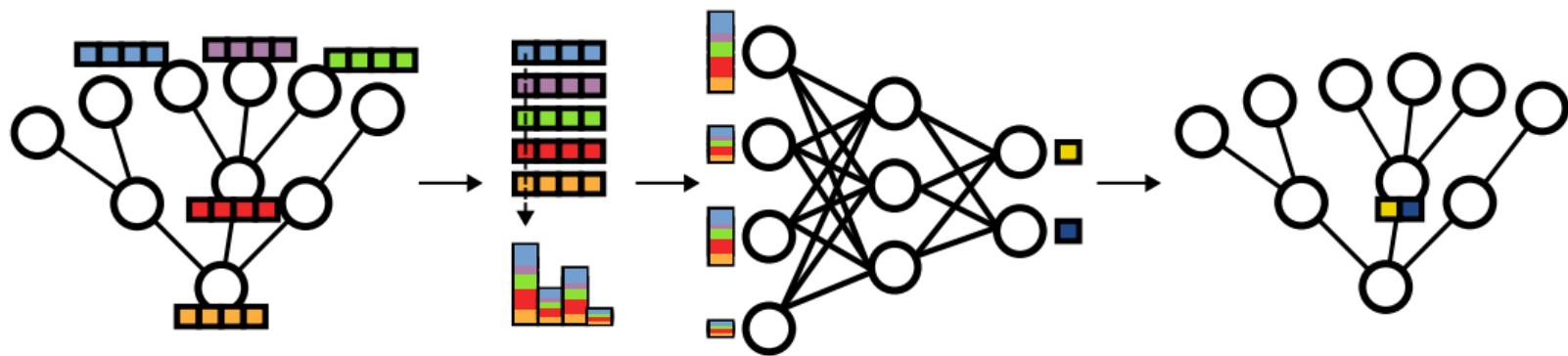
Graph convolutional networks learn node embeddings for downstream analysis



Graph convolutional networks learn node embeddings for downstream analysis

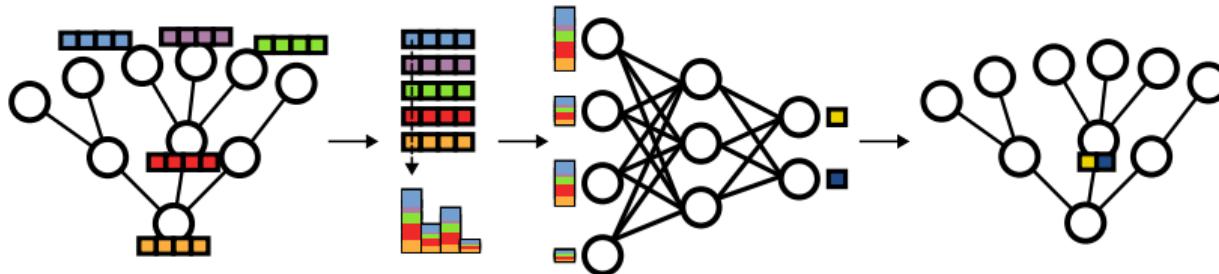


Graph convolutional networks learn node embeddings for downstream analysis

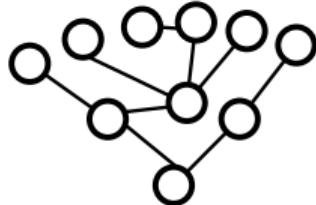
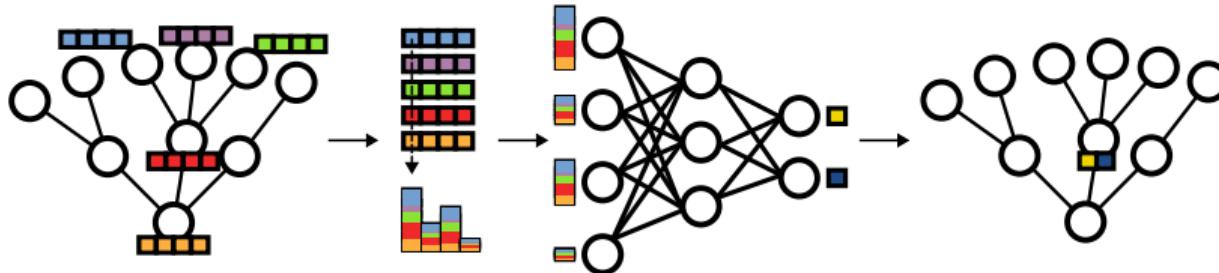


How do we learn without a training set?

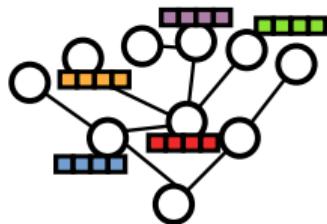
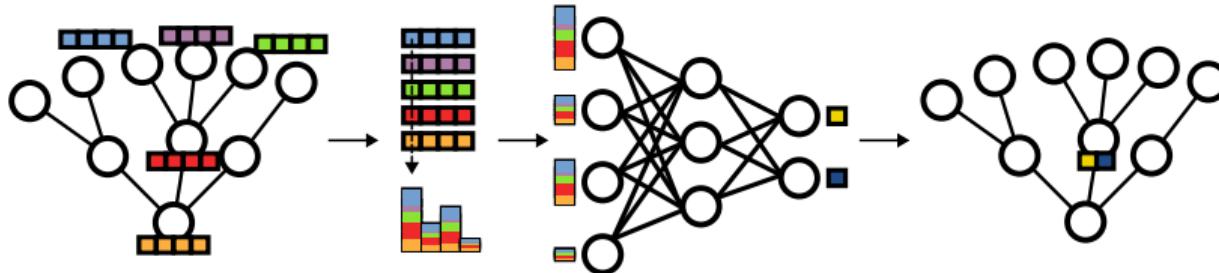
Graph convolutional networks learn node embeddings for downstream analysis



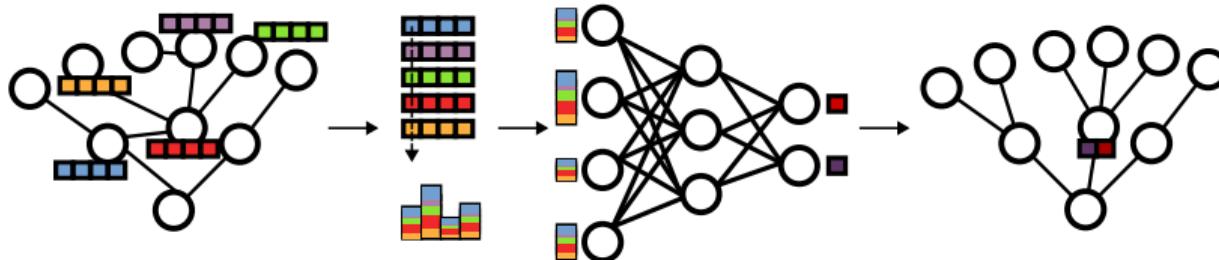
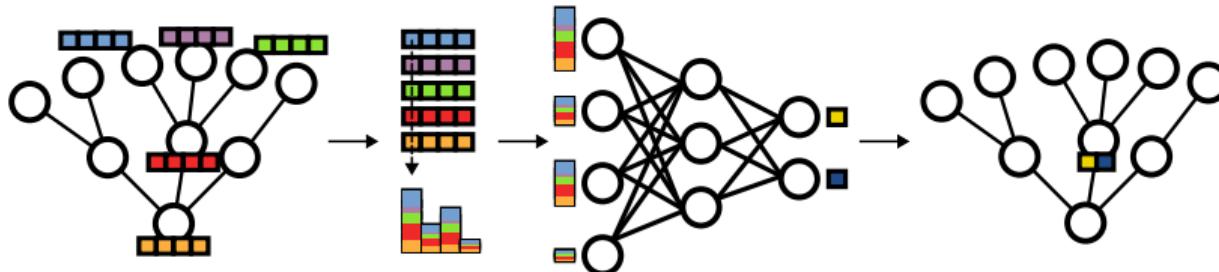
Graph convolutional networks learn node embeddings for downstream analysis



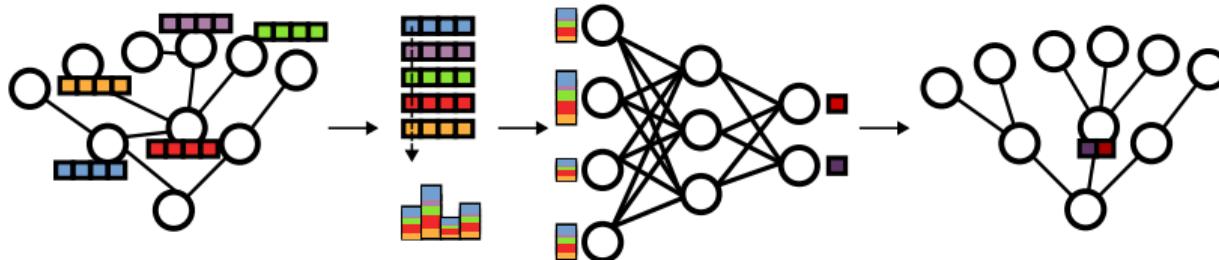
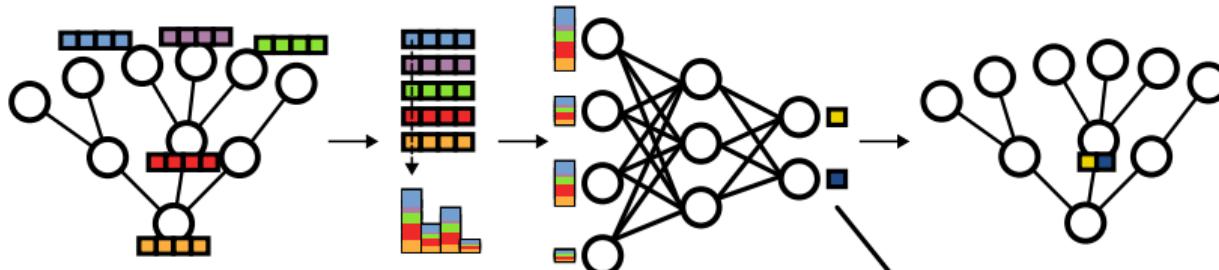
Graph convolutional networks learn node embeddings for downstream analysis



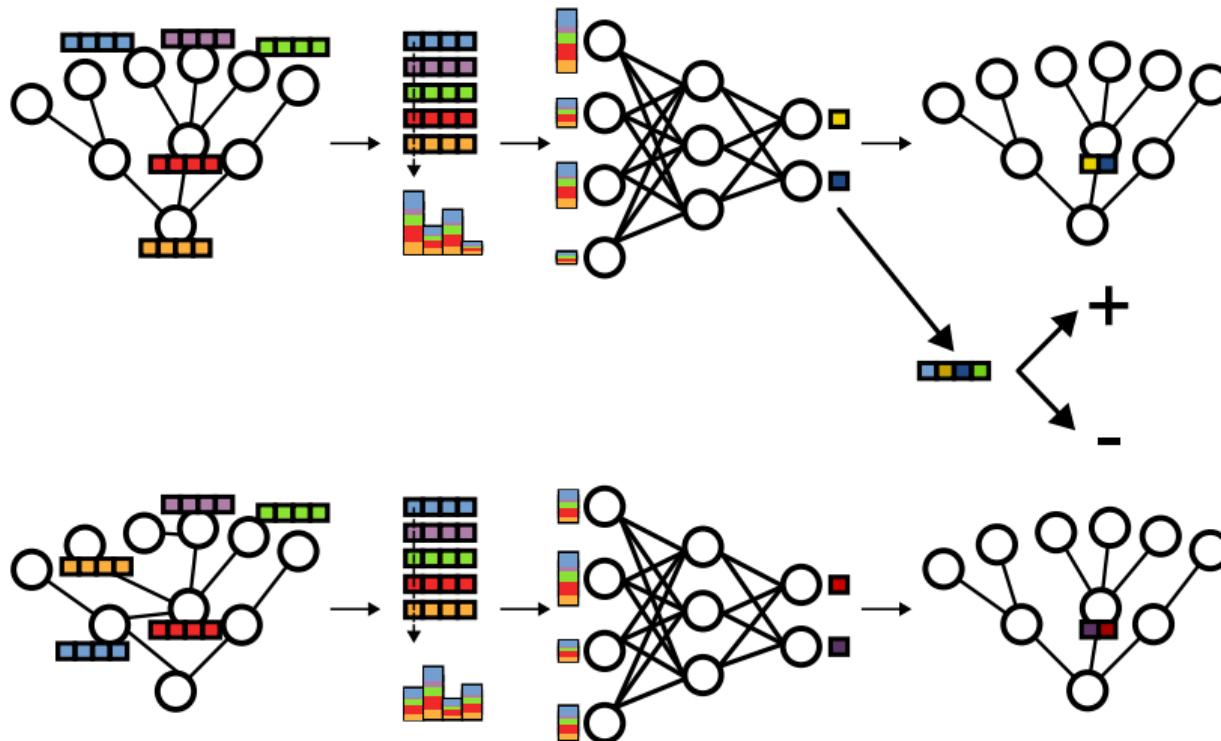
Graph convolutional networks learn node embeddings for downstream analysis



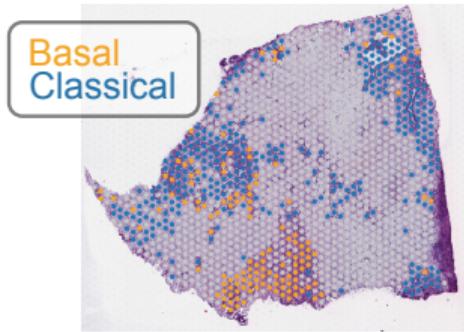
Graph convolutional networks learn node embeddings for downstream analysis



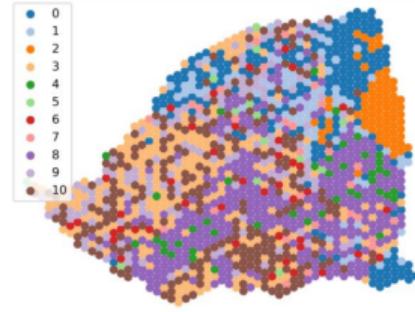
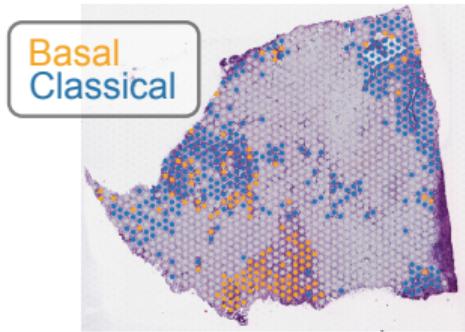
Graph convolutional networks learn node embeddings for downstream analysis



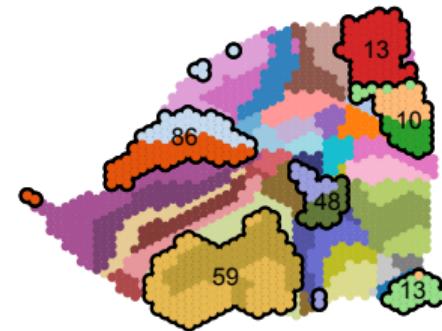
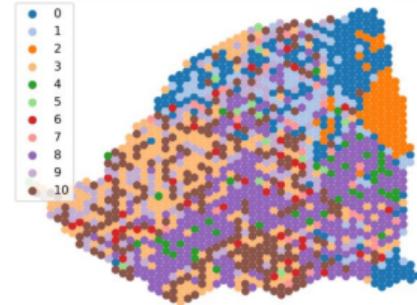
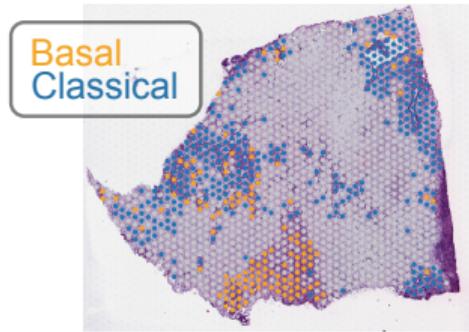
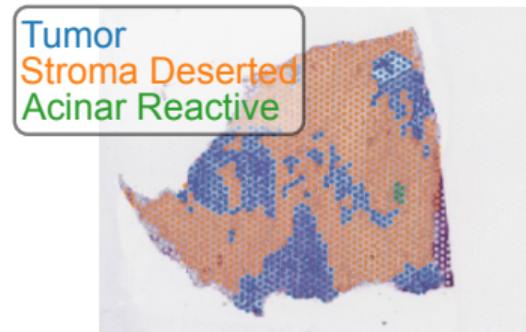
Our model better incorporates spatial cell organization



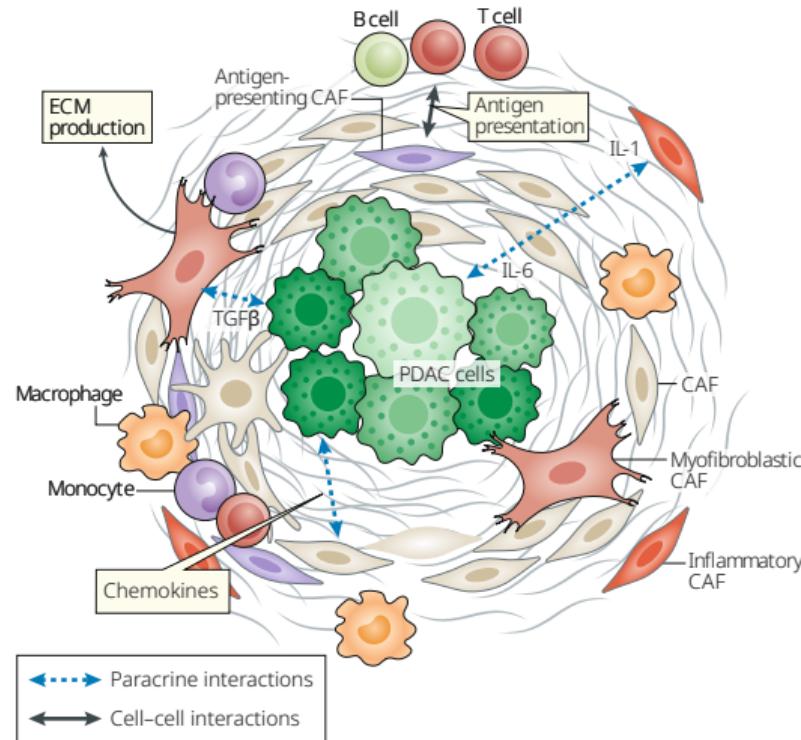
Our model better incorporates spatial cell organization



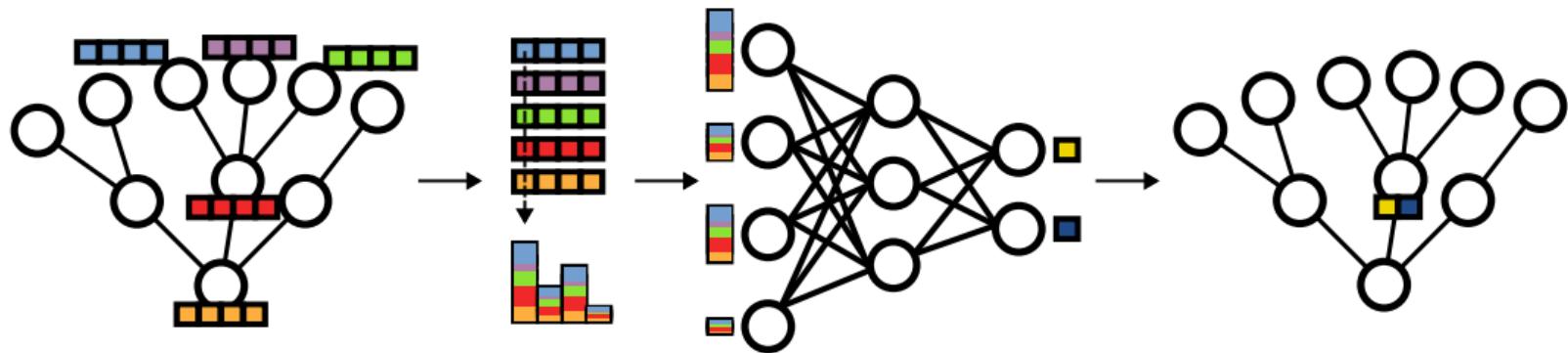
Our model better incorporates spatial cell organization



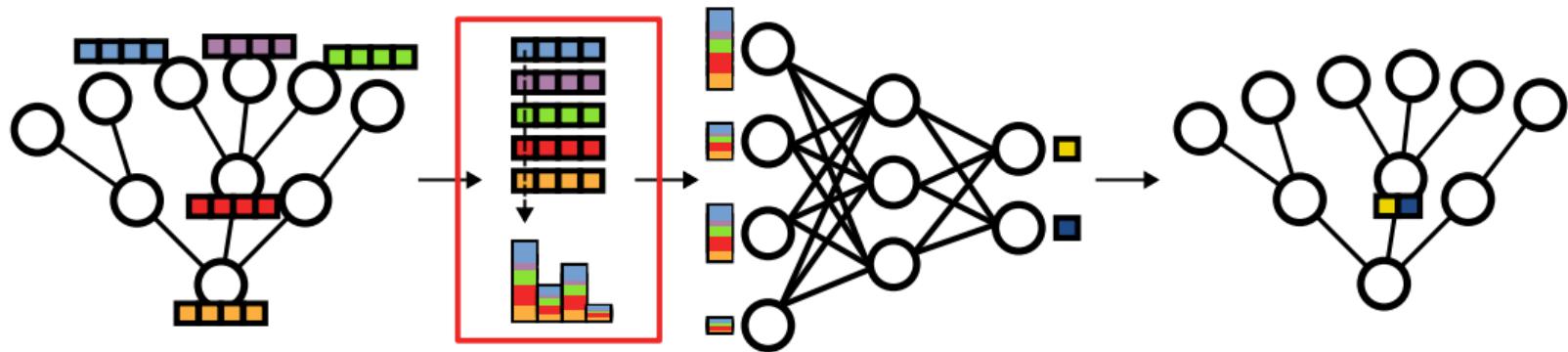
The microenvironment may influence subtype



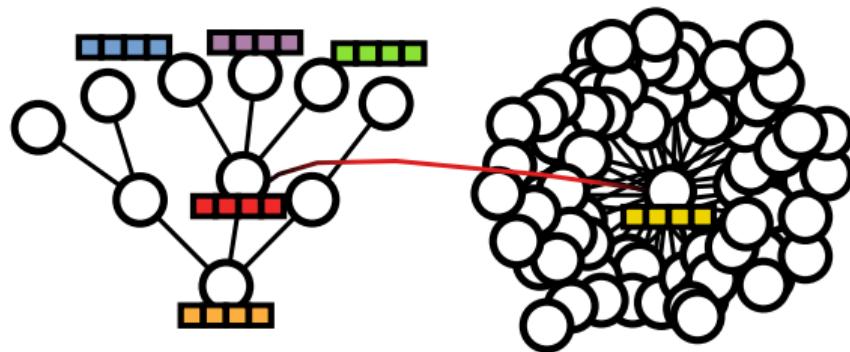
Graph attention networks learn communication



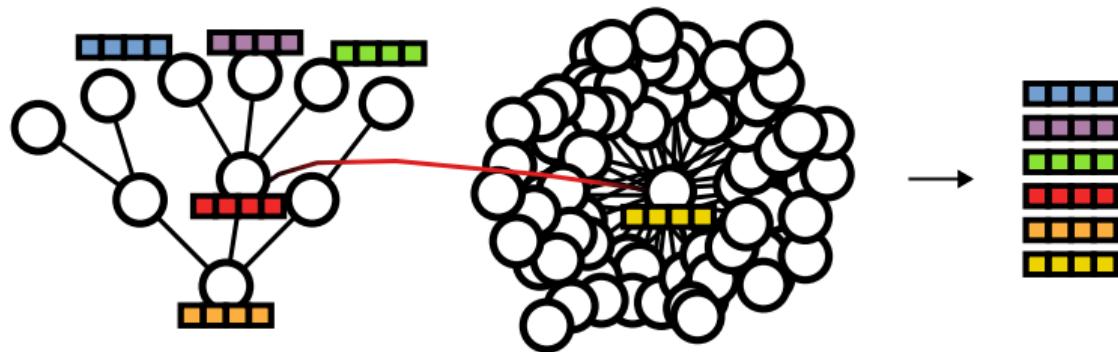
Graph attention networks learn communication



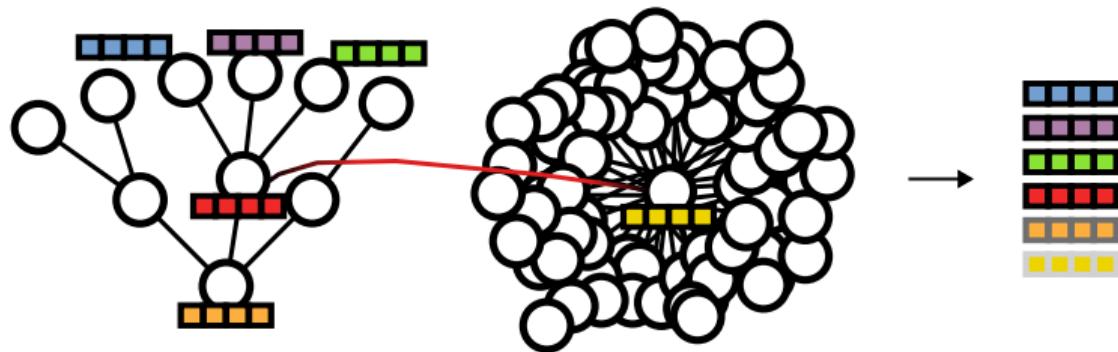
Graph attention networks learn communication



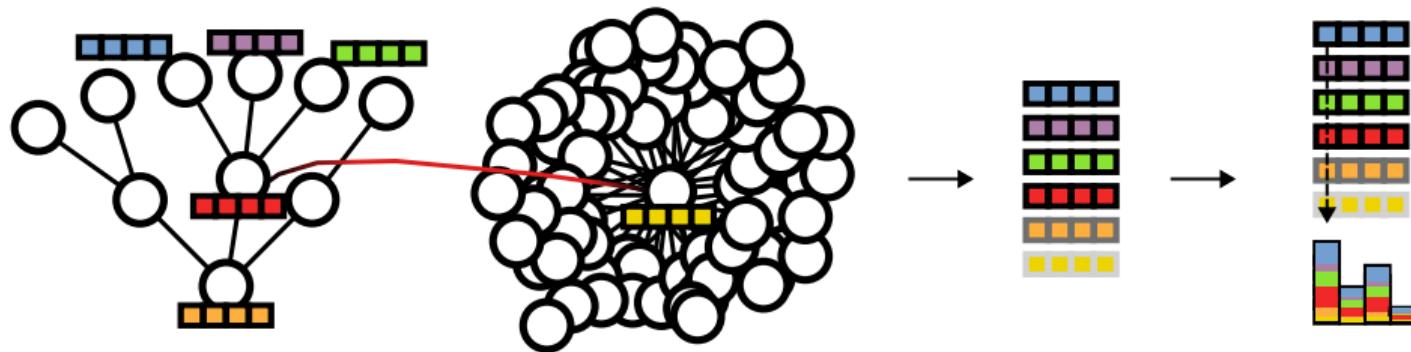
Graph attention networks learn communication



Graph attention networks learn communication



Graph attention networks learn communication



G

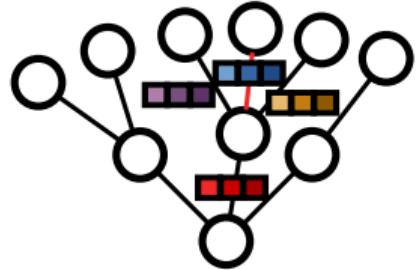
Do you use an attention model?



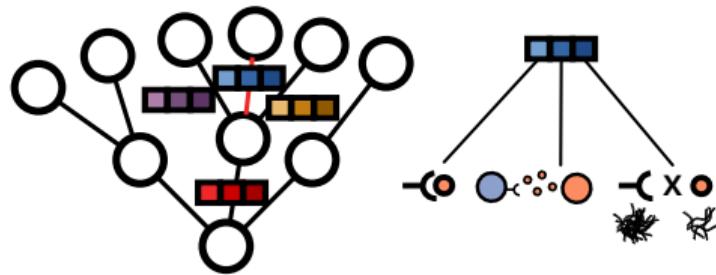
Yes, as a language model, I use an attention mechanism to help me understand the relationships between different words and phrases in a given text sequence. The attention mechanism allows me to assign varying levels of importance to different parts of the input text, which helps me generate more accurate and meaningful responses. This attention mechanism is a key component of many advanced language models, including the GPT (Generative Pre-trained Transformer) family of models, which I am a part of.



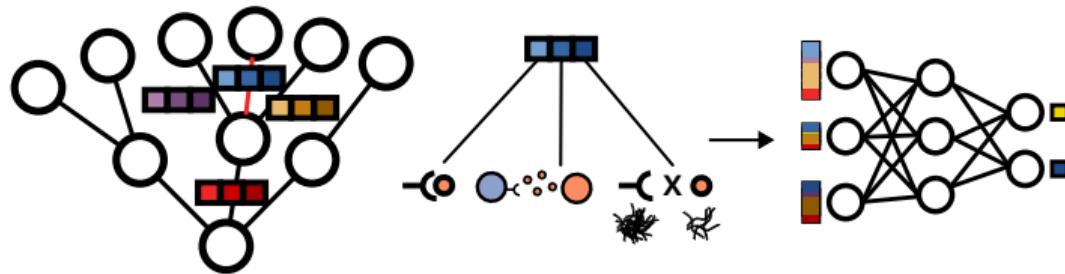
Graph attention networks learn communication



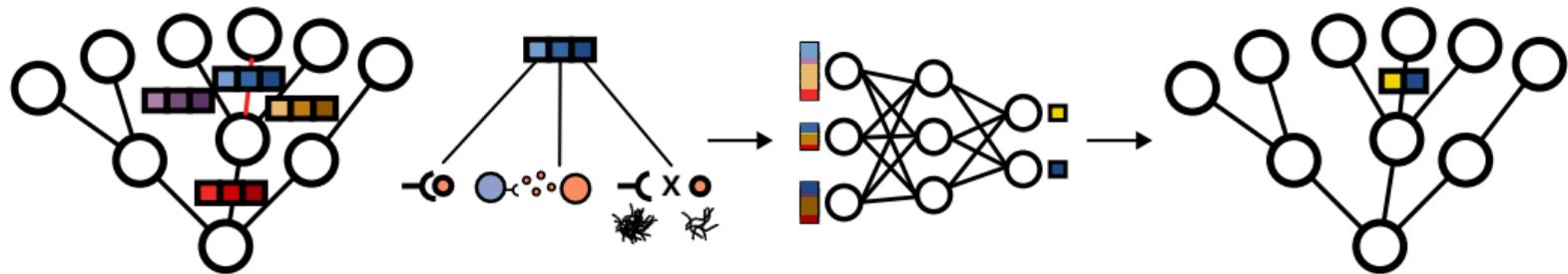
Graph attention networks learn communication



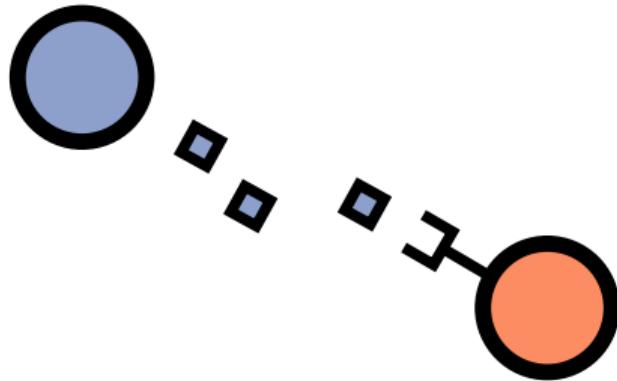
Graph attention networks learn communication



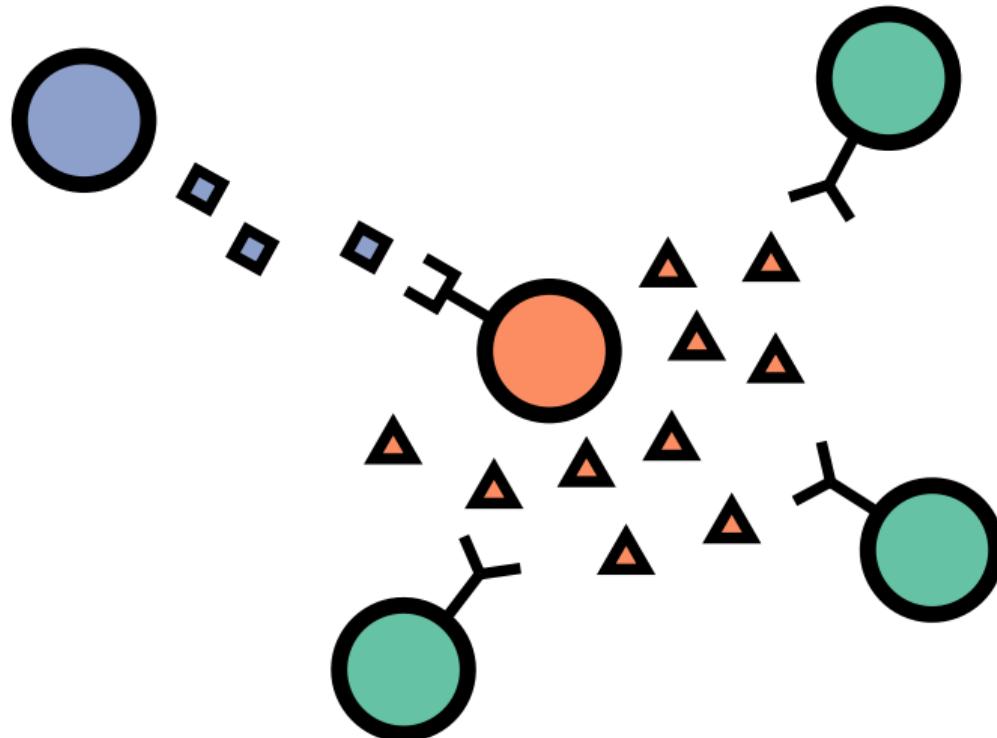
Graph attention networks learn communication



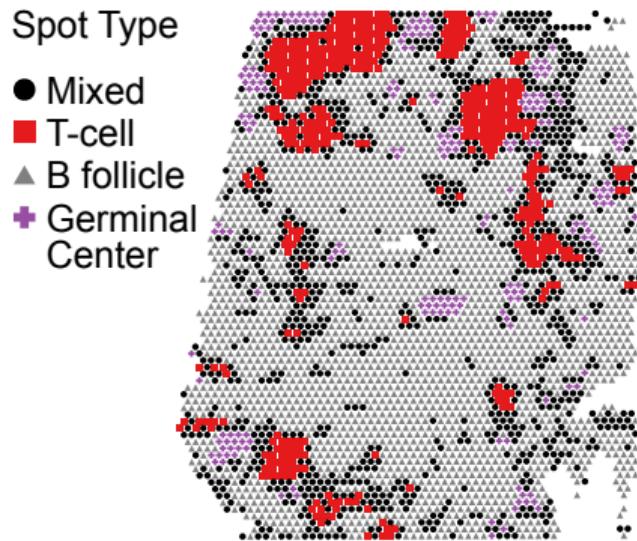
Our model detects cell-cell communication patterns



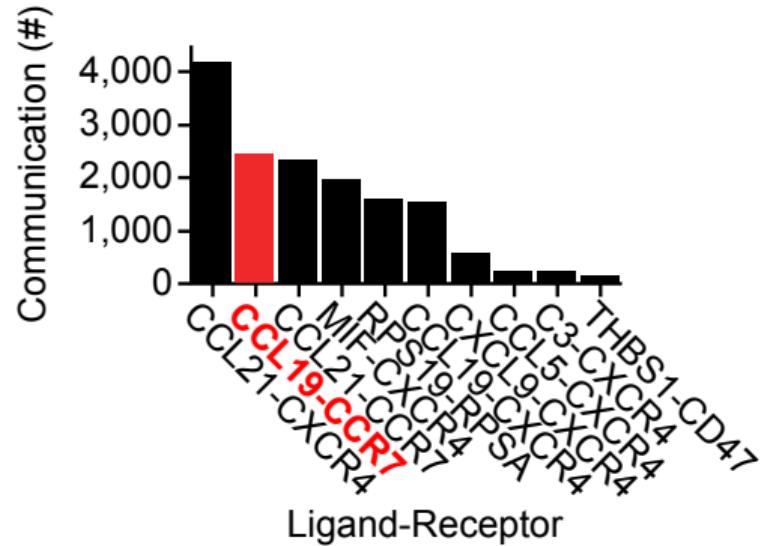
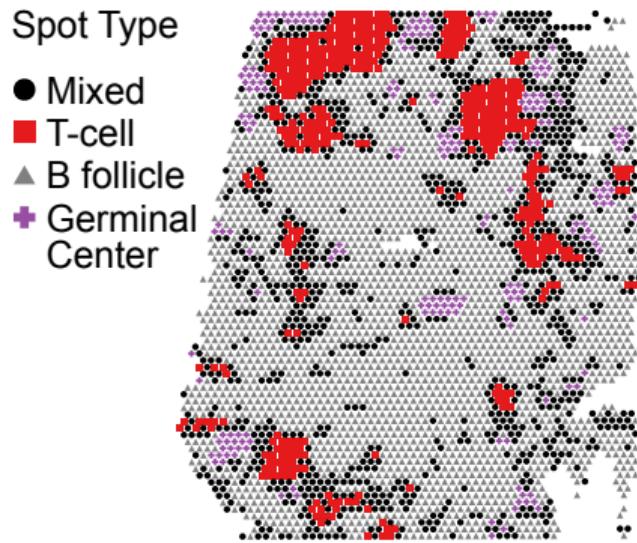
Our model detects cell-cell communication patterns



NEST identifies localized *CCL19-CCR7* in lymph node



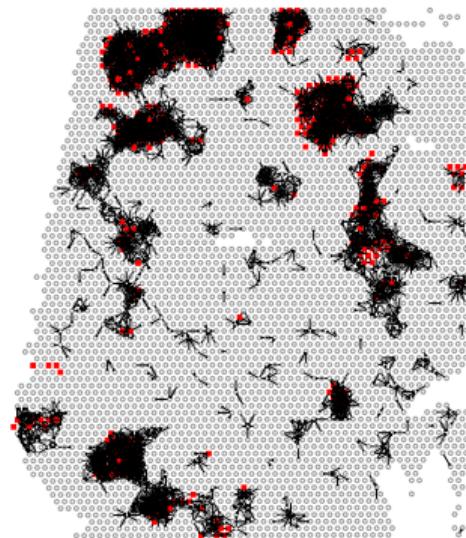
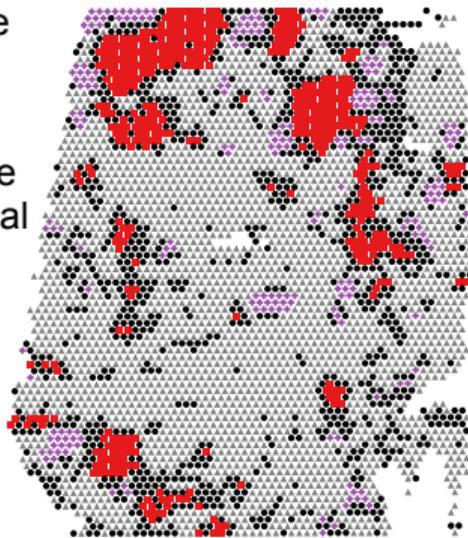
NEST identifies localized *CCL19-CCR7* in lymph node



NEST identifies localized *CCL19-CCR7* in lymph node

Spot Type

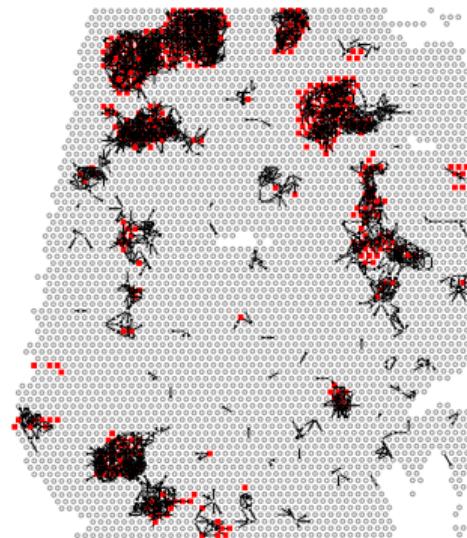
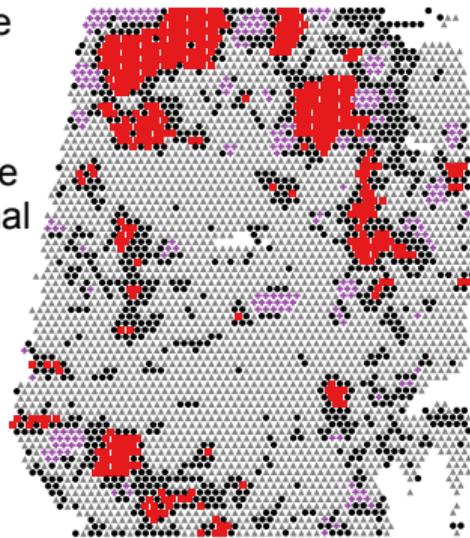
- Mixed
- T-cell
- ▲ B follicle
- + Germinal Center



NEST identifies localized *CCL19-CCR7* in lymph node

Spot Type

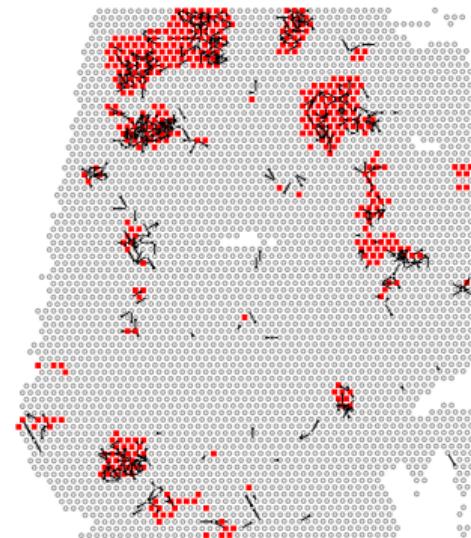
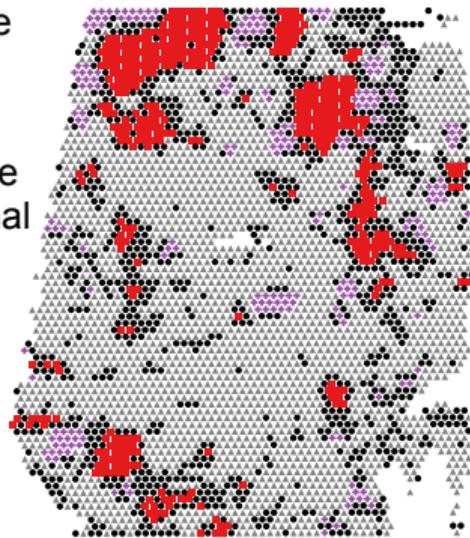
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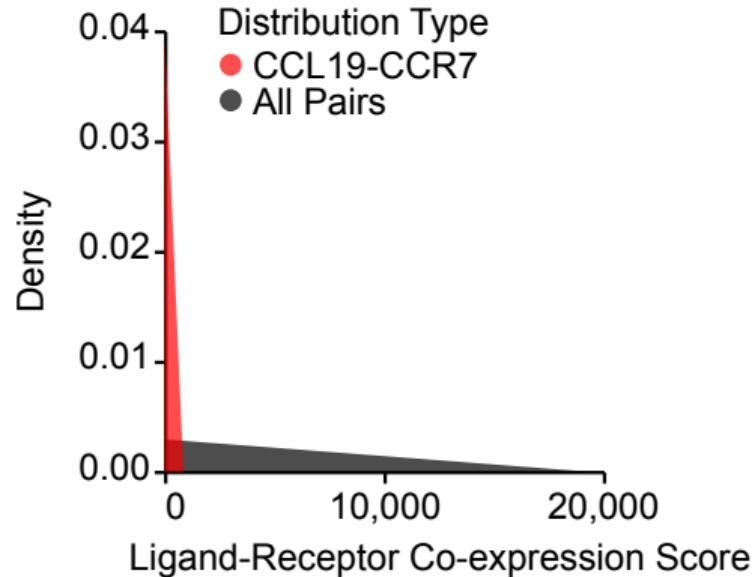
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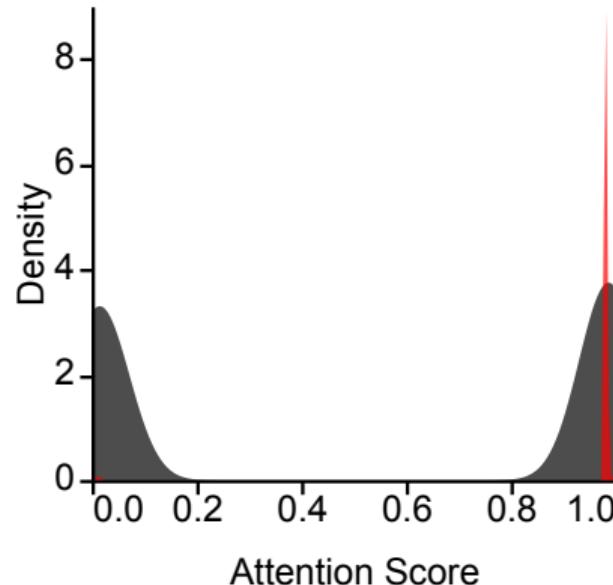
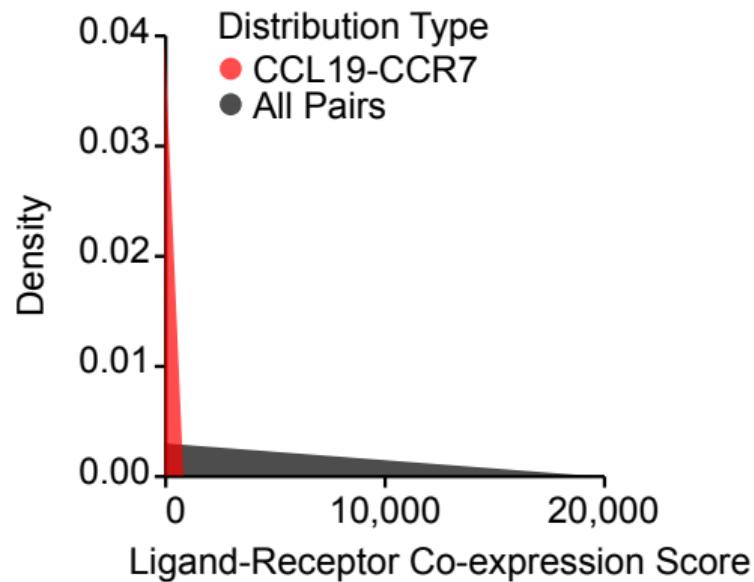
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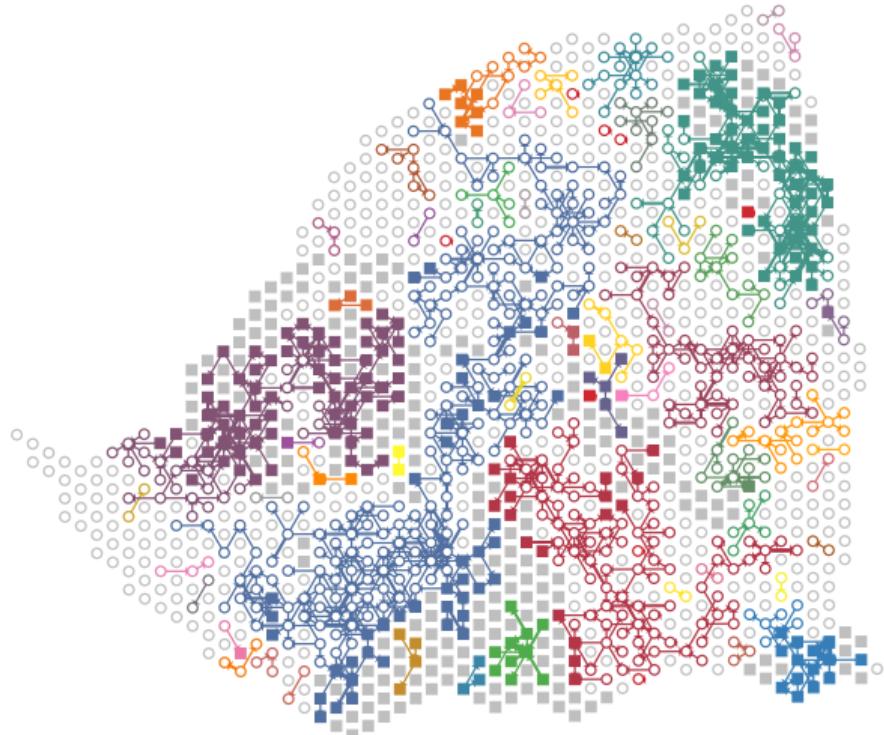
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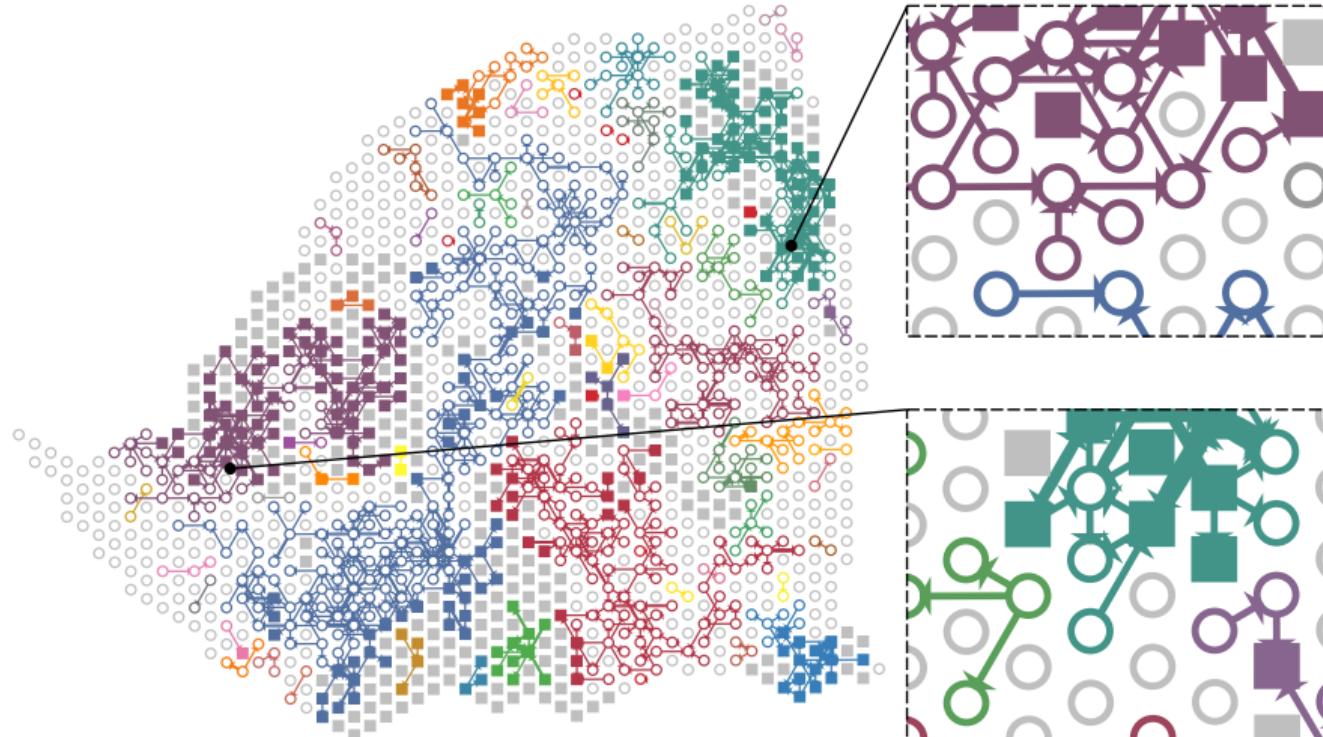
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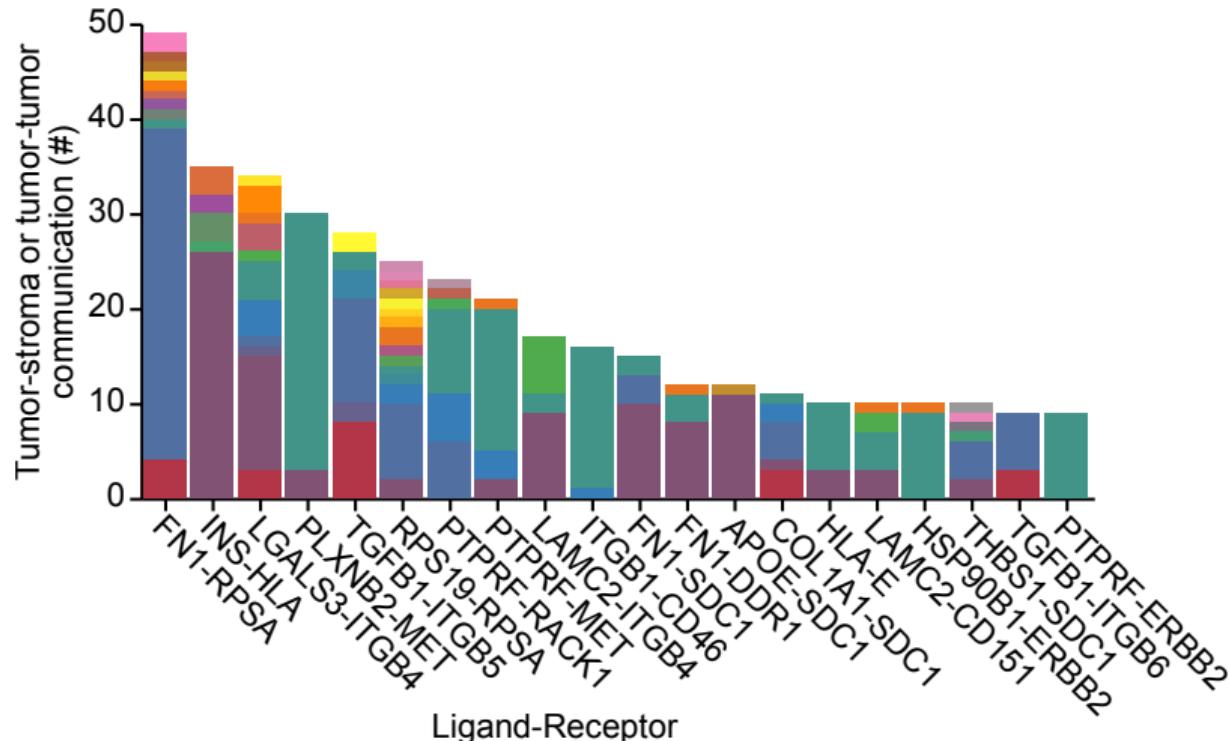
NEST reveals spatially dependent communication *in situ*



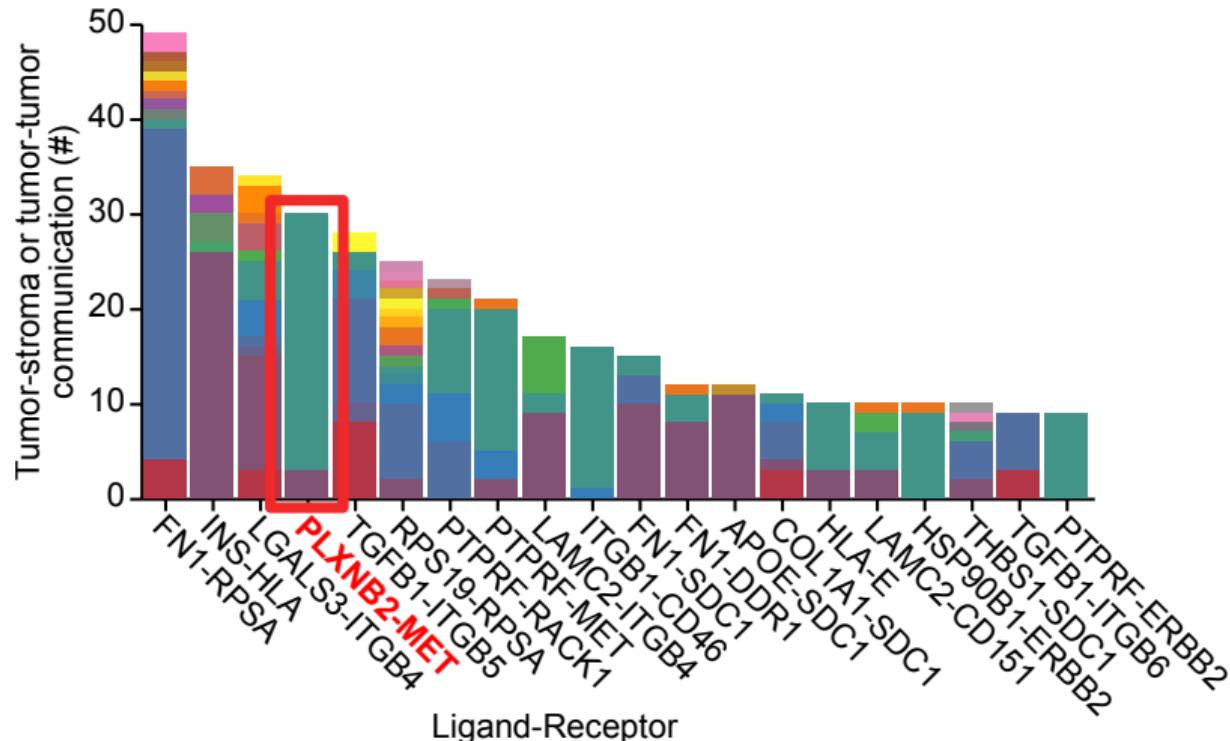
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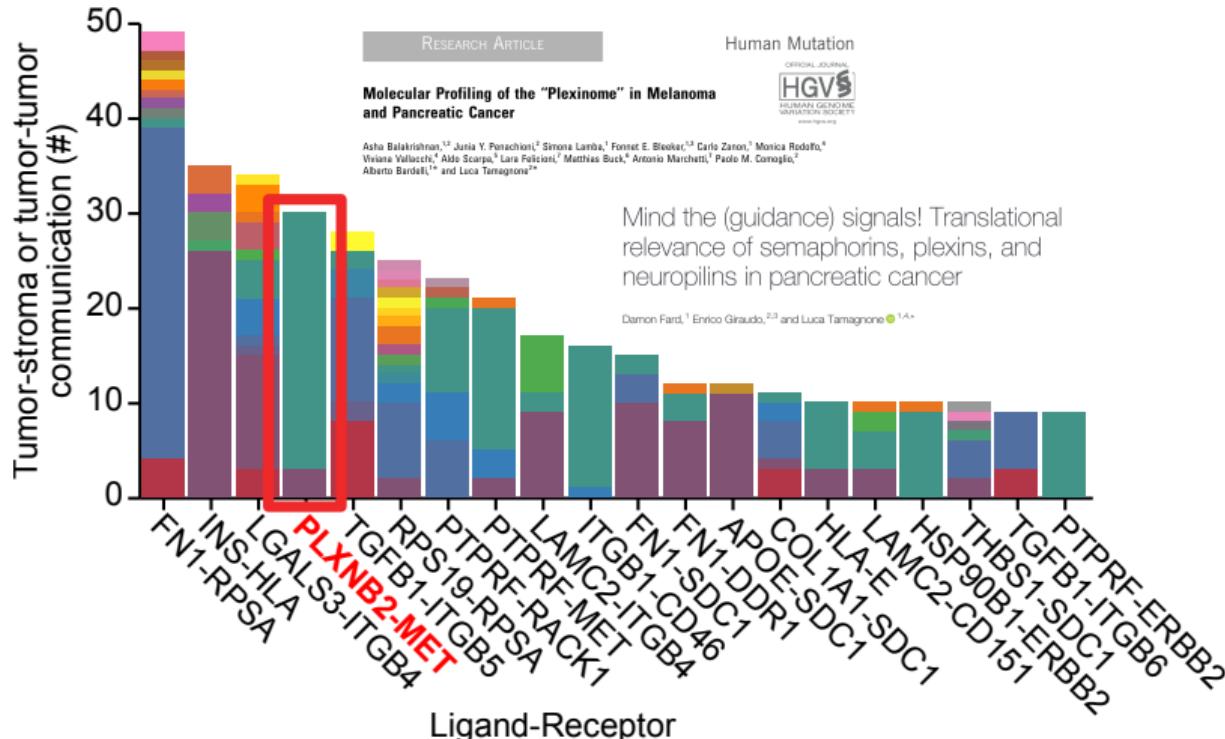
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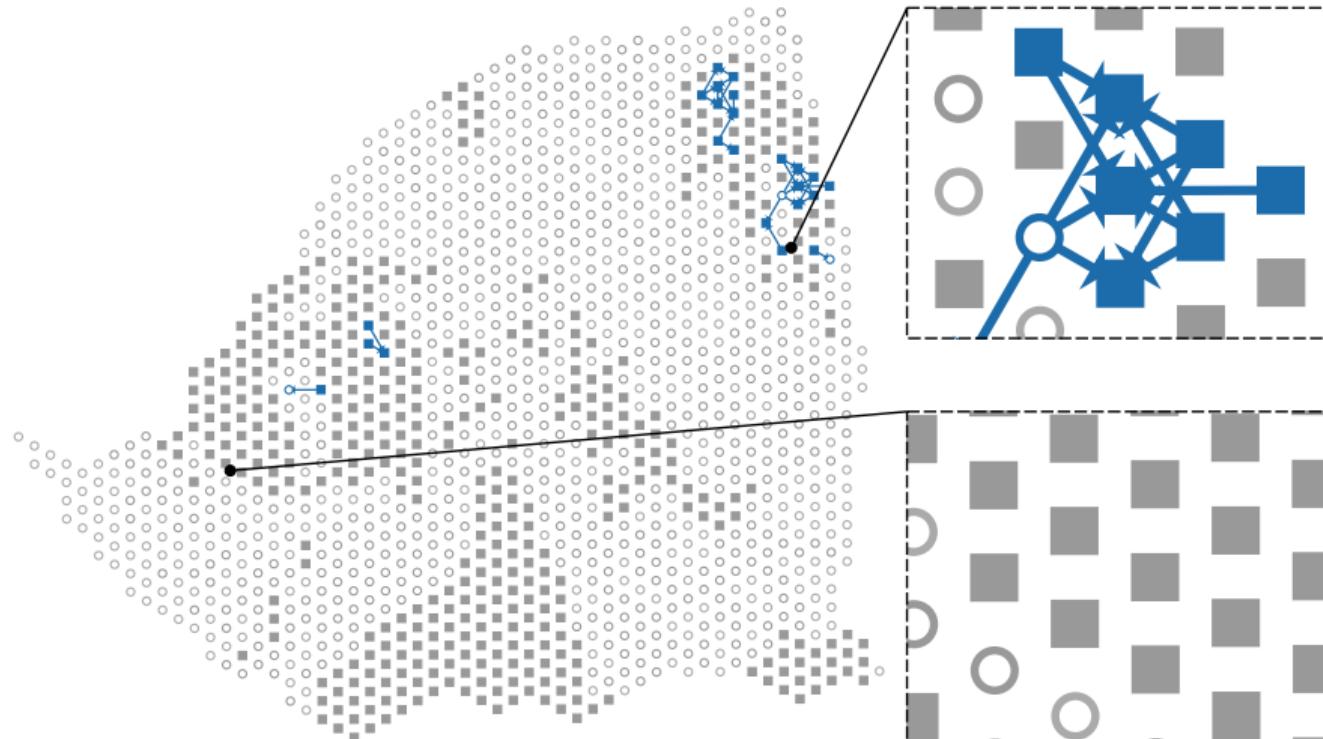
NEST reveals spatially dependent communication *in situ*



NEST reveals spatially dependent communication *in situ*



NEST reveals spatially dependent communication *in situ*



What was covered within this module

The role and context of cell-cell communication

Single-cell RNA sequencing for cellular transcriptomes

General principles of cell-cell communication detection

Inner workings of select single-cell RNA sequencing methods

Spatial transcriptomics for communication detection

Spatially-resolved cell-cell communication method

We are on a Coffee Break & Networking Session

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