Biology is Spatial: A Primer on Spatial Biology and Applications to Oncology and Immunology

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Precision Medicine and Computational Biology
Sanofi US R&D

Thank you to Emanuele and Magnus!

Agenda

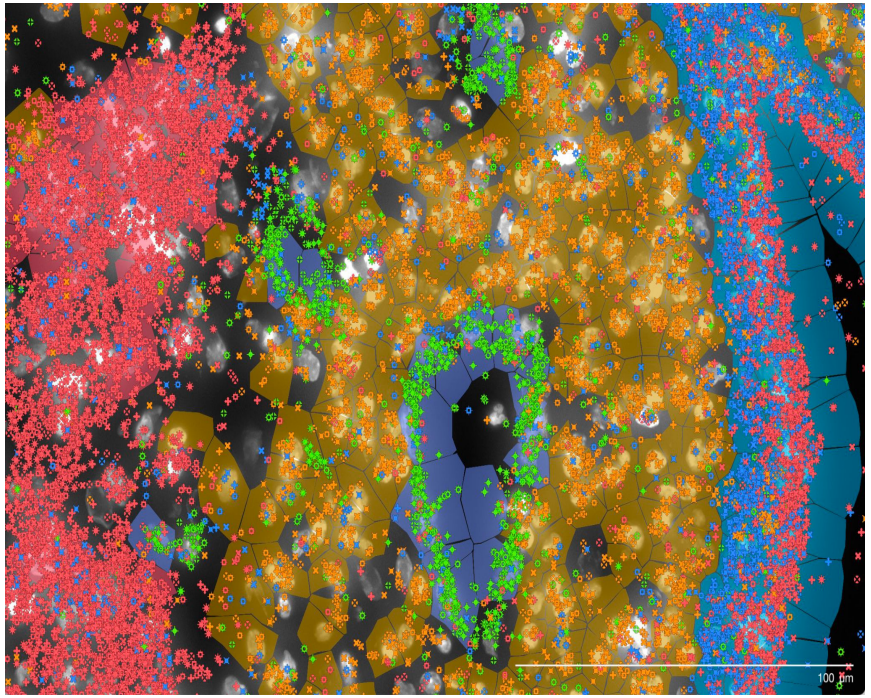
- Introduction to spatial biology technologies
- Overview of spatial biology data analysis

- Deep dive into oncology case

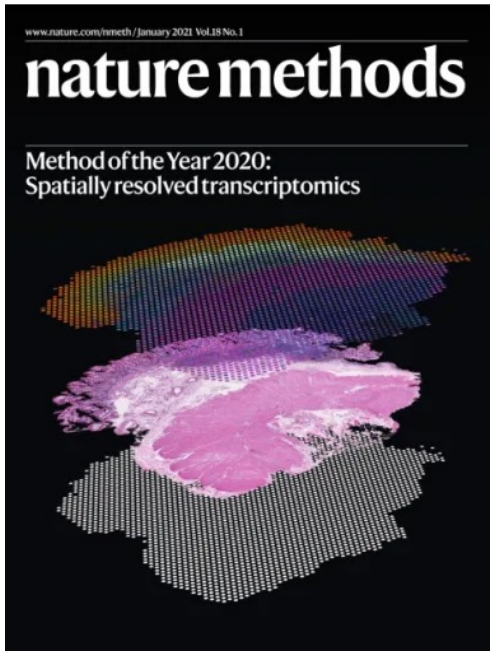
- Deep dive into Immunology case

Learning objectives:

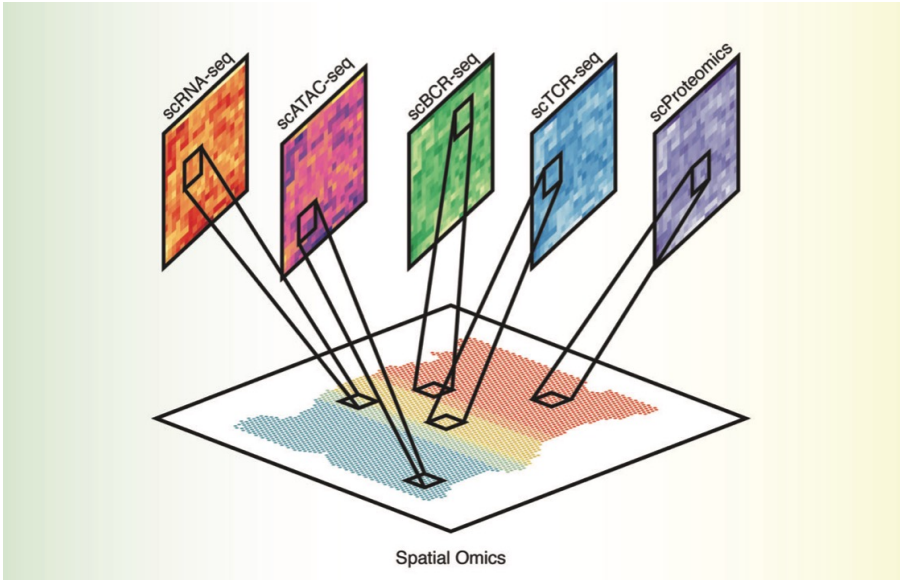
- What is spatial biology?
- Similarities and differences in spatial technologies
- High level analytics being developed for spatial
- Questions spatial biology can answer
- Current limitations to the technologies.

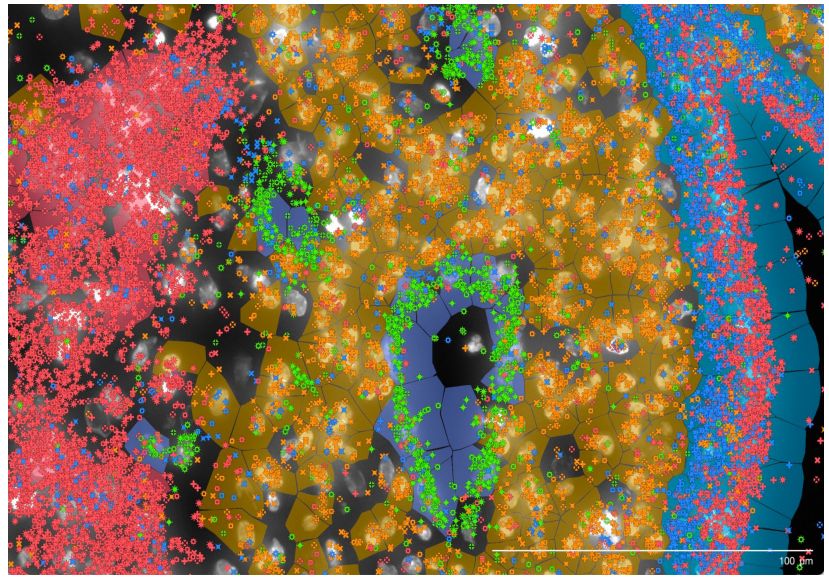


The Next Frontier: Spatial Biology



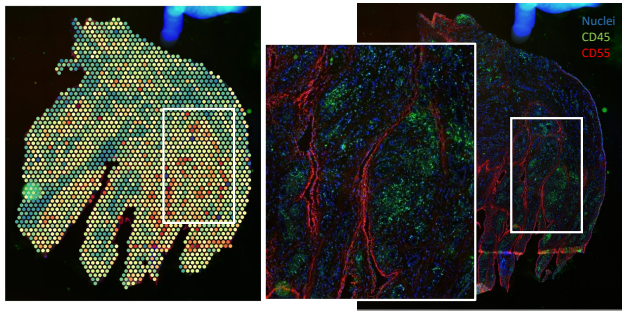
"We're in the middle of one of the biggest revolutions in life science biology," says Beechem. "Spatial multiomics is changing the way life science research gets done."

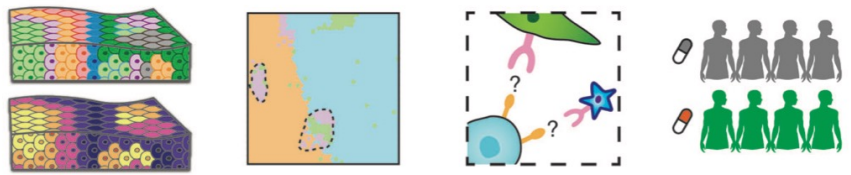


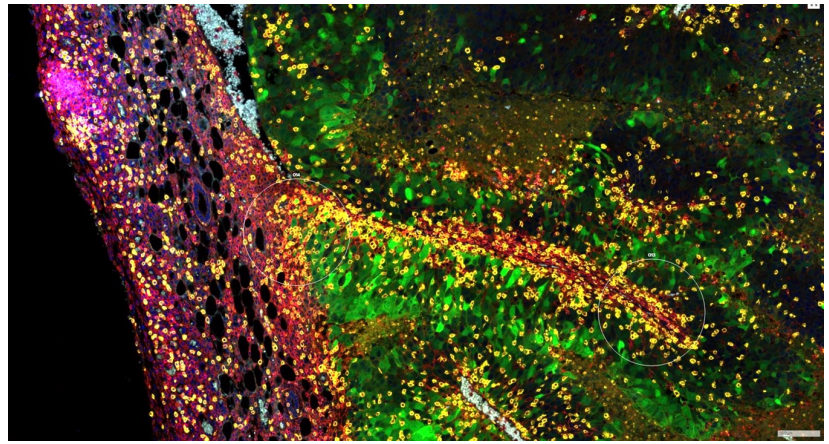


Human ER+ breast Xenium

Spatial transcriptomics of the synovium of RA patients reveals tissue heterogeneity



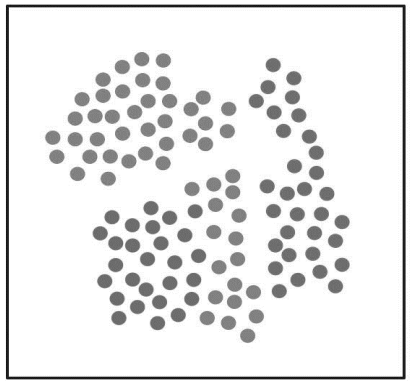




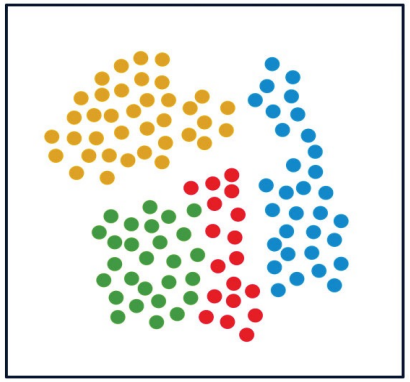
MC38 treated with IO

Wu et al Clinical Translational Medicine 2021

Where does Spatial Biology Fit? Adding the “where” to “what”



Bulk



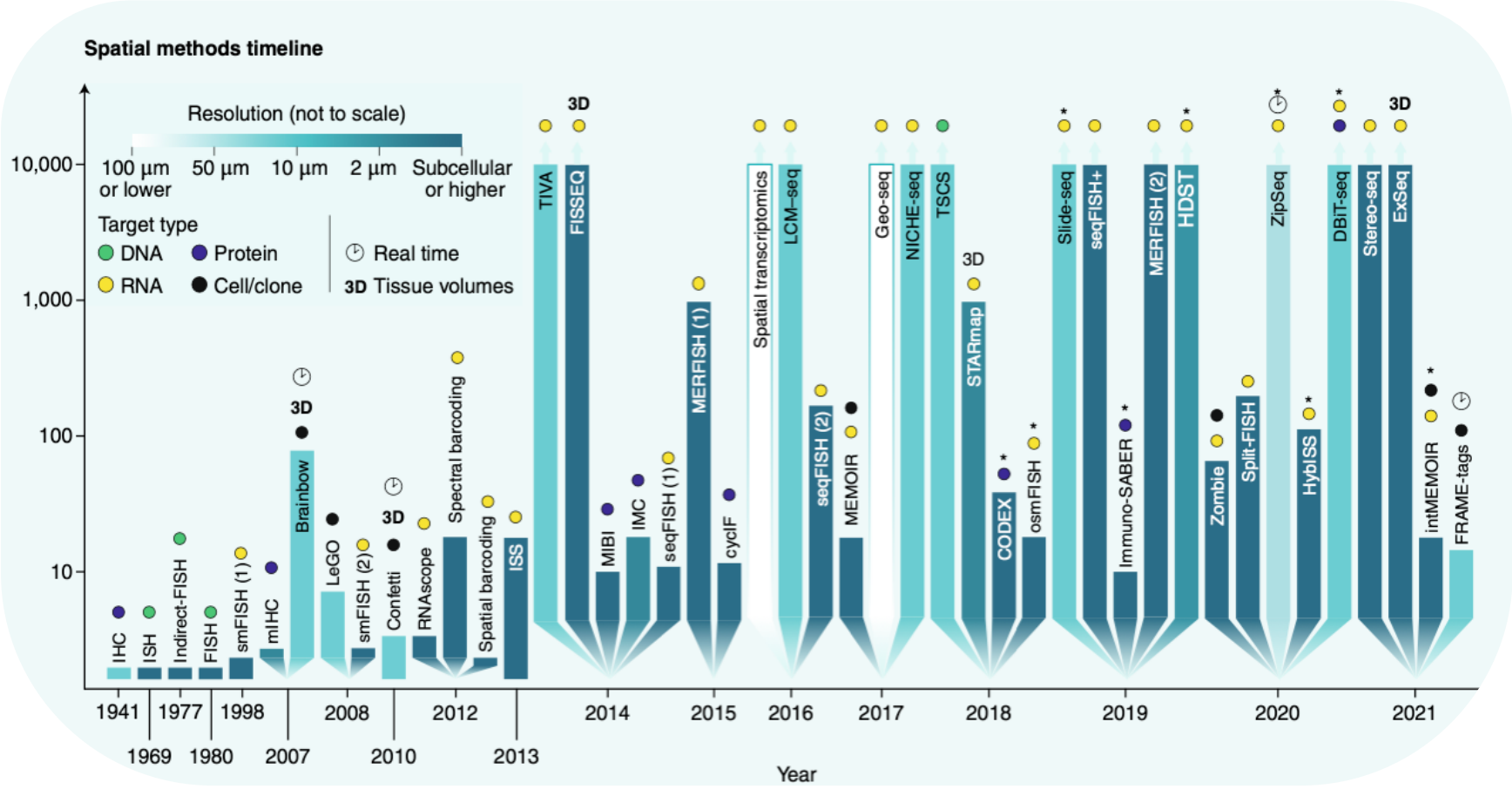
Single Cell

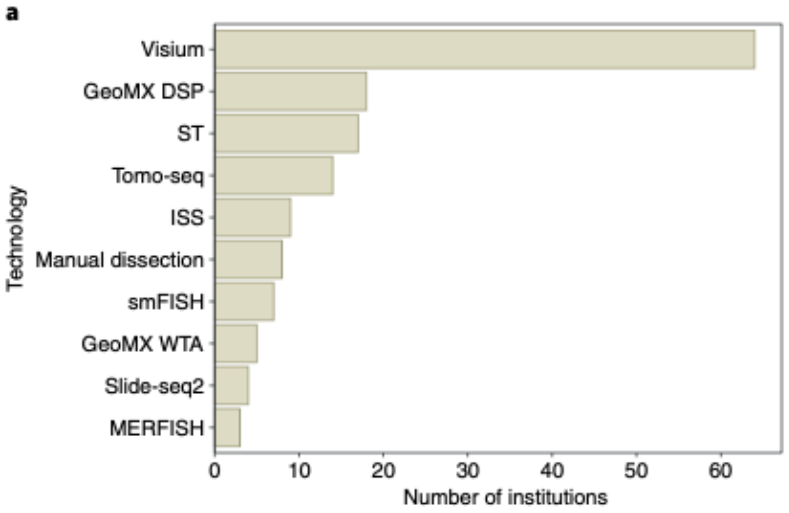
Spatial

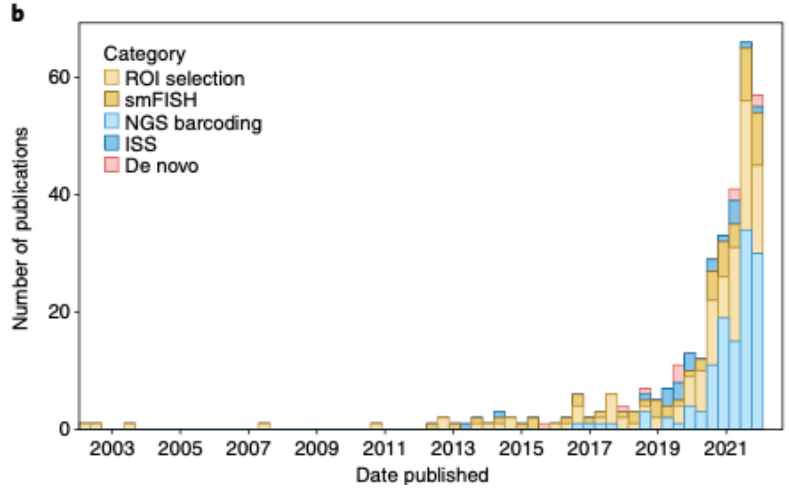
Spatial omics technologies uncovers biology that is important for drug discovery and validation pipelines

- Characterize Disease Biology
- Credentialing Targets & Validation
- Identify Mechanism of Action of a compound
- Identify cell states involved Drug Resistance
- Stratify patients in biopsy tissues

Spatial Technologies are on the Rise





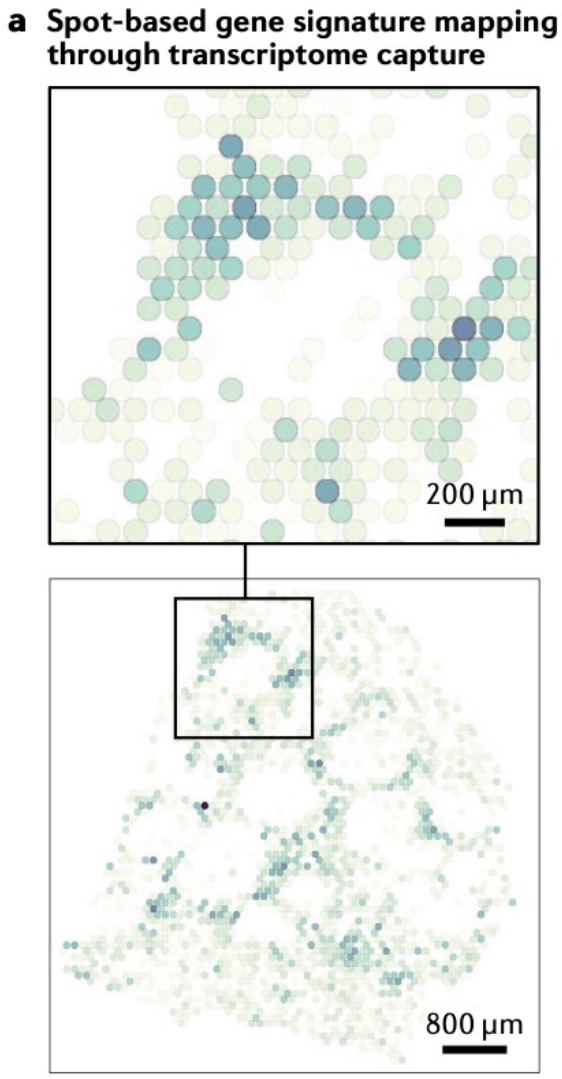


- Not as many techniques as single cell
- Spatial biology is 5-10 years behind single cell
- Analytical solutions are exploding but challenges are emerging

Lewis et al. Nature Methods 2021
Moses and Pachter Nature Methods 2021

Different Types of Spatial Readouts

Spot-based, RNA



ROI-based, RNA

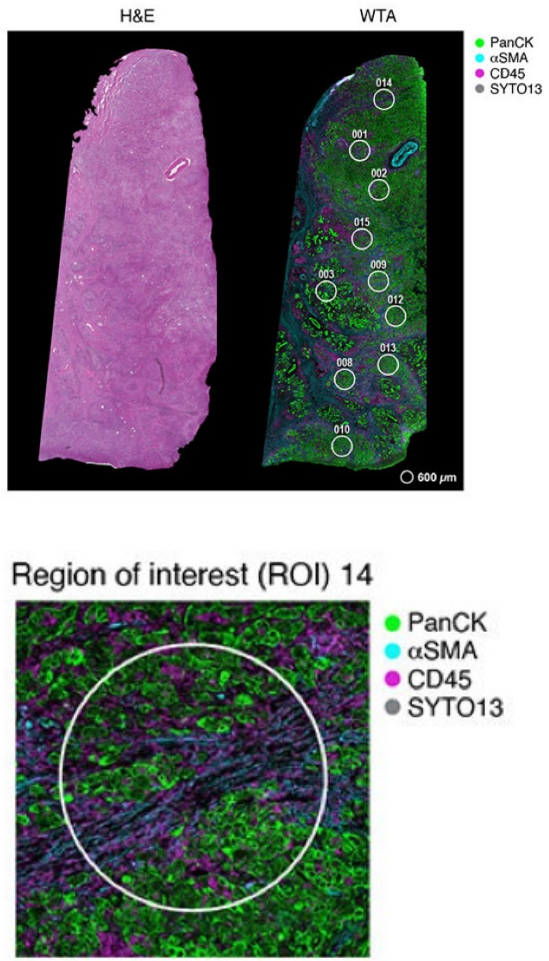


Image-based RNA subcellar

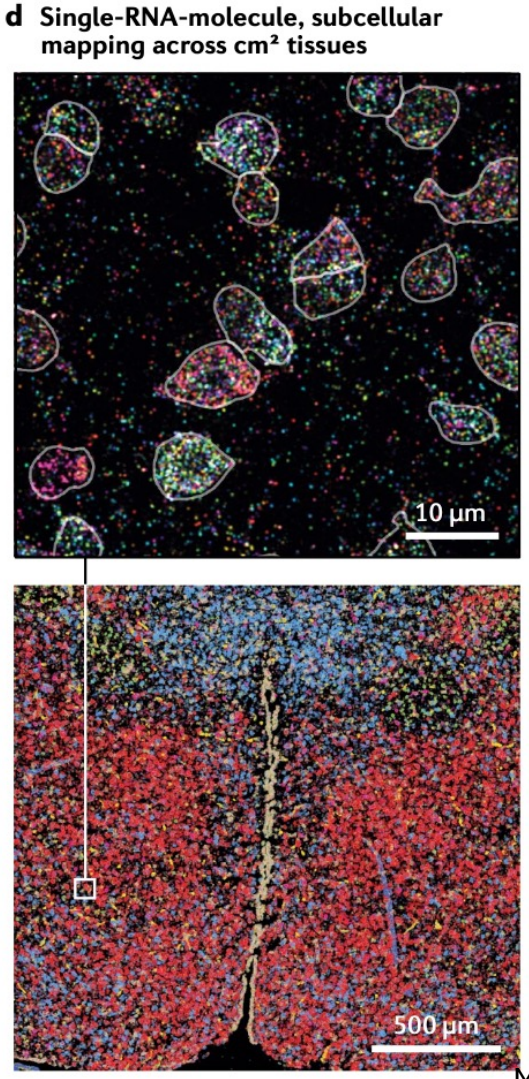
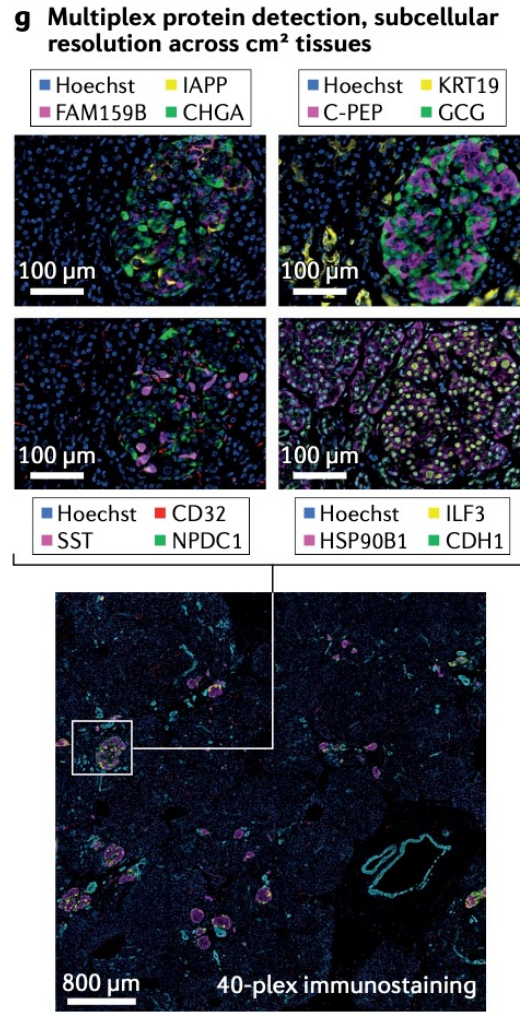


Image-based Protein subcellular

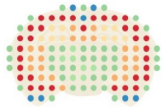


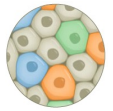
Moffit et al Nature Rev Genetics 2022
Kleino et al Computational and Structural Biology 2022

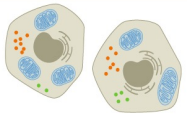
Examples of Spatial Technologies and their Features Along the Spectrum

Technology

ROI/Spot | Single-cell | Sub-cellular







Sample Throughput → Cell Resolution

Current Implementation

nanoString GeoMx Digital Spatial Profiler	10X GENOMICS Visium Spatial Gene Expression	AKOYA BIOSCIENCES Phenocycler-Fusion	10X GENOMICS Xenium
RNA: Whole Transcriptome Protein: 500+	**RNA:** Whole Transcriptome Protein: 31+	**RNA:** upcoming Protein: 65+	**RNA:** 350+ Protein: upcoming
Throughput: Samples/week = 8	**Throughput:** Samples/week = 2	**Throughput:** Samples/week = 2	**Throughput:** Samples/week = 2

Applications & Specific outcomes

Hypothesis Generation
Exploratory Analysis

Selection of co-expressed genes
Identification of co-localize cell type
Endotype specific spatial signature
at spot resolution

Hypothesis Validation
Target Credentialing

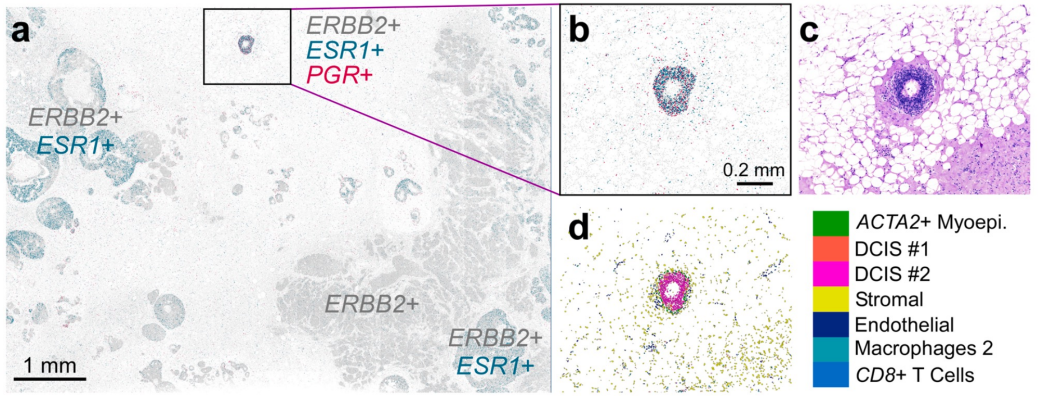
Validation of co-expressed genes
Validation of cell-cell interactions
Signature for activation/co-activation
Endotype specific spatial signature
at cellular resolution

Current Challenges

Sample Access | **Data processing Data integration** | **Throughput** | **Cost** | **Targeted Panel**

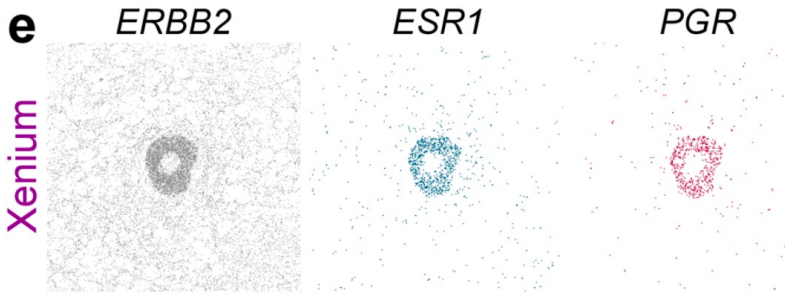
An Example of Resolution: Chromium vs Visium vs Xenium

- Use serial sections to explore tech and heterogeneity
- Breast cancer sample, Stage II-B, ER+/PR-/HER2+ 2x 25um FFPE curls.
- Adjacent tissue sections for Visium and Xenium (human breast + 33 add ons)

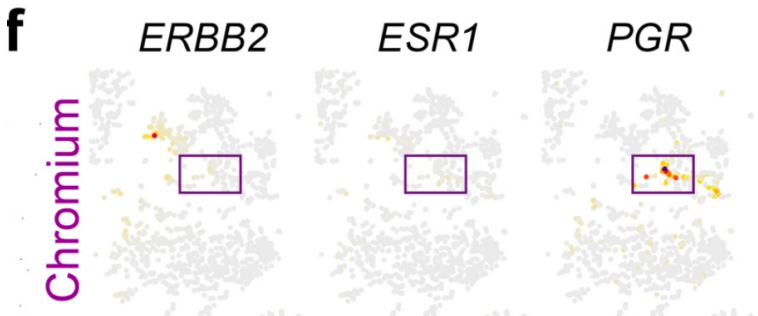


a) Xenium spatial plot HER2+(ERBB2) ER+ (ESR1) PR+ (PGR) decoded transcripts.
b) Closer view of triple-positive ROI.
c) Corresponding H&E image.
d) Cell types contained within ROI reveal that this is a DCIS #2 tumor epithelium

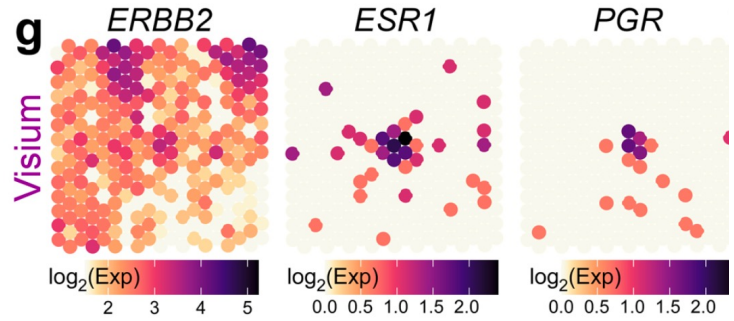
Xenium spatial plot



Chromium scFFPE-seq



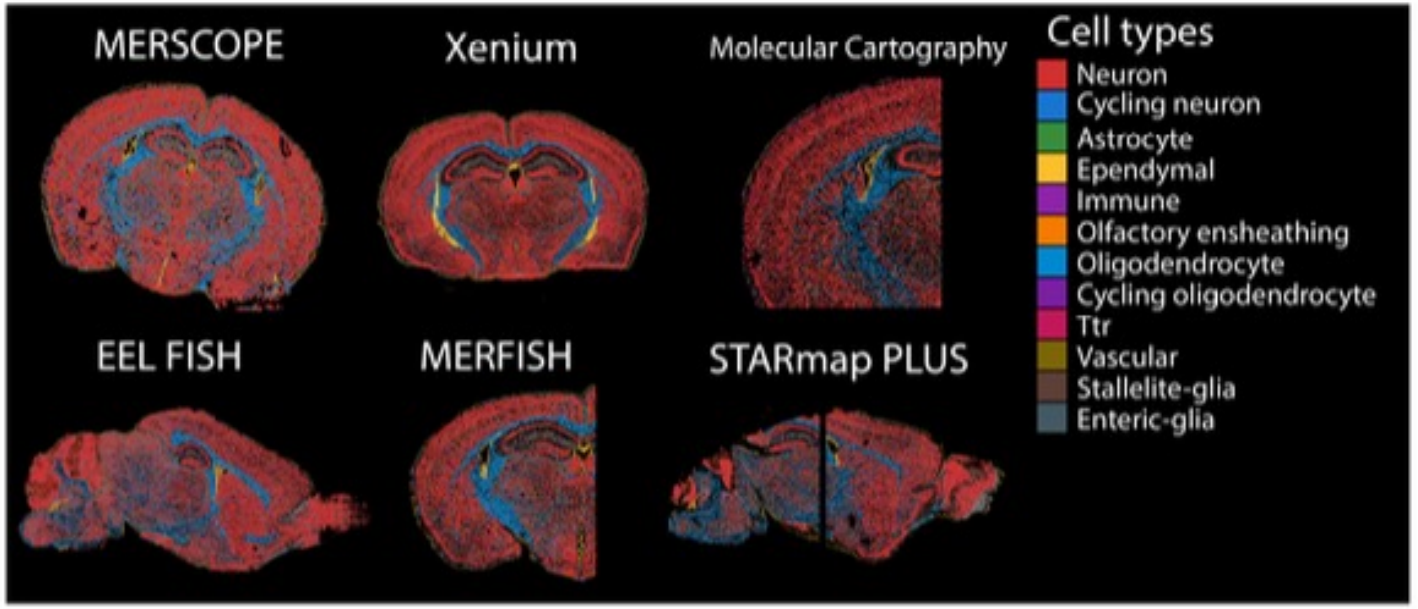
Visium spatial plot



Janesick et al Nature Communications 2021

An Example of Resolution Across In situ methods

a

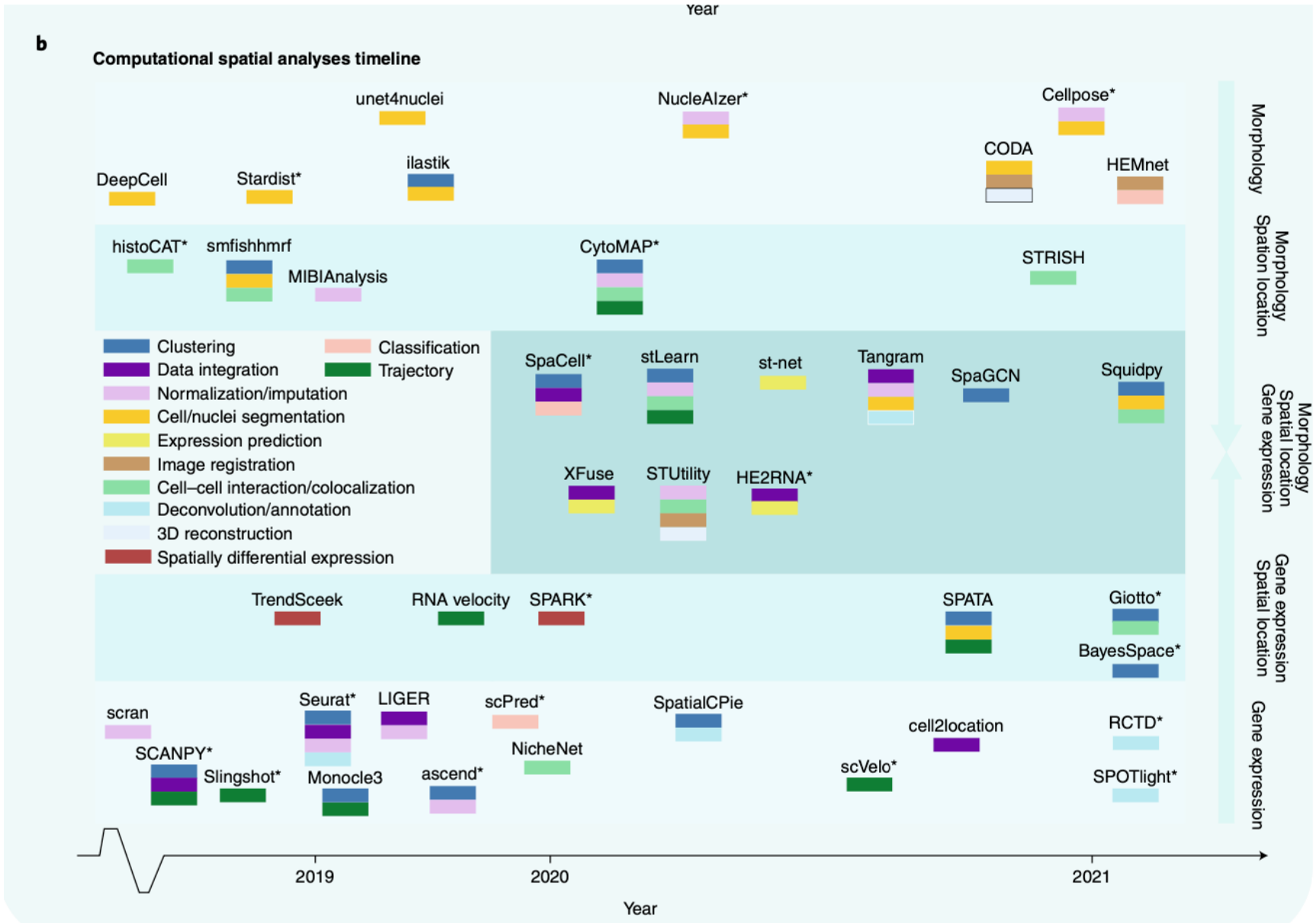


b

	Section profiled	Panel size (num. genes)	Avg. counts per cell	Avg. features per cell
MERSCOPE	Full coronal section	483	294	68
Xenium	Full coronal section	248	297	81
Molecular Cartography	Half coronal section	99	195	24
EEL FISH (Borm et al.)	Full sagittal section	440	42	29
MERFISH (Zhang et al.)	2 half coronal sections on same slide	1147	183	92
STARmap PLUS (Zeng et al.)	Full sagittal section	1022	123	64

Hartman and Satija Biorxiv 2024

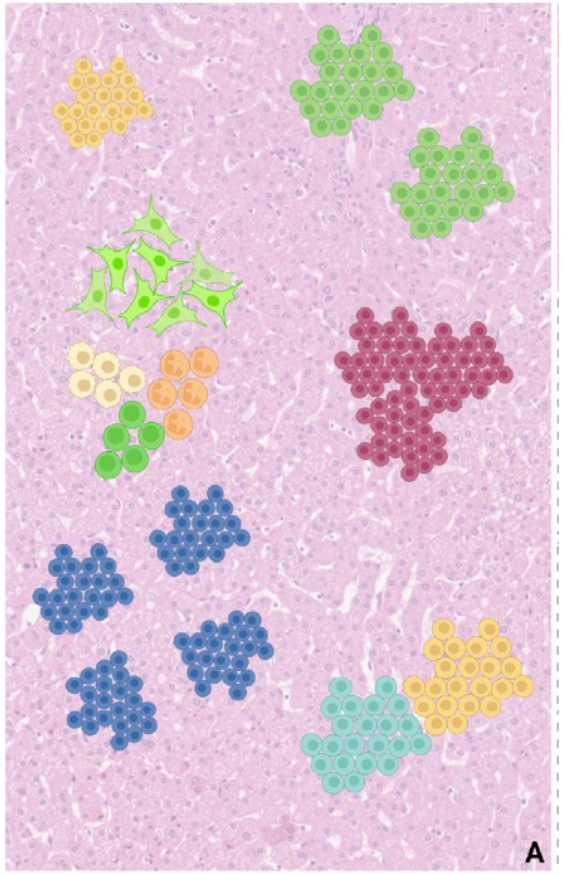
Spatial Analytics Emerging and Rapidly Rising like Single cell Analytics

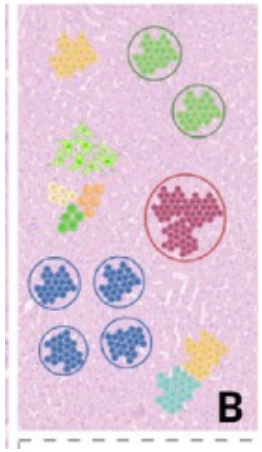


- Similar analytics to single cell but some notable differences like morphology-based analytics and cell-cell interactions/communications

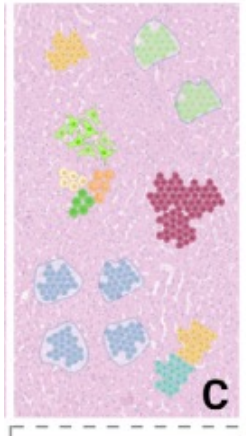
Lewis et al. Nature Methods 2021

What Information Can Be Extracted from Spatial Data ?

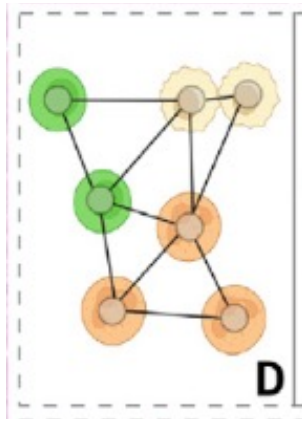




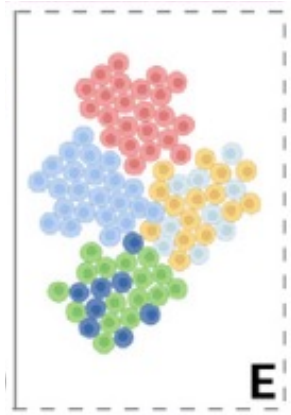
Cell type based spatial clustering



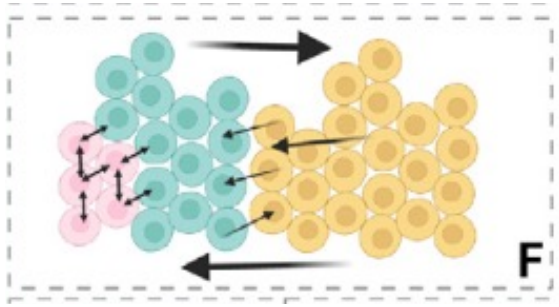
Spatial Clustering detects domains



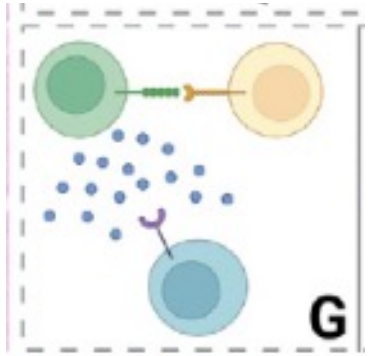
Identify cellular proximity depicted by spatial graphs



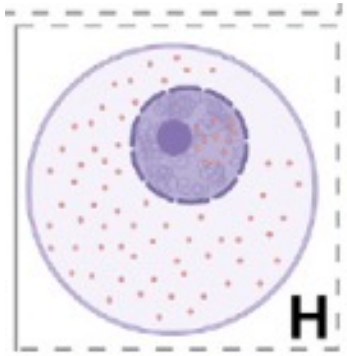
Cellular neighborhood analysis identifies spatially connected cells



Discover cell-cell communications, at different lengths



Map inter cell-cell communication between receptor ligand curated lists



Subcellular distribution of transcripts to understand cellular scop

Kleino et al Computational and Structural Biology 2022

Q&A of Introduction?

Let's get to Science!

Papers we will review!

Biology is Spatial: A Primer on Spatial Biology and its Applications in Oncology and Immunology

- Single cell and spatial transcriptomics of non-small cell lung cancer (10X Genomics sc + visium). https://pubmed.ncbi.nlm.nih.gov/38782901/

- Charting the cellular bidgeography in colitis reveals fibroblast trajectories and coordinated spatial remodeling. (Merfish in situ spatial). https://pubmed.ncbi.nlm.nih.gov/38569542/

Paper #1: 10X scRNASeq + 10X Visium

nature communications

Article

https://doi.org/10.1038/s41467-024-48700-8

Single-cell and spatial transcriptomics analysis of non-small cell lung cancer

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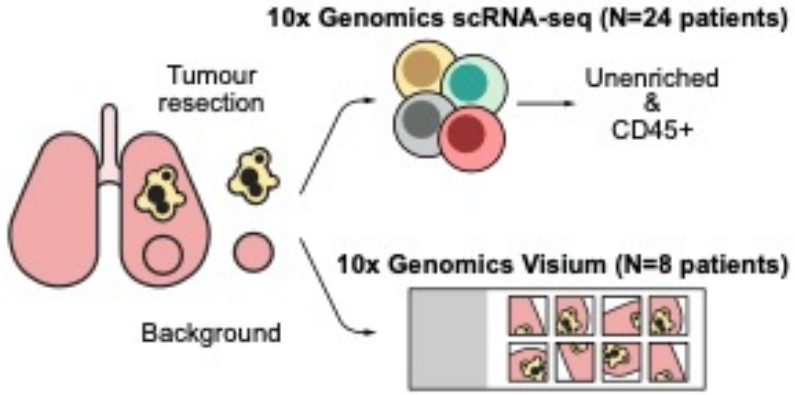
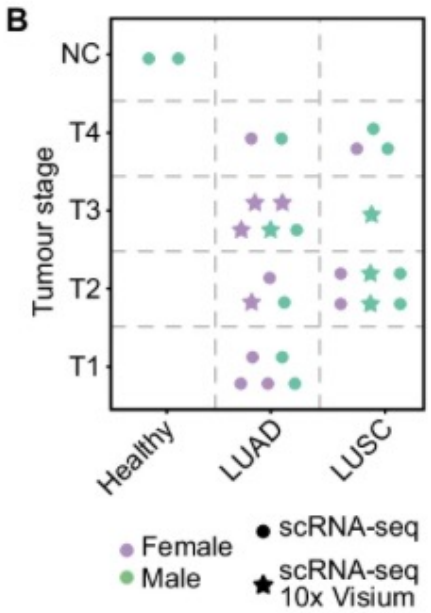
Check for updates

Marco De Zuani[1,2,3,4,11], Haoliang Xue[1,2,3,4,11], Jun Sung Park[1,2,5], Stefan C. Dentro[5,6], Zaira Seferbekova[5], Julien Tessier[7], Sandra Curras-Alonso[8], Angela Hadjipanayis[7], Emmanouil I. Athanasiadis[2,9], Moritz Gerstung[2,5,6], Omer Bayraktar[1,2] & Ana Cvejic[1,2,3,10]





scRNAseq data (10x)

26 samples:

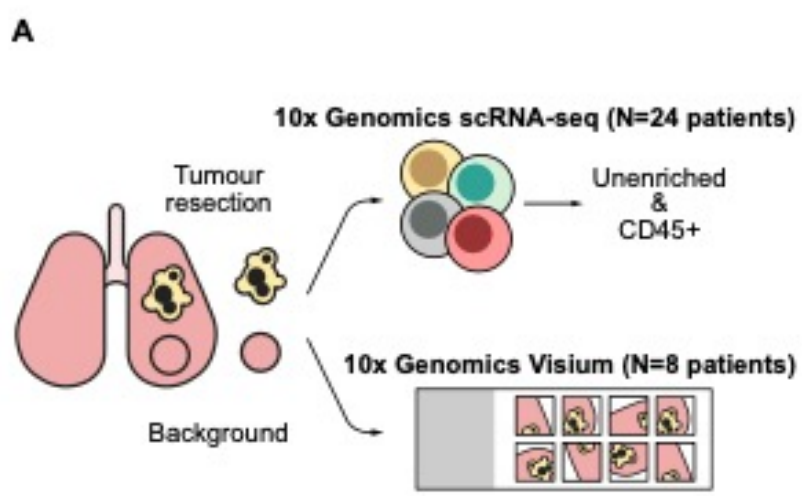
- Samples: 24 patients tumor + background (14 LUAD + 10 LUSC) + 2 healthy donors.
- Total number of cells: 503,549 cells tumour + 392,257 cells background/healthy.

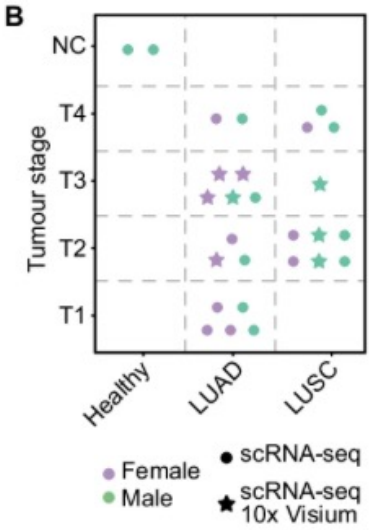
Spatial transcriptomics data (Visium – 10x)

10 samples:

- 8 NSCLC samples (7 matched scRNAseq samples (4 LUAD + 2 LUSC) + 1 LUAD) + 2 healthy.
- 36 sections in total (20 tumour + 16 background).

Analytical Study Workflow





Single cell

Spatial

Clustering and annotation of sc

De-convolution of 10X Visium using scRNA-Seq

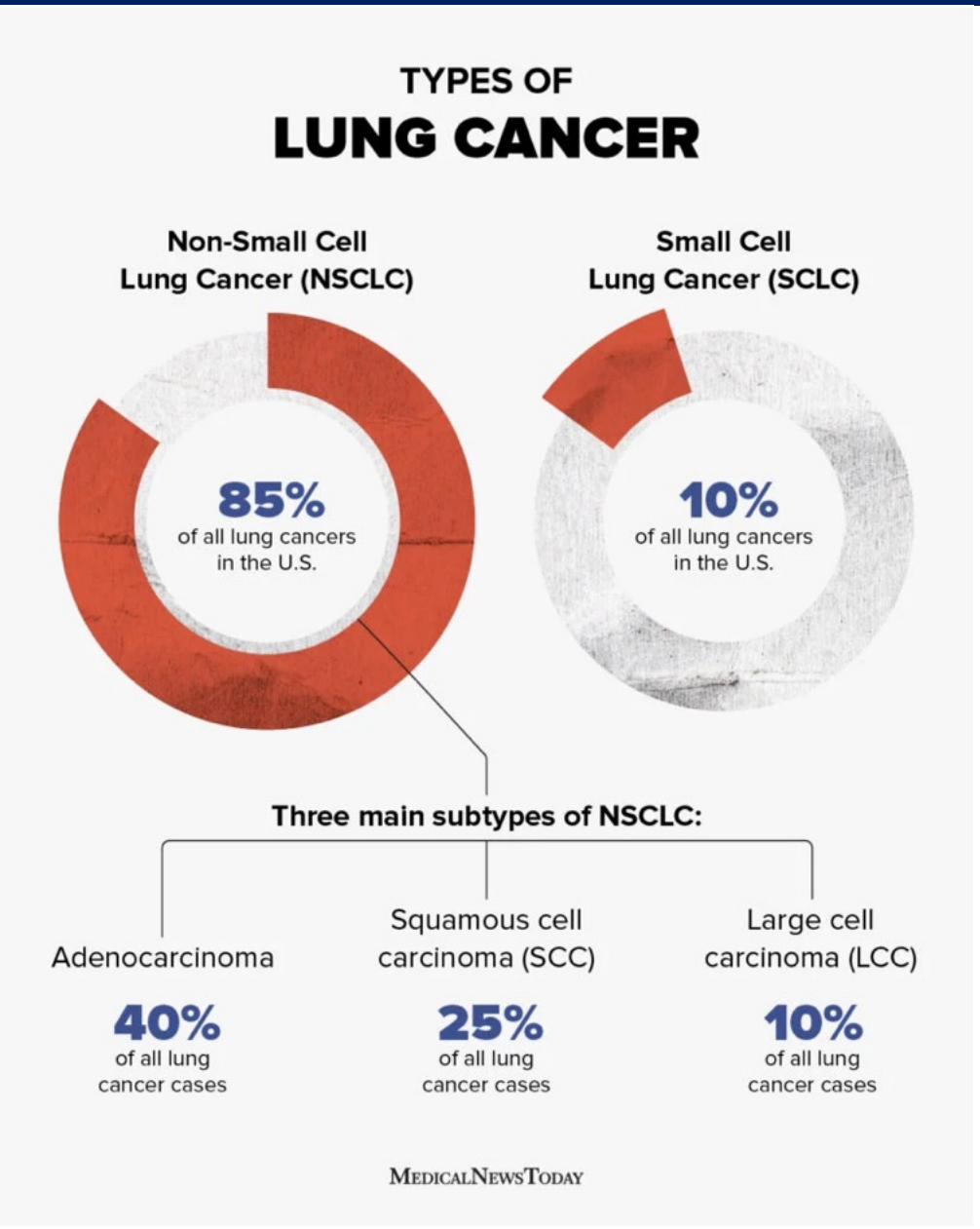
Cellular co-localization of receptor-ligand Interactions

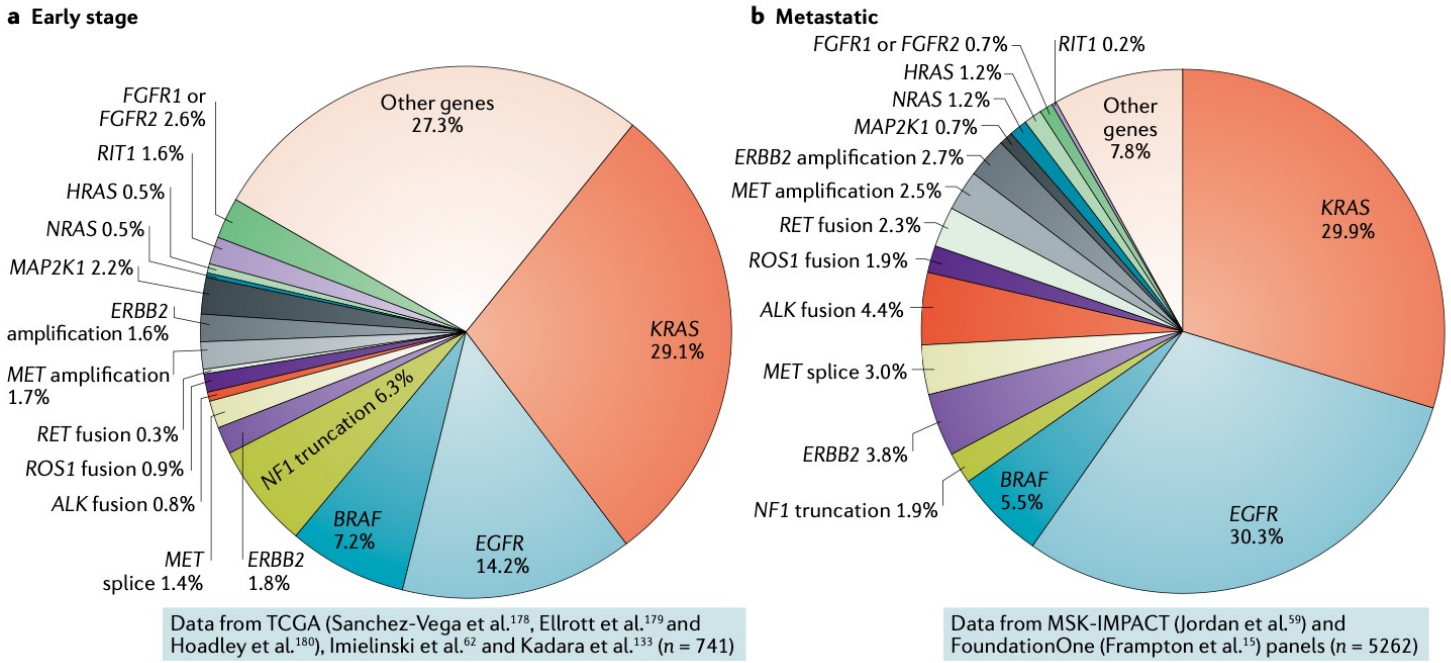
Identification of ligand-receptor interactions

Copy Number Identification of Tumor cells

Correlations of distance between cells

Non-Small Cell Lung Cancer

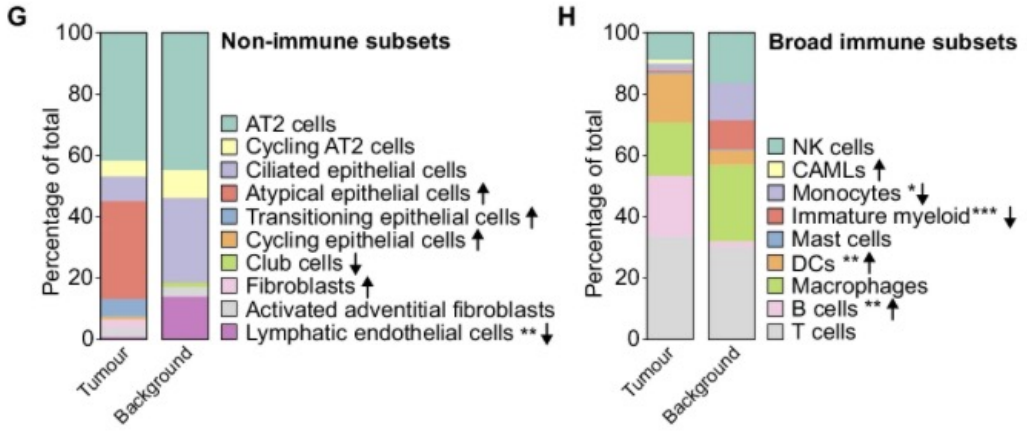




What we don't know is the cellular interactions driving disease

Immune and Non-immune Composition in LUAD and LUSC similar

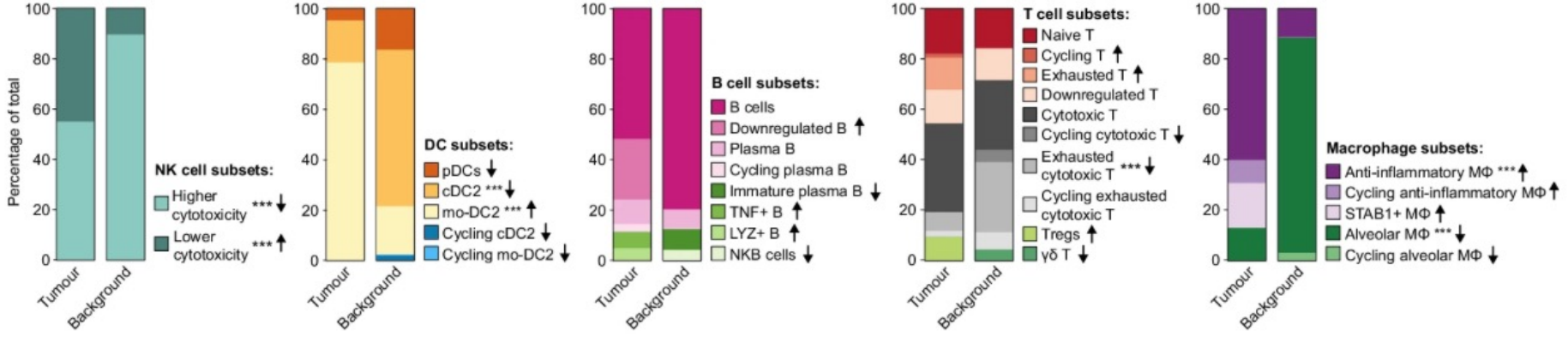
Broad Clustering



Increase in Fibroblasts and Epithelial subtypes (Atypical, Transitioning, Cycling), and expanded B cells and DCs

Decrease in fraction of lymphatic endothelial cells, monocytes and immature myeloid cells

Sub-Clustering



46 cell states

Increase in low cyto NK cells, moDC-2, **Bcells** (LYZ+ and TNF+), **T cells** (Treg, cycling, exhausted), **Macs** (anti-inflame, STAB1+),

Decrease in high cytotoxic NK cells, **DC** subtypes (pDCs, cDC2, cycling DC2, cycling moDC2), **B cells** (NKB cells, immature plasma B), **T cells** (gDT, exhausted cytotoxic, cycling cytotoxic), alveolar Macs, cycling alveolar Macs

Predicting Ligand Receptor Interactions in scRNASeq: CellPhoneDB

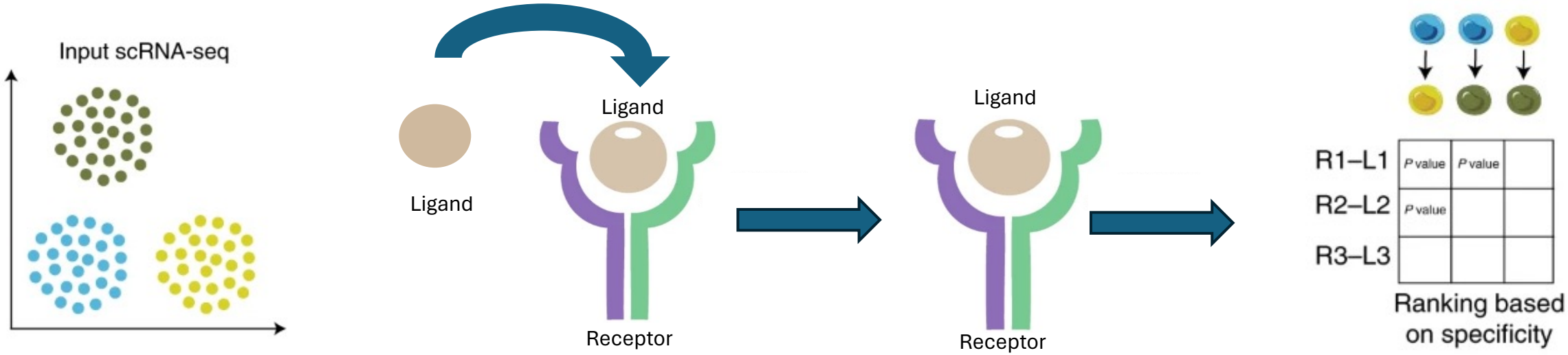
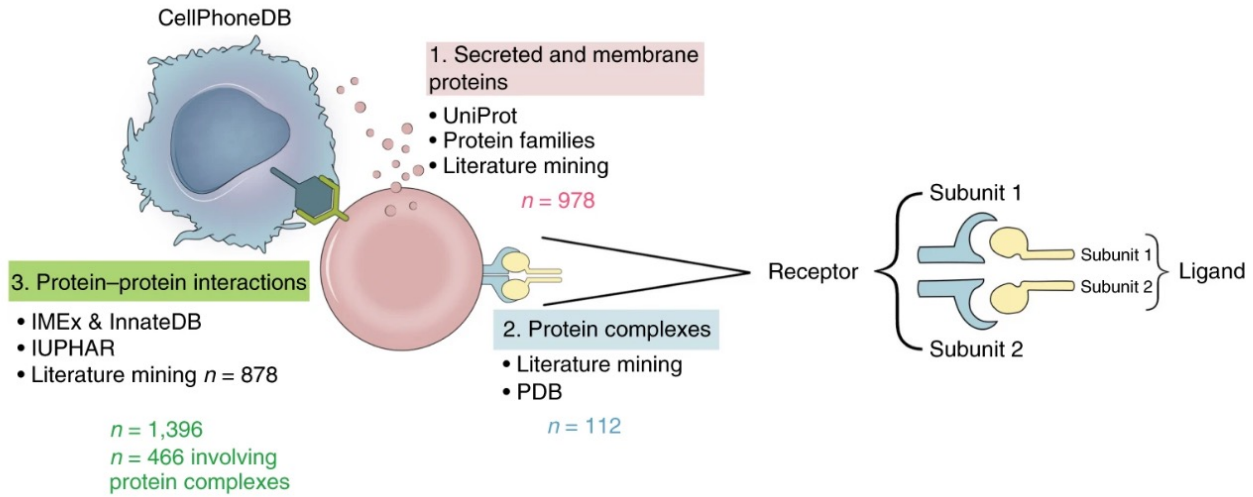
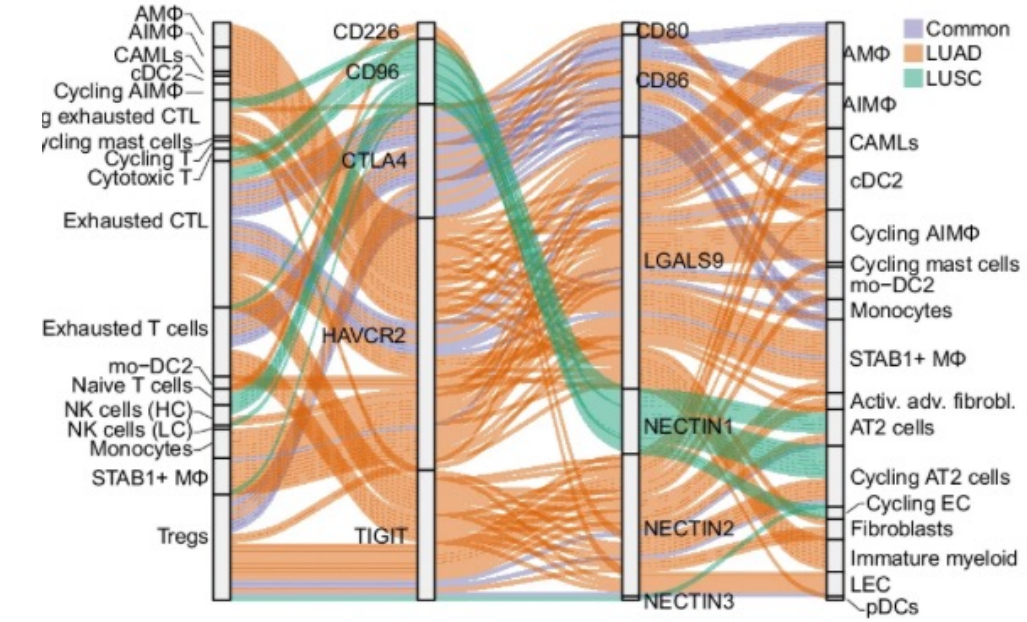


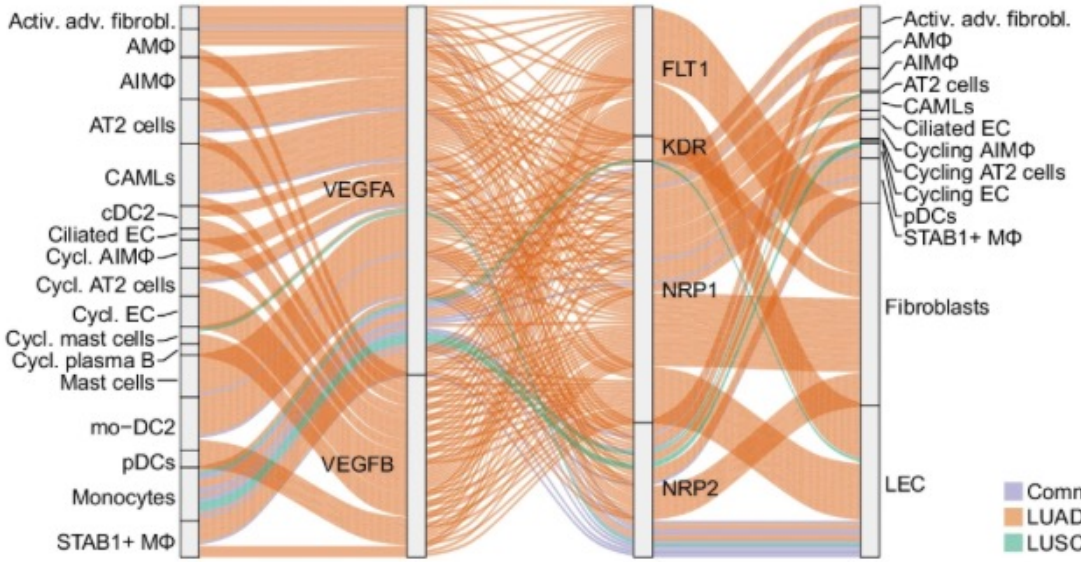
Fig. 1: Overview of the database.

From: CellPhoneDB: inferring cell–cell communication from combined expression of multi-subunit ligand–receptor complexes



LUAD vs LUSC mostly similar ligand-receptor interactions by scRNASeq





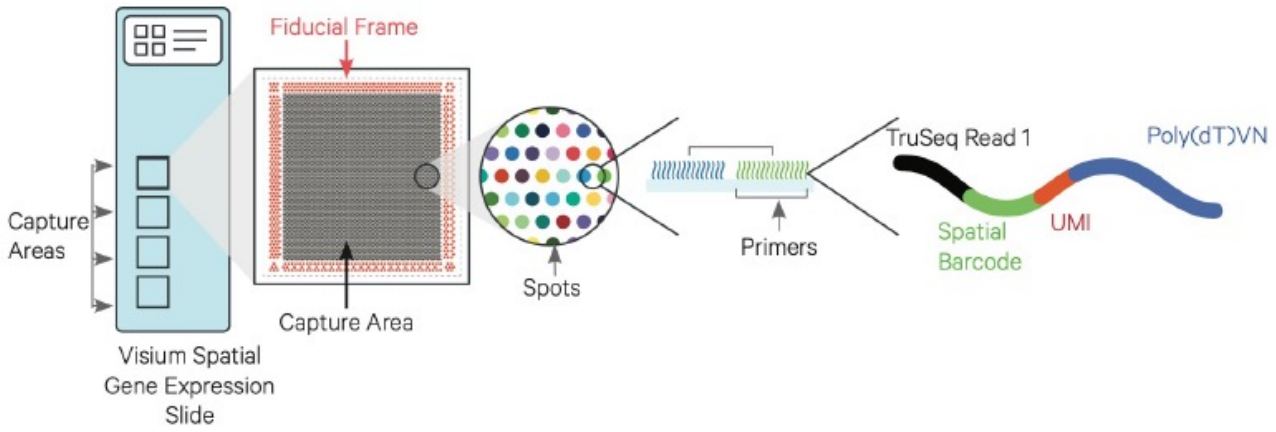
- Similar interaction network between non-immune cells, AIM(Phi) and Tcells.
- Immune checkpoint ligand-receptor interactions different between LUAD vs LUSC.
- Higher number of L-R pairs in LUAD vs LUSC.

Notable differences

- **LUAD** (*LGALS9-HAVCR2 (TIM3), NECTIN2-CD226 (DNAM1)* and *NECTIN2/NECTIN3-TIGIT)*
- **LUSC** (*CD96-NECTIN1*)
- **Both** (*CD80/CD86-CTLA4* and *HLAF-LILRB1/2*)

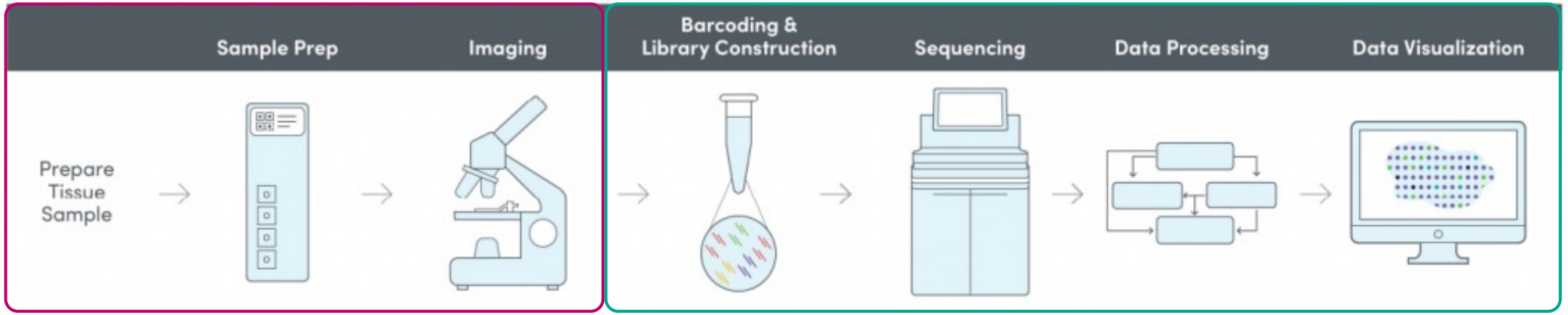
Expressed in both LUAD and LUSC (more frequent LUAD)

Deep Dive into 10X Genomics Visium

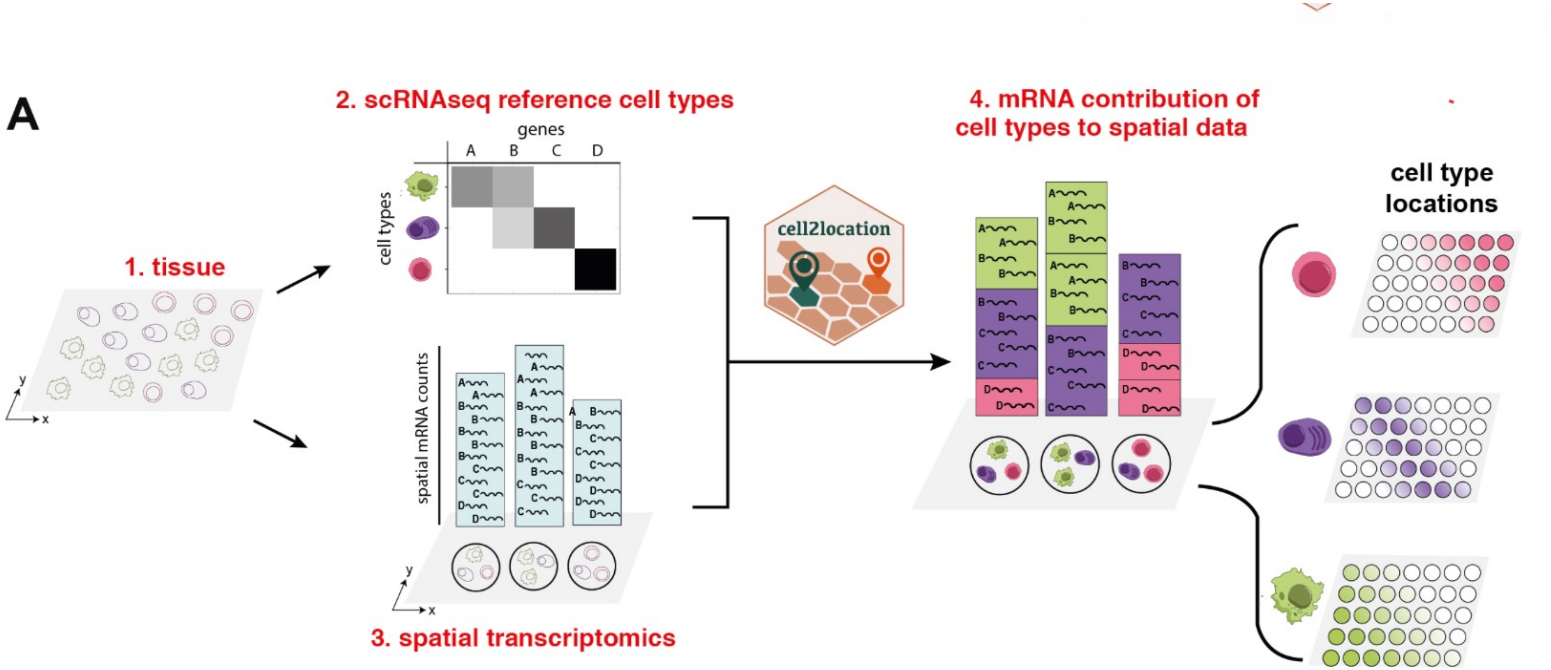


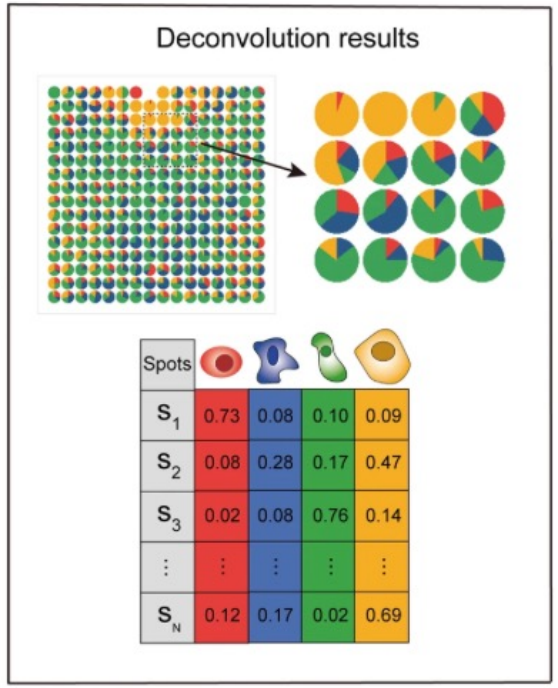
Visium Spatial Gene Expression Slides:

- 4 Capture Areas per Slide
  - 6.5 mm x 6.5 mm Capture Area
  - ~5,000 spots per Capture Area
  - 55 µm spot diameter
    - Millions of oligos per spot



First Order Metrics: Deconvolution of Spot-based Spatial data



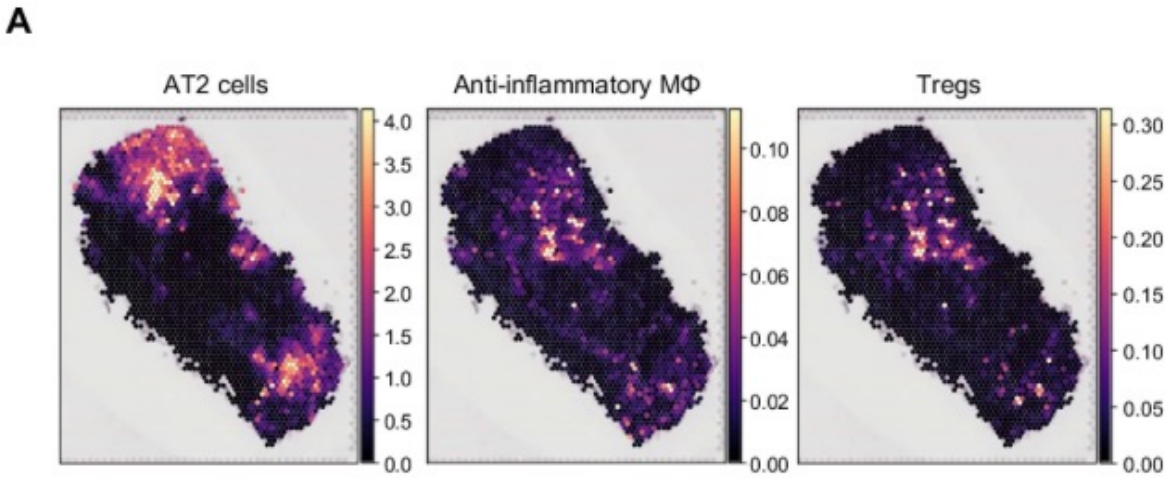


Cell type abundance in tumor and background by summing up q05 cell abundance across spots.

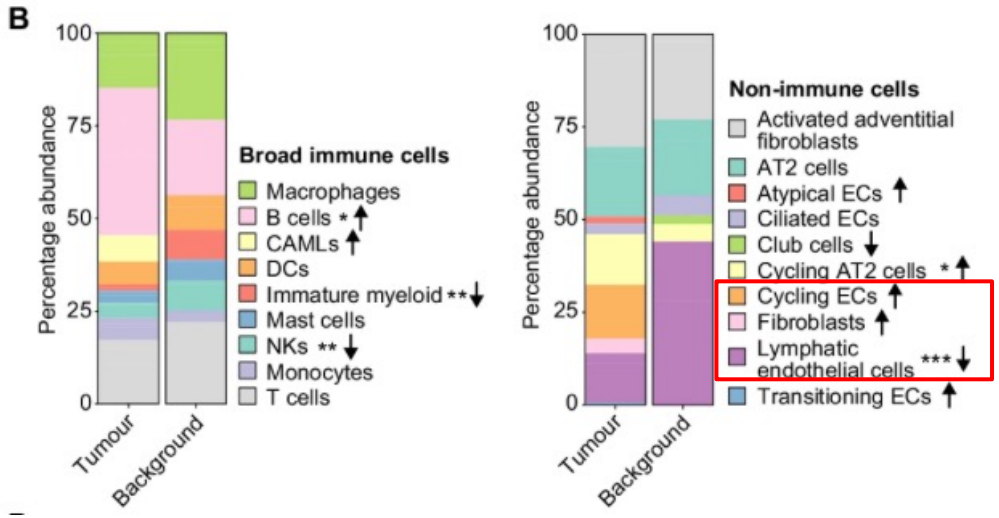
Gene Expression: Seurat, SpatialCPie, RCTD, SPOTLight, Cell2Location

Broad Cell Deconvolution Identifies AT2, anti-Inflammatory Macs, and Tregs in 10X Visium

Two consecutive 10um sections, n=8 patients
(N_{tumour}=20, $N_{background}$=16)



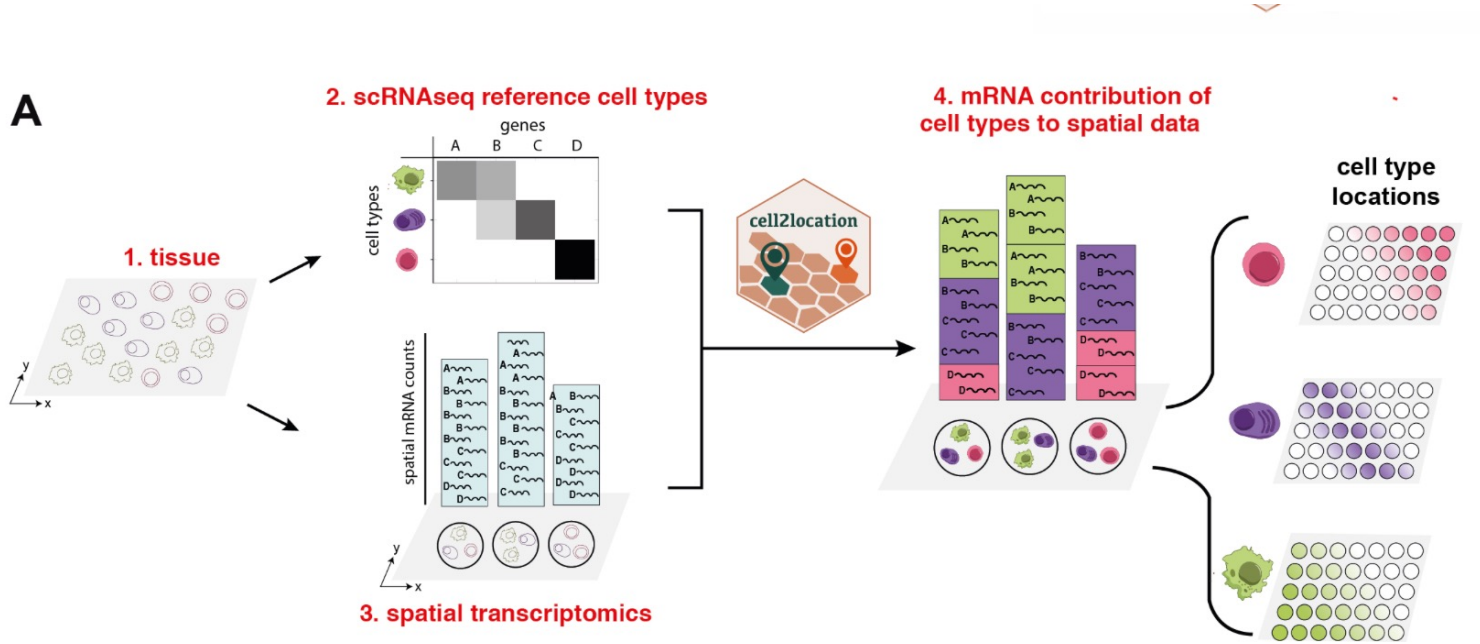
Average UMI count 6894/spot in tumour and 3350/spot in background

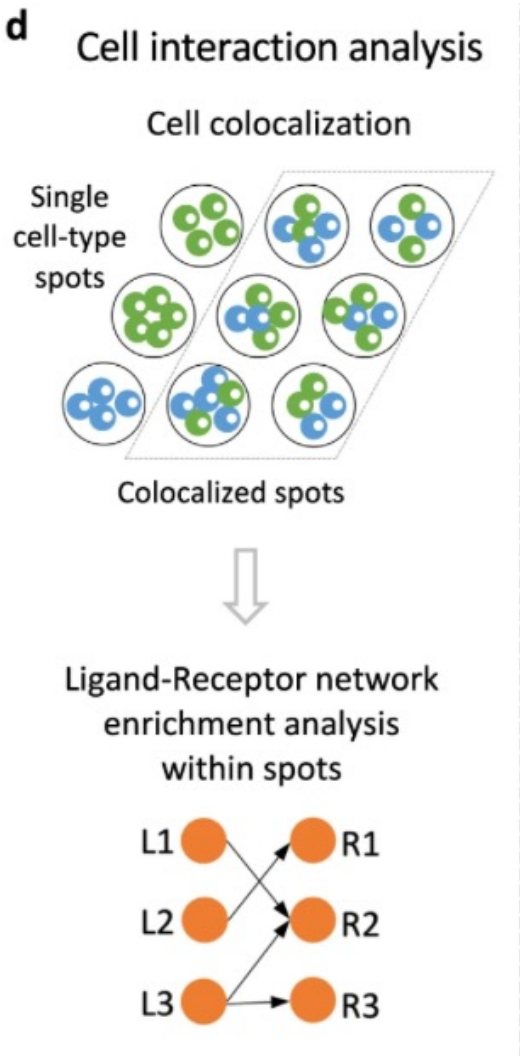


Increase in CAMLs, B cells and cycling AT2,
Decrease in immature myeloid cells, NK cell, LECs

Some discrepancy spatial proportions vs single cell data, higher in non-immune populations

Second Order Metrics: Mapping Cell-Cell Communication through LR Pairs

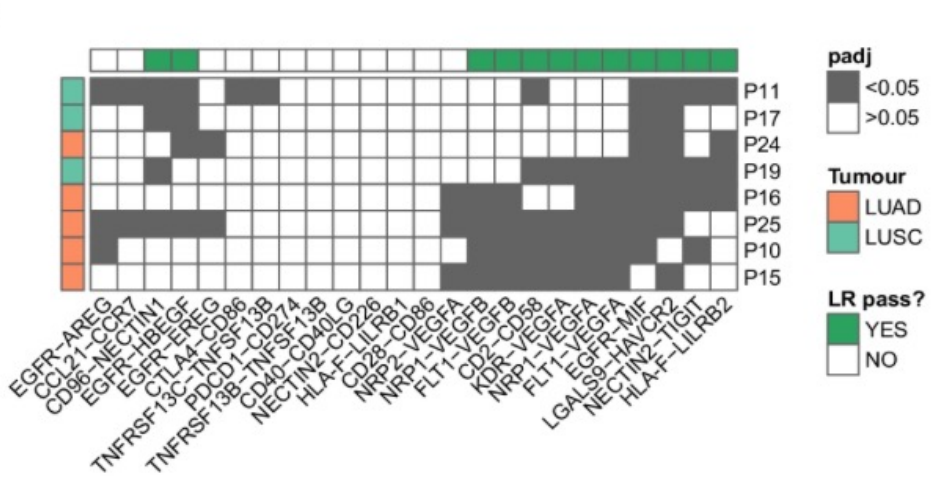


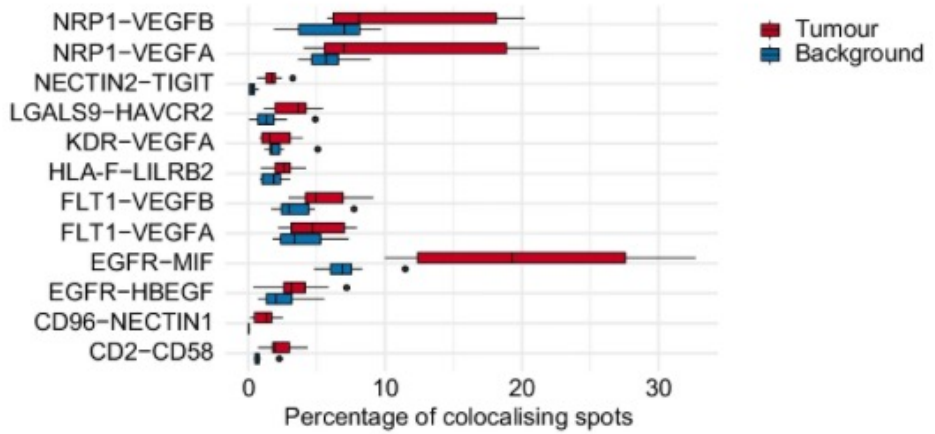


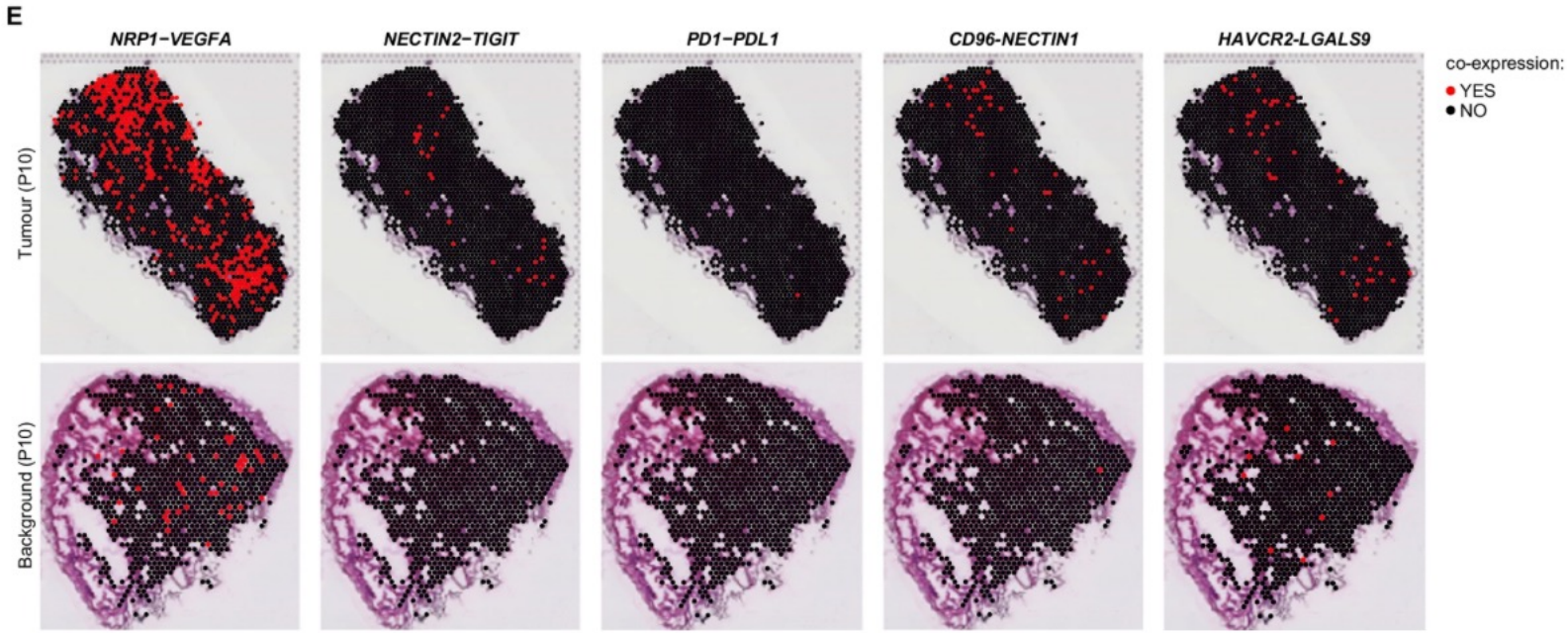
- Measure co-occurrence of cell types across spots and co-occurrence of LR pairs--> validation of ligand receptor interactions.

Beibei Ru et al Nature Communications 2023

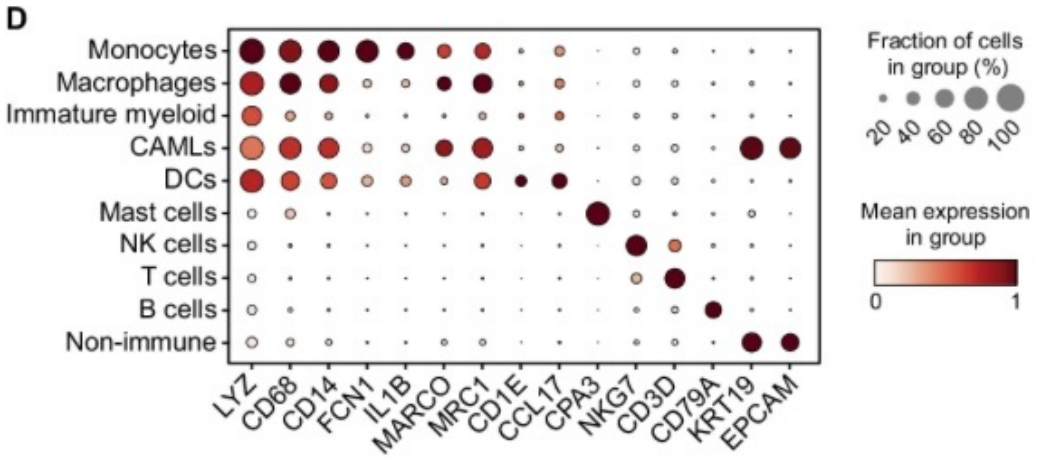
10X Visium confirms spatial colocalization of key ligand-receptor pairs

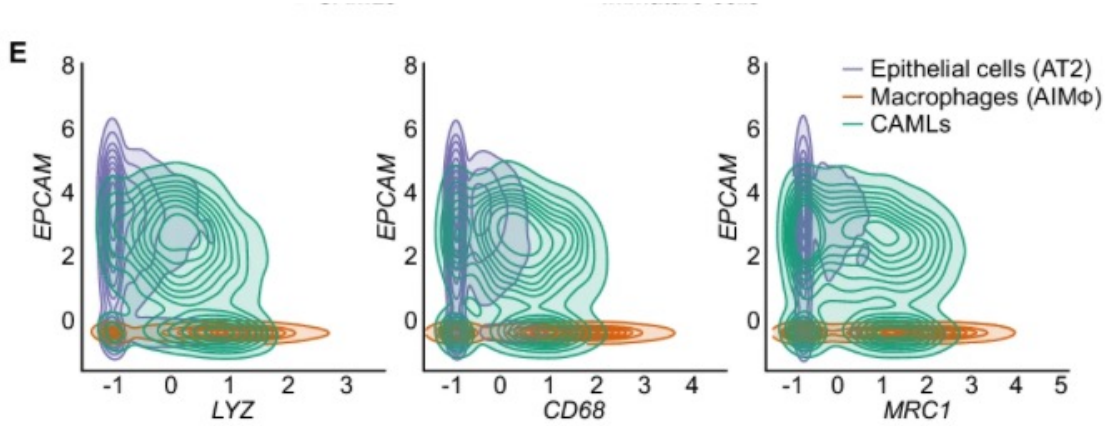


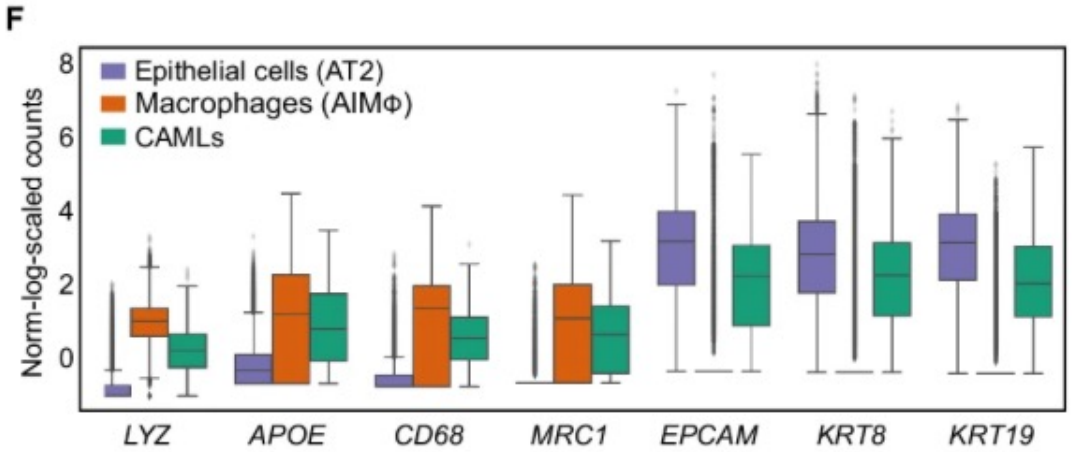


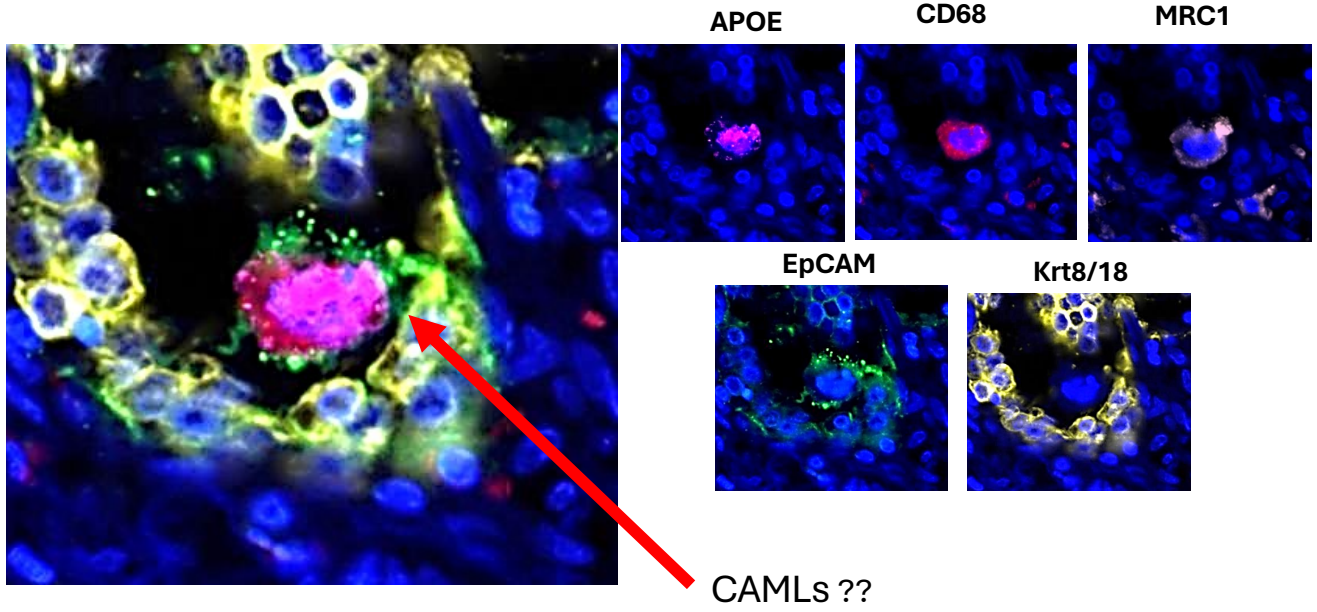


Identification of CAML cells: To be or Not to be a Duplet?



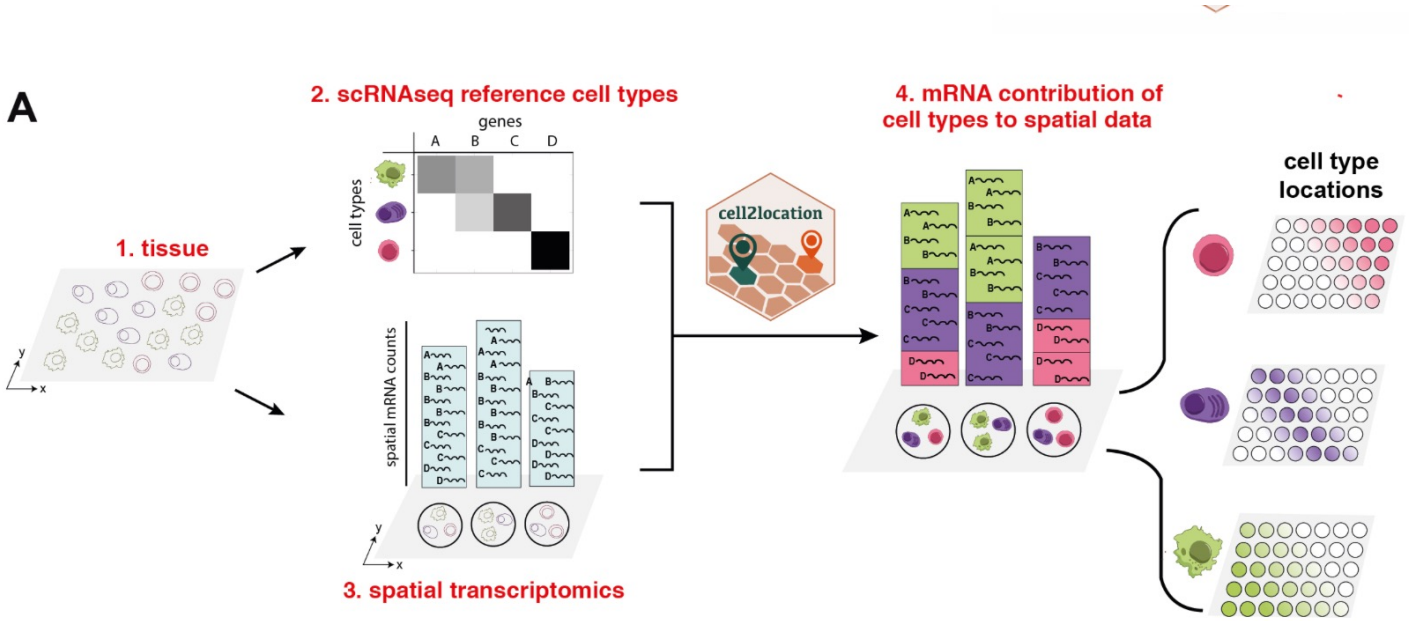




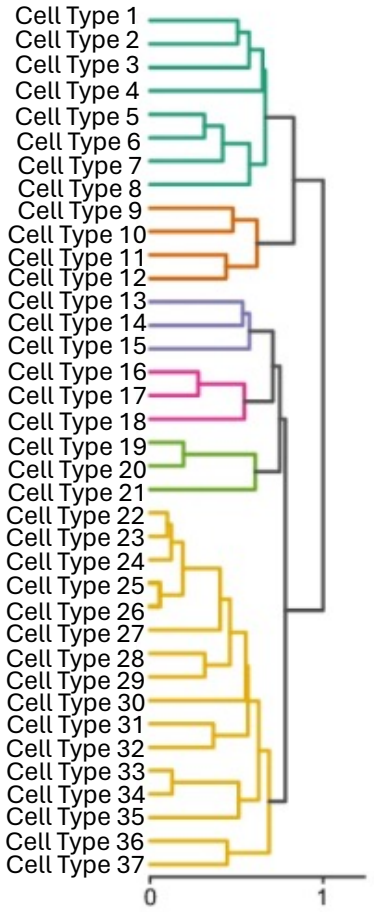


Subset of macrophages attached to a cancer cell

Third Order Metrics: Distance between cells from Spatial Data



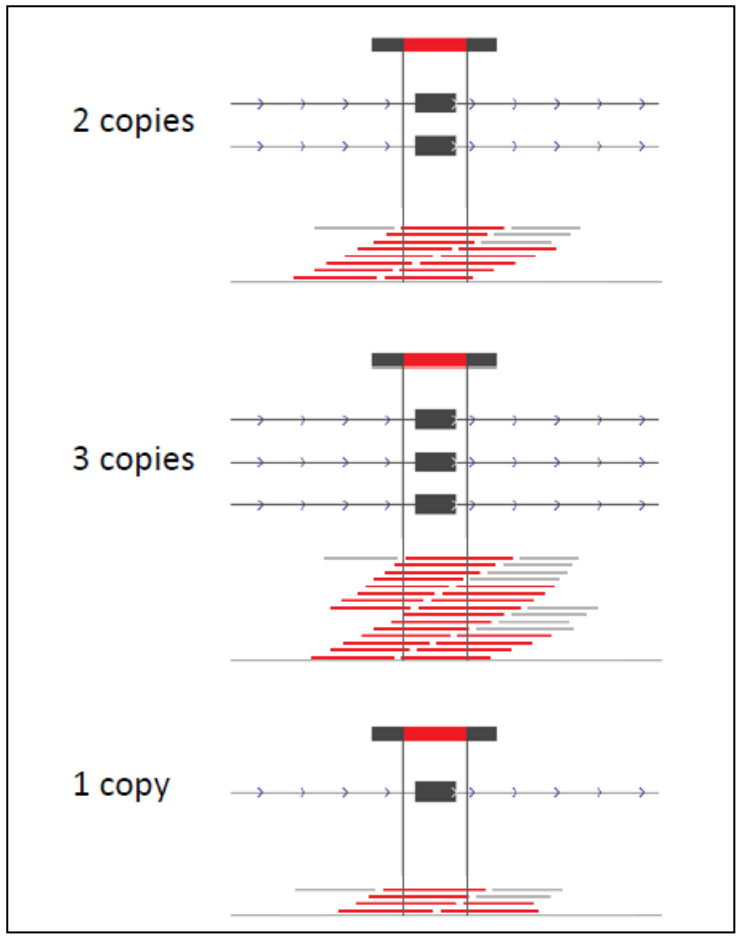
Hierarchical clustering



The correlation of the cell type abundances between two cell types indicates the degree of co-localization.

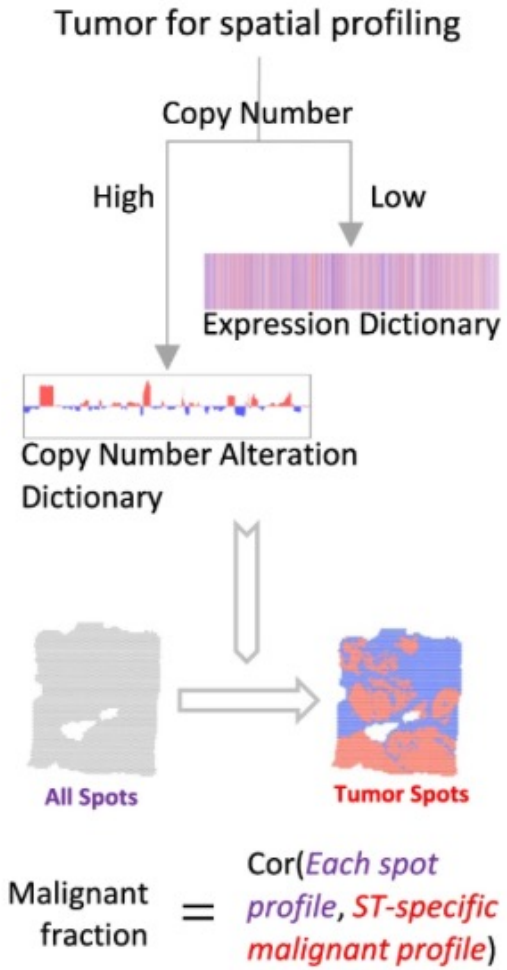
Copy Number Identification in Spatial Data

General view



- The number of aligned reads on a given regions is proportional to the number of starting DNA copies of that region
- This information can be used to infer DNA copy number variations (CNVs).
- Healthy vs Tumor CNV.
- CopyKat application used for single cell datasets.

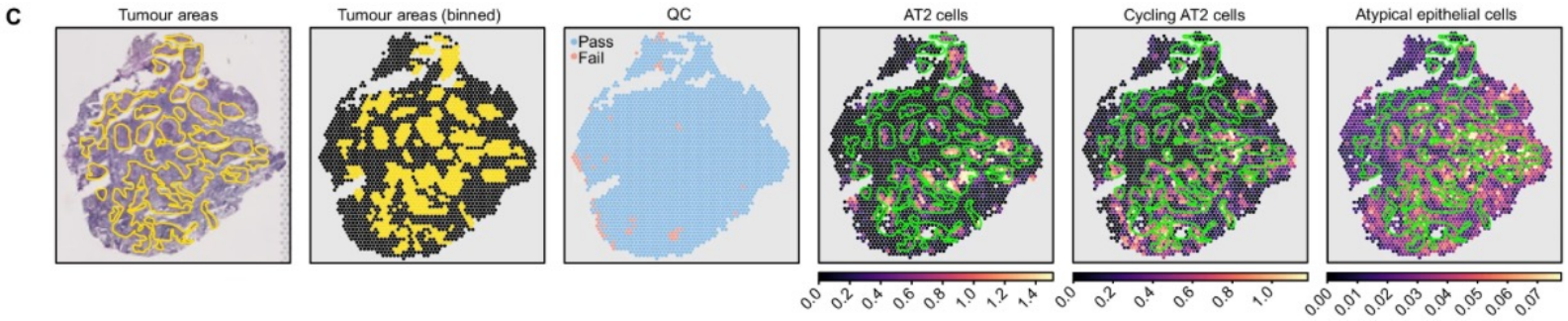
Infer malignant copy fraction



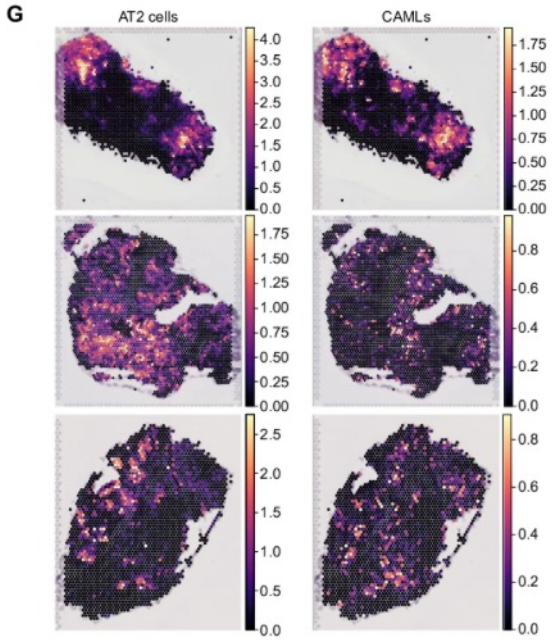
- CNV dictionary + transcriptome= ST malignant expression profile
- ST malignant profile correlated with gene expression of each spot
- Expression dictionary can come from single cell RNASeq or copy number changes from that indication from previous data.

CAMLs co-localize to Tumor cells and Share Copy Number

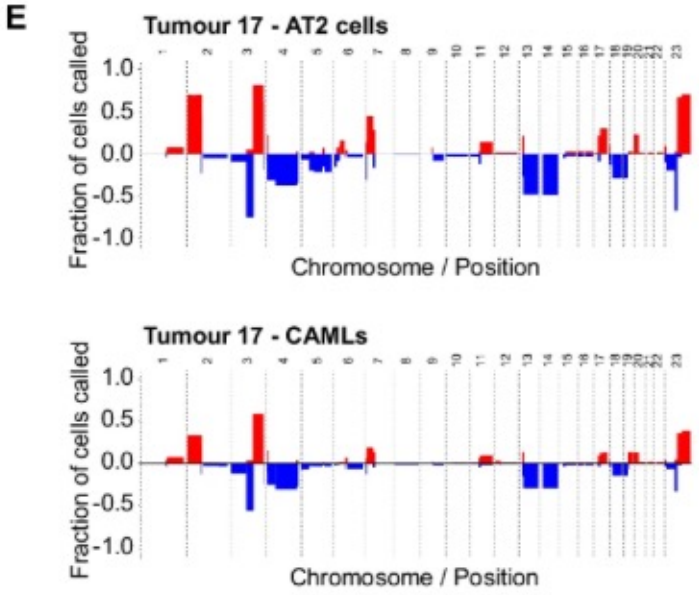
Pathology annotation of tumor regions matches cell2location mapping of tumor cells



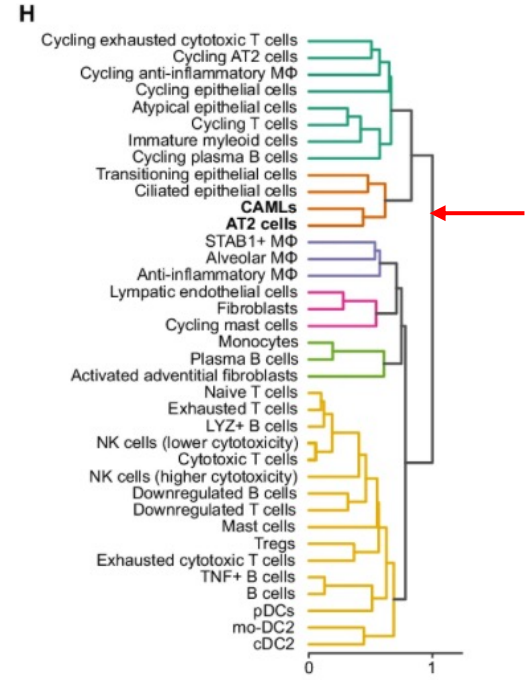
Spatial Images depicting abundance of CAML and AT2 on three tumor sections



CAML show CNAs similar to AT2 cells



Hierarchical clustering of correlation distance calculated by cell type composition



CAML and AT2 cells close together

Hypothesis that CAMLs are macrophages tightly attached to a cancer cell undergoing phagocytosis or fusion

Study Summary

- ❑Built largest single cell multi-omics analysis of treatment niave non small cell lung cancer.
- ❑Focused on the role of myeloid cells within NSCLC tumours through integration of single cell RNASeq and spatial transcriptomics.
- ❑Direct comparisons of NSCLC subtypes for potential subtype specific therapeutic targets.
- ❑Identified Cancer associated myeloid cells (CAMLs) and their association to tumor cells.

Key Spatial Take Home Messages

- ❑Integration of single cell RNASeq with 10X Visium provides deeper insights into cell-cell interactions in TME.
- ❑Spatial cell typing/Deconvolution of 10X Visium performed by using single cell data from same patient or single cell reference data with algorithms like cell2location.
- ❑Ligand-Receptor co-localization analysis can be validated in spatial transcriptomics data through co-expression of ligand-receptor above median in the same spot.
- ❑Copy Number analysis can be performed on single cell RNASeq data and inferred on 10X Visium data.

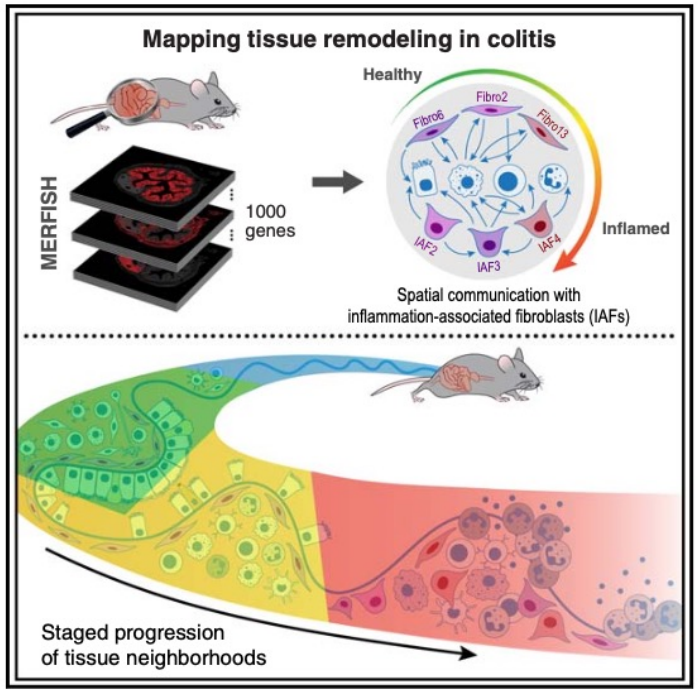
Paper #2: In Situ Merfish example

Cell

Resource

Charting the cellular biogeography in colitis reveals fibroblast trajectories and coordinated spatial remodeling

Graphical abstract



Authors

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Correspondence

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In brief

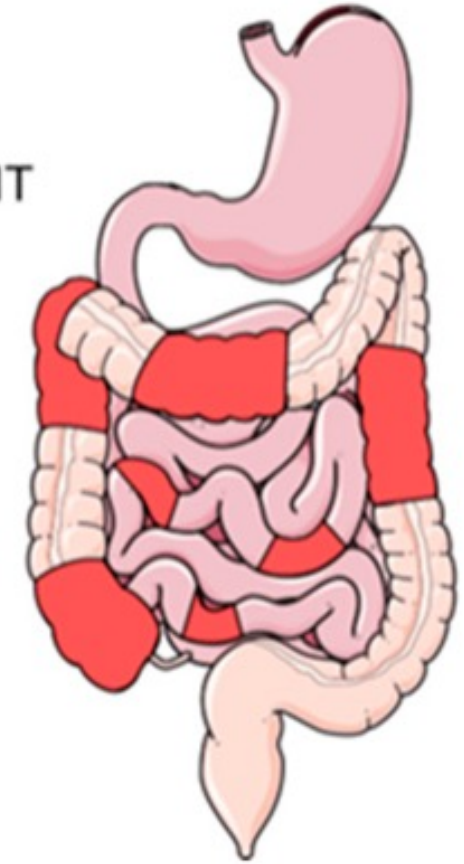
The cellular and spatial remodeling associated with induced inflammation and recovery in a mouse colitis model yields invaluable insights into inflammatory fibroblast states in human ulcerative colitis.

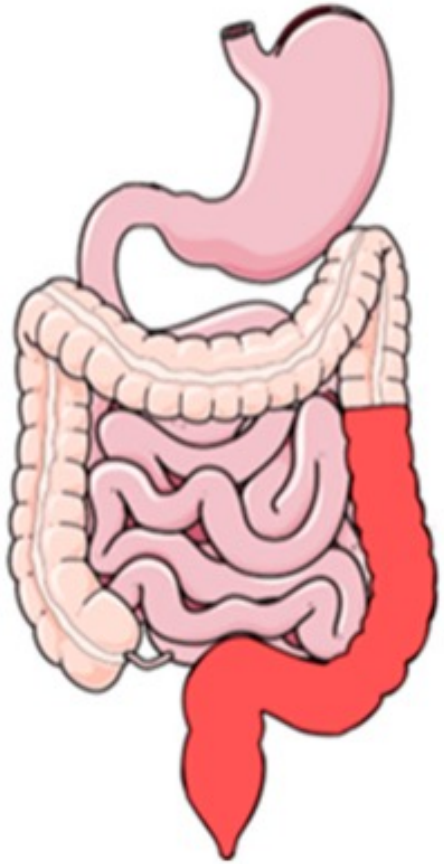
- ❑ Merfish method of choice (Multiplex Error-Robust Fluorescence In situ Hybridization)
- ❑ Mouse DSS colitis model
- ❑ Profile onset of disease and recovery of disease

Crohn’s vs Ulcerative Colitis

Crohn's Disease

- May affect any part of the GIT
- Discontinuous patchy inflammation
- Transmural (affects the full thickness of the bowel wall)

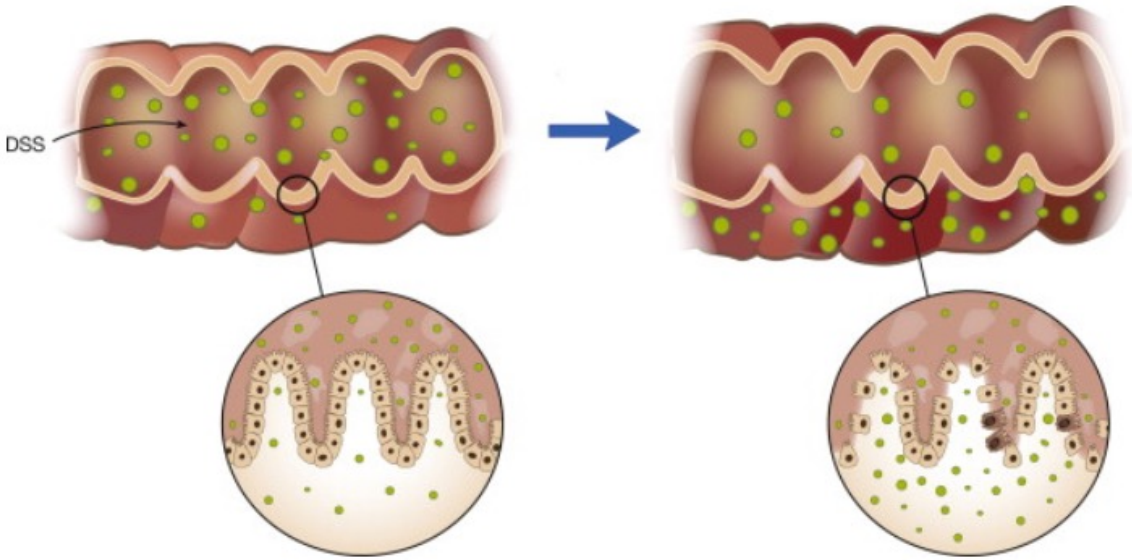




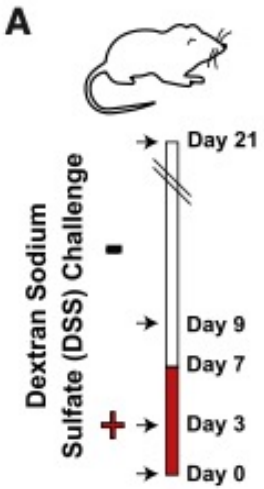
Ulcerative colitis

- Affects only large intestine
- Continuous inflammation
- Mucosal and submucosal layers are affected

Dextran Sodium Sulfate Challenge



- ❑ Colitis due to loss of epithelial barrier and entry of organisms or their products into lamina propia
- ❑ Stimulation of innate and adaptive lymphoid elements
- ❑ Secretion of pro-inflammatory cytokines and chemokines.
- ❑ Influx of cells with cytotoxic potential, neutrophils and inflammatory macrophages.



2.5% dextran sulfate sodium salt
Treated for 7 days ad libitum
Sacrificed at Day 0, 3, 9, 21

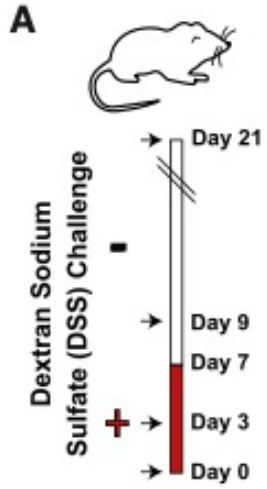
Table 1

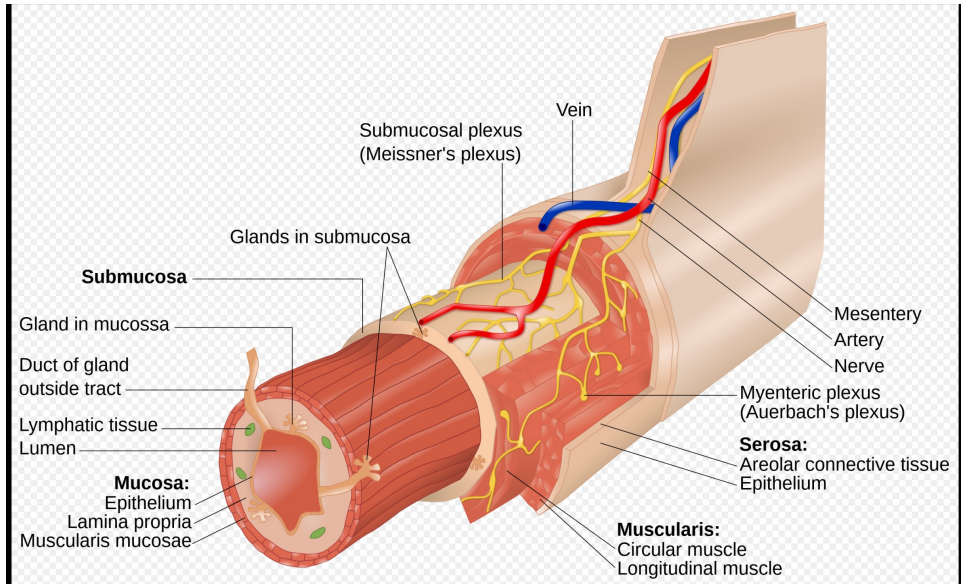
Factors that influence effectiveness of dextran sodium sulfate to induce colitis

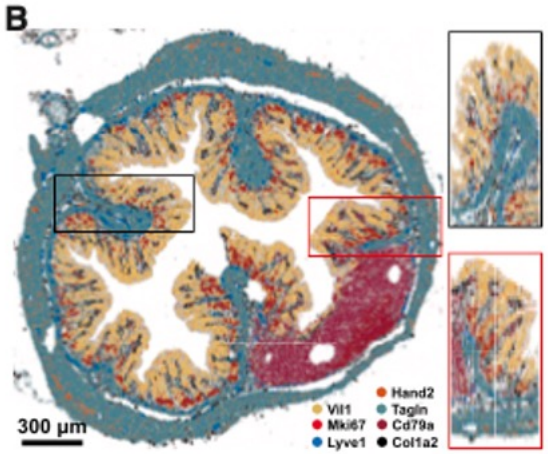
Factors	Variables	Description
DSS	Molecular weight	40-50 kDa for tissue penetration (larger molecule does not penetrate colonic tissue well and smaller molecule has poor distribution)
	Dosage concentration	Ranges from 1.5%-3% used most frequently (1% with mild symptoms and delayed onset)
	Duration of therapy	Acute: 5-10 d administration
	Manufacturer/batch	Chronic: 4-5 repeating cycles of DSS and sterile water
		Various manufacturers with differing potency
Host	Genetically susceptible strain of animal	Certain strains are more susceptible to DSS colitis than other strains.
		Susceptible strains: C3H/HeJ, C57BL/6, BALB/C
Environment	Housing Conditions	Group *vs* individual unit, frequency of cage changes alters coprophagy by host
	Microbial State	Germ-free *vs* specific pathogen free *vs* wild type

DSS: Dextran sodium sulfate.

Analytical Study Workflow







Gastrointestinal wall of Gastrointestinal Tract

Spatial

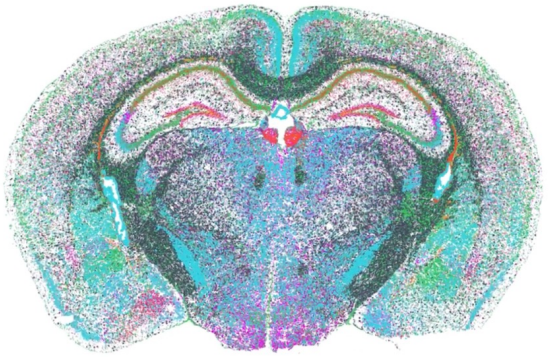
Partitioning RNAs into cells

Spatial clustering of in situ data

Spatial Neighborhood analysis

Spatial Prioritized receptor-ligand analysis

Multiplex Error Robust Fluorescence In situ Hybridization

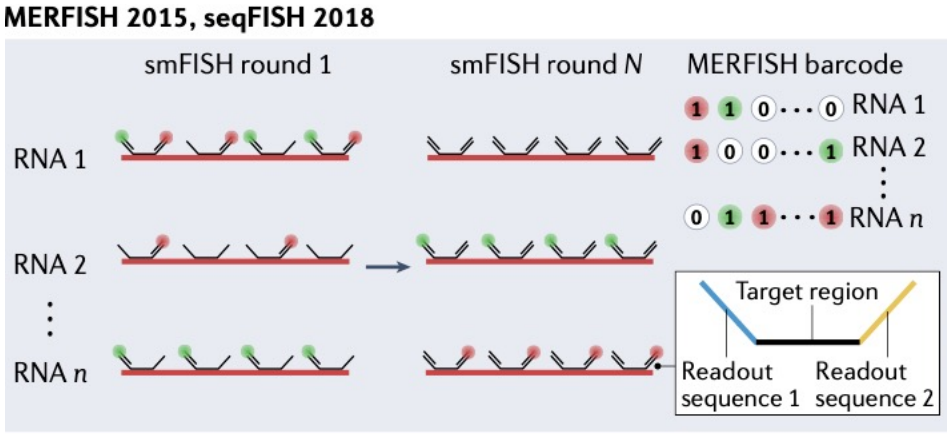


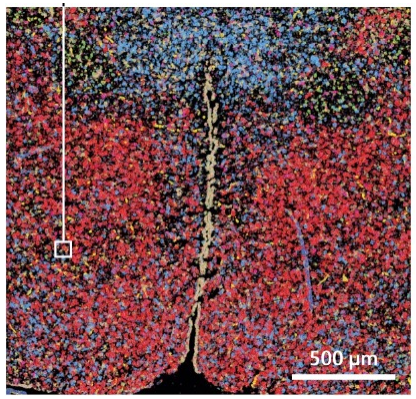
smFISH

Binds specifically to target transcript

- ❑ Directly count targets with high detection efficiency
- ❑ Only measure a few molecules at a time

MERFISH



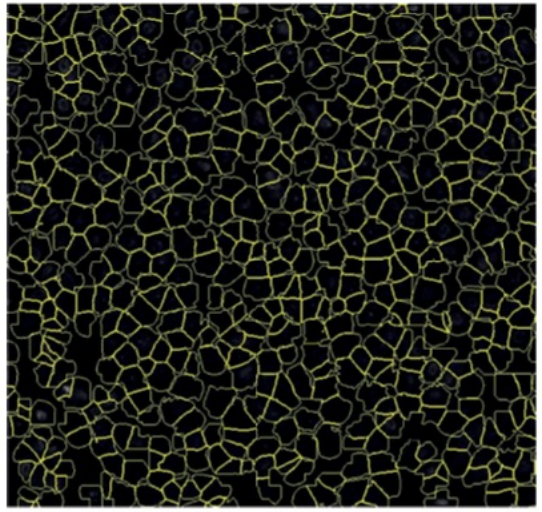


- ❑ Built upon idea of FISH
- ❑ Measure tens to thousands of transcripts
- ❑ Combinatorial barcoding (paper uses 30-bit barcodes, hamming distance of 4)

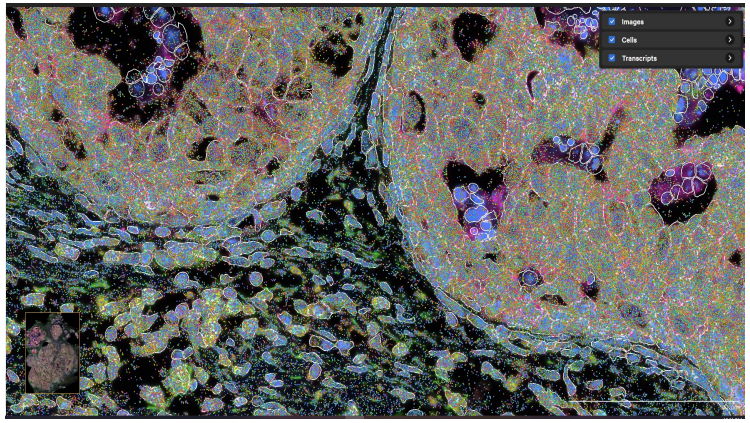
First Order Metrics: Segmentation of Cells is required for In Situ Methods

Segmentation approaches

h Cell segmentation and type or state classification



Morphology based

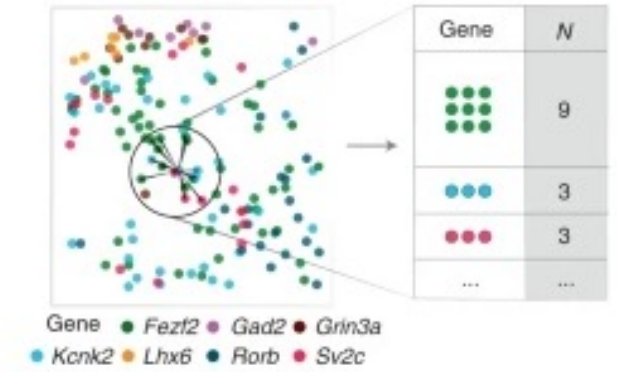


Boundary (ATP1A1/ CD45/E-Cadherin
Protein
RNA
DAPI

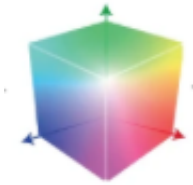
Cell Segmentation-free (Pseudo cell approaches): Partitioning RNAs into cells

k-NNs

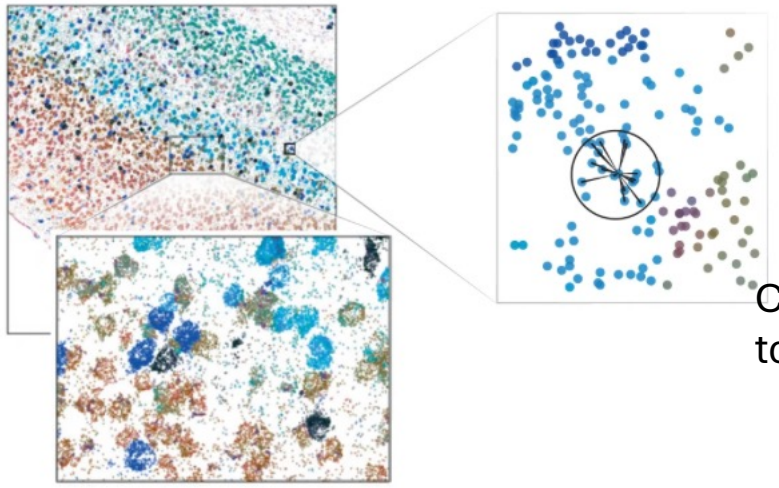
Neighborhood Composition vector



Embed into 3D space



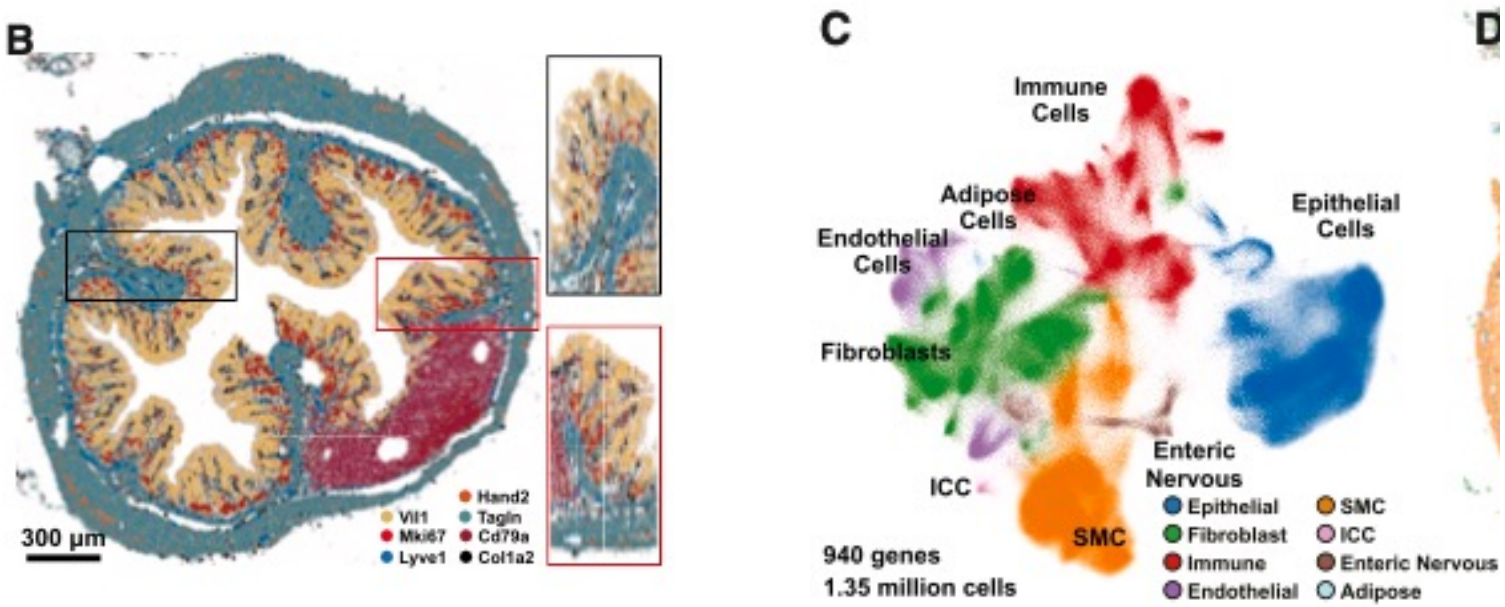
Affected by number of neighbors
Dimensional reduction resolution



Cell specific markers selected to estimate accuracy of Baysor

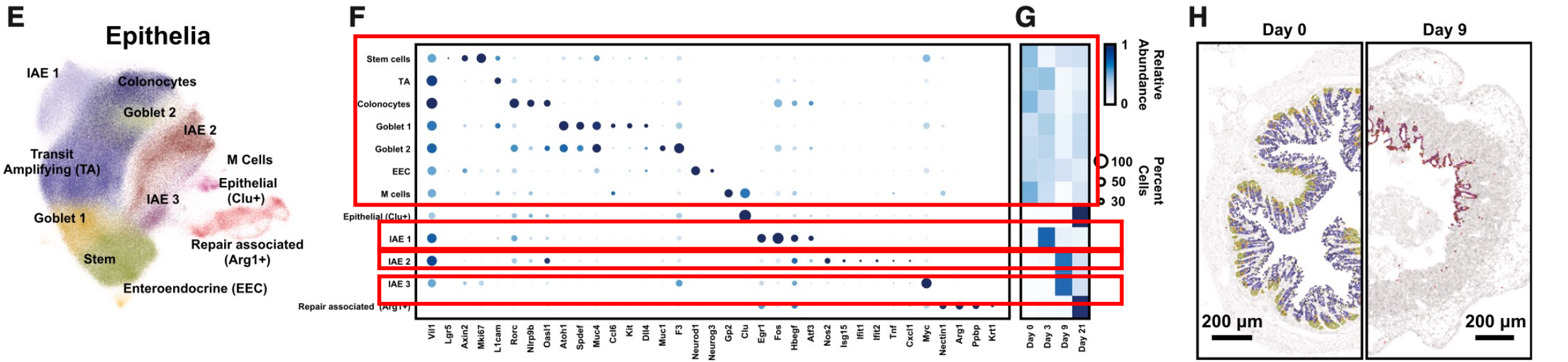
Petukhov et al, Nature Biotechnology, 2022

Spatial Distribution of 940 RNAs at Day 0 in DSS model



- Profiled 52 slices from 15 mice and partitioned these RNAs into cells, 139 million RNA, 1.3 million putative cells.
- Key marker genes of expected gut structures identified Mucosa, submucosa, musclaris externa, submucosal plexus, and myenteric plexus, occasionally lymphoid follicle and stray serosa.
- 72 cell clusters, 8 major classes, in agreement with published single cell, Smillie 2009.

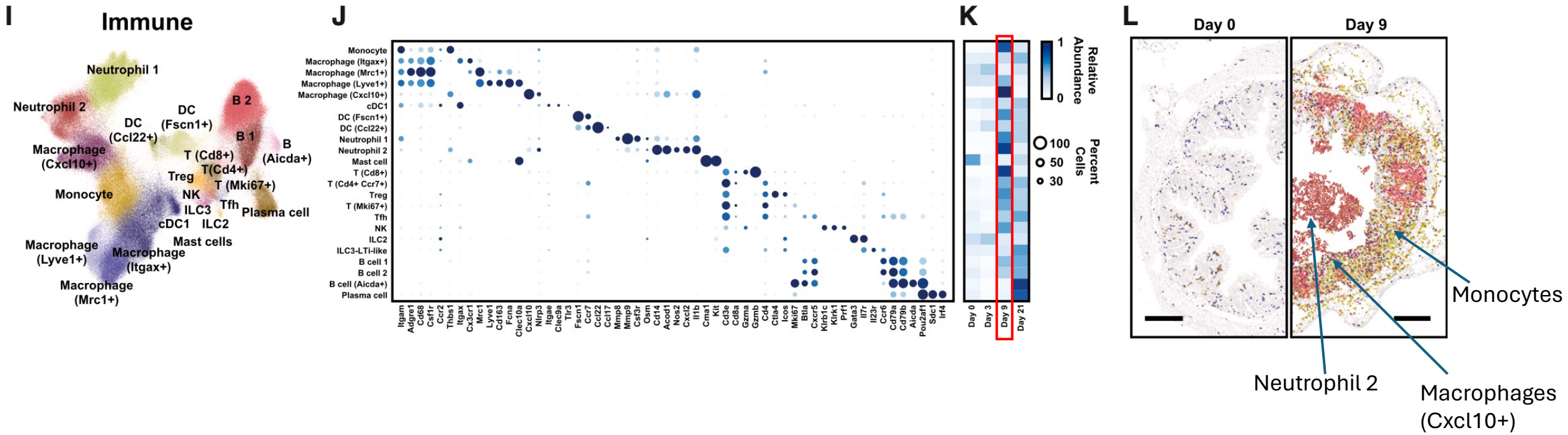
Cellular Remodeling in Epithelial Populations: IAE1, IAE2, IAE3



- ❑ Expression of expected colonic cells (stem cells, Trans activating, colonocytes, Goblet 1and 2, EEC, and M cells).
- ❑ Identification of multiple inflammation associated epithelial populations (IAE1, IAE2, IAE3).

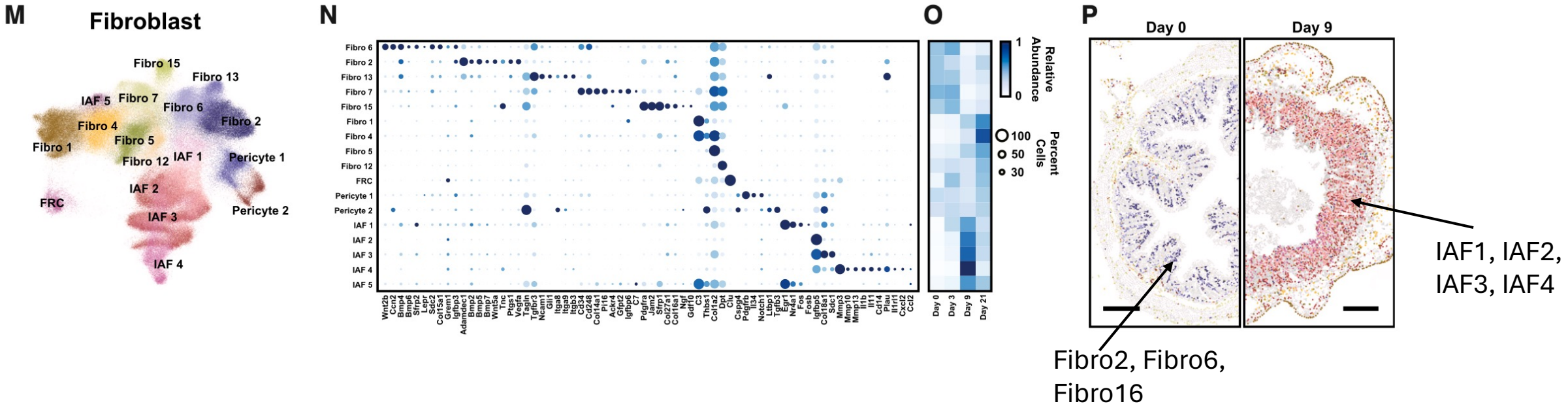
- ❑ IAE1 present at Day3 after DSS treatment, expression of genes associated with transcriptional remolding.
- ❑ IAE2 present at Day 9, expression of interferon stimulated genes, inflammatory cytokines.
- ❑ IAE3 present at Day 9, expression of Myc and stem markers role in epithelial proliferation, repair and renewal.

Large Increase of Immune Cell Populations at Day 9 after DSS



- ❑ At Day 0, Dendritic cells, Macrophage (Itagx+), Macrophage (Mrc1+), and Macrophage (Lyve1+); Itagx+ and Mrc1+ (LP) and Lyve1+ (sub-mucosa).
- ❑ Large increase of Immune cells into Lamina Propia, and other locations (ie mucosa, follicles, lumen).
  - ❑ Day 9
    - ❑ Monocytes and Macrophage CXCL10+ (LP).
    - ❑ Dendritic cells (cDC1, Fscn1+, Ccl22+, mucosa), Neutrophil 1 (LP) and Neutrophil 2 (lumen).
    - ❑ Tcells (cytotoxic Tcells,CD4+ Ccr7+ helper cells, Tregs, LP), T follicular helper cells (follicle).
    - ❑ NK cells (LP), ILC2 and ILC3 (LP), Bcell 1 and Bcell 2 (LP and lymphoid follicles).
  - ❑ Day 21
    - ❑ B (Aicda+, follicular dark zones and plasma cells), rare population of Mast cells.

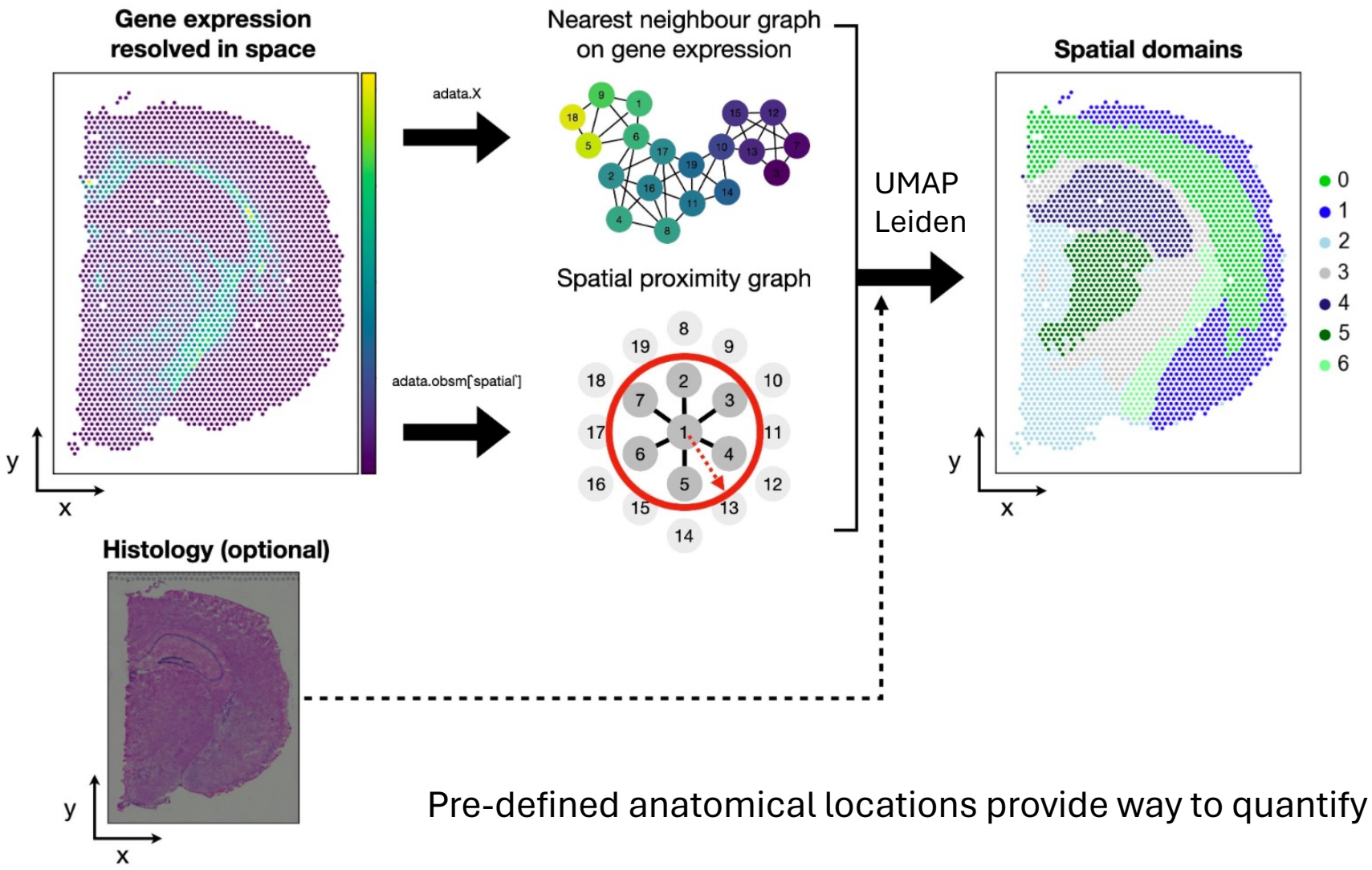
Cellular Remodeling in Fibroblast Populations



- ❑ Diversity of fibroblast populations with distinct spatial locations.
- ❑ Leverage markers and spatial location clear associations to previous described populations.
- ❑ Fibro6 (crypt base, Pdgfra-low), Fibro 13 (myofibroblasts, Tagln, Gli1), Fibro7 (Pi16+ or Ackr4+), Fibro 15(between muscle layers), Fibro15 ENS homeostasis. Fibro4 (near edges of follicles), fibroblast reticular cells (FRCs)
- ❑ Fibro 5 and 12 not seen in scRNA-seq.

- ❑ Presence of Immune activated fibroblast (IAFs) populations at Day 3 and Day 9

Second Order Metrics: Cellular Neighborhood Analysis



Two types of methods:

- KNN (nearest neighbor graph) in low dimensional representation→ community detection in coordinate space.

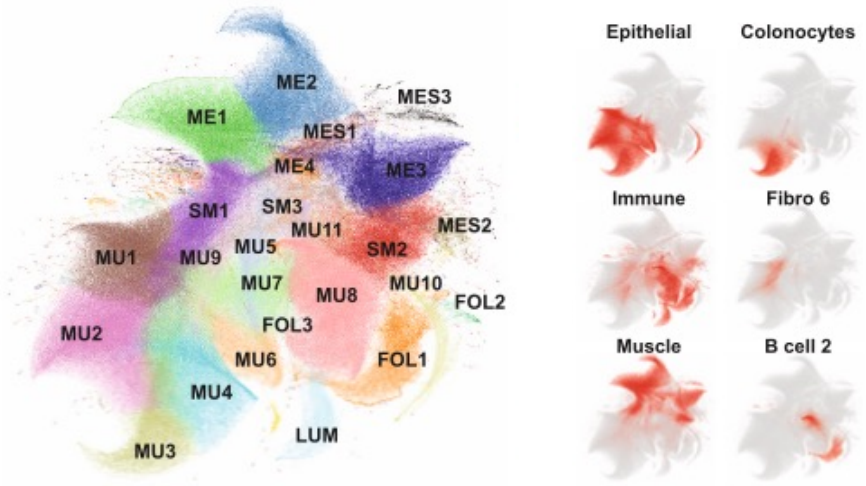
- Modeling spatial dependencies of gene expression
- Modeling spatial dependencies of gene expression + histological images

Pre-defined anatomical locations provide way to quantify organization in tissues

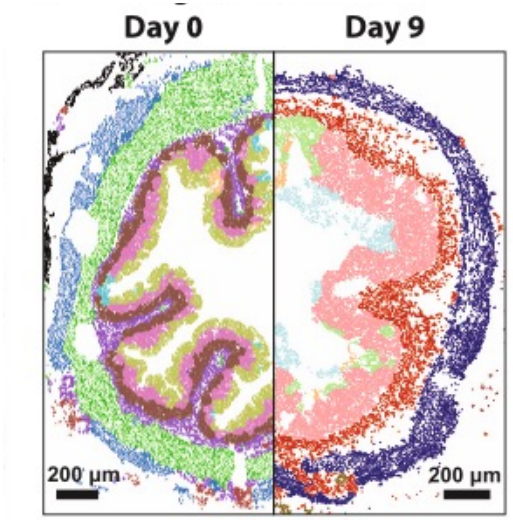
https://www.sc-best-practices.org/spatial/domains.html

Cellular Neighborhoods Define Spatial Remodeling After DSS Treatment

UMAP of cell neighborhoods and abundance



Spatial distribution of all cells colored by neighborhood

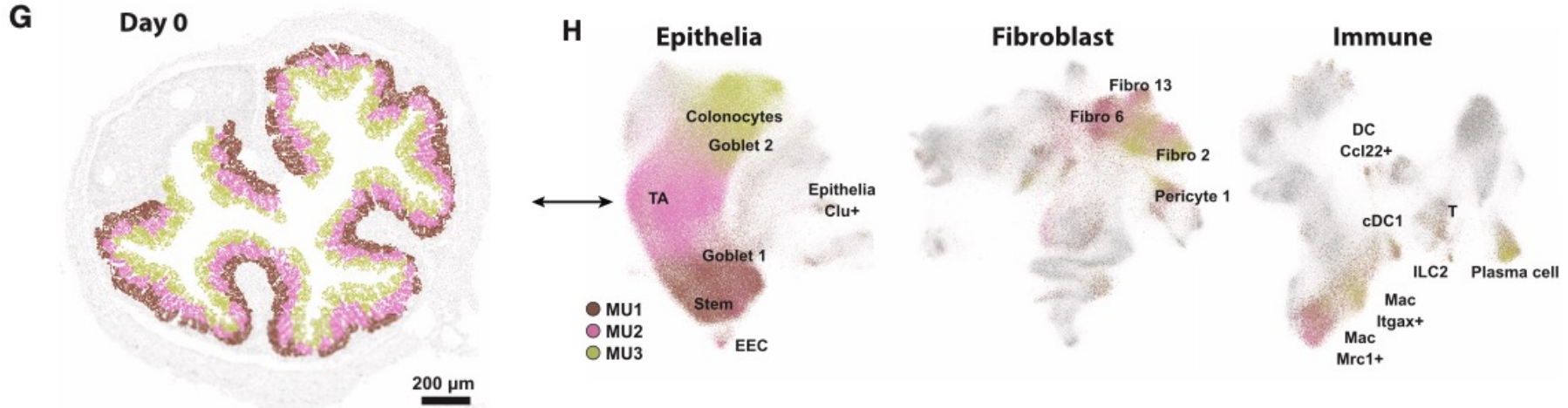


- Anatomical and coordinate free method
- Recurrent neighborhoods
- Names of neighborhoods based on anatomical location and order of emergence in disease
- Neighborhood analysis highly reproducible in healthy mice

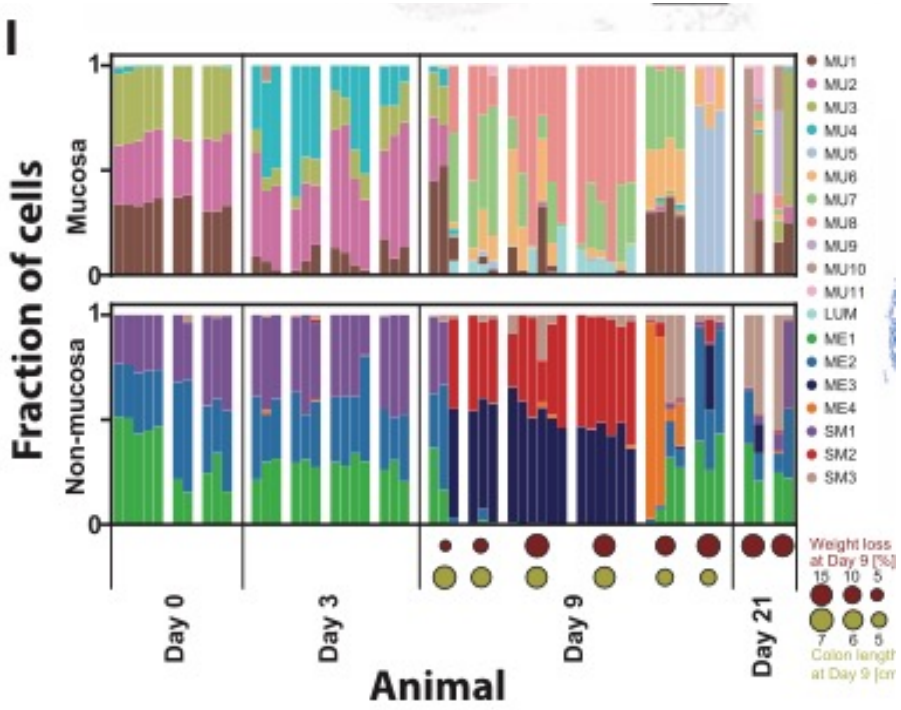
25 cellular neighborhoods, 99.5% of cells

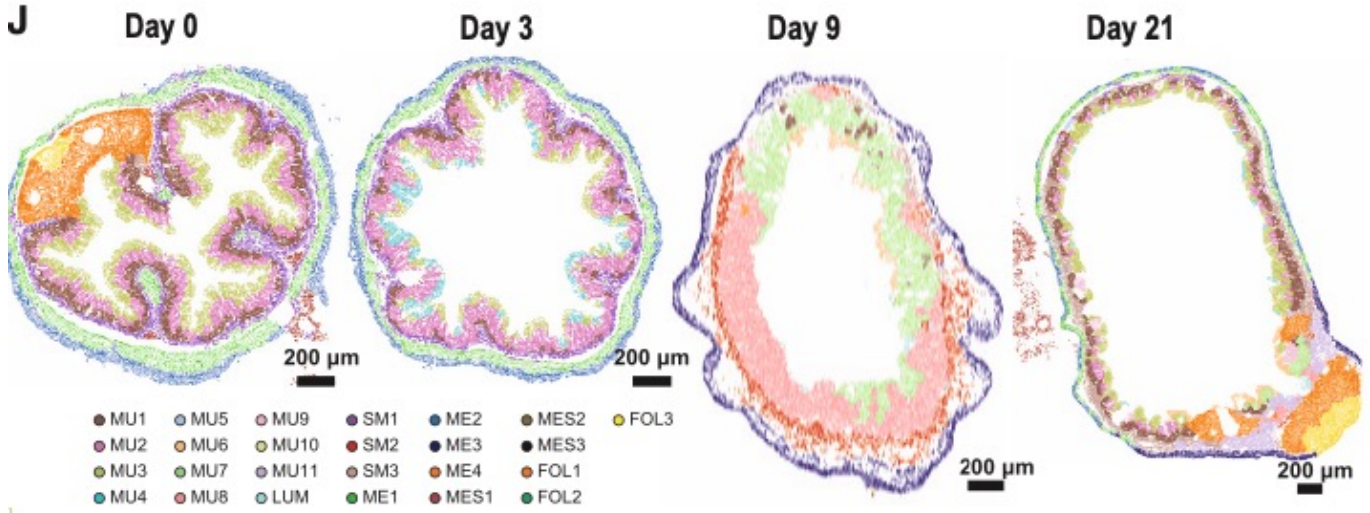
Mucosa (1-11)
LU (Lumen)
Sub-mucosa (SM1-3)
Musclaris externa (ME1-4)
Follicles (FOL1-3)
Mesentery (MES1-3)

Features of healthy mucosa supported by analysis of data



Entire Gut Disrupted at Day 9 following DSS Treatment





Day 0

Organization conserved from crypt to crypt within and between mice.

Day 3

See inter slice variation in mucosa but not non-mucosa.

Day 9

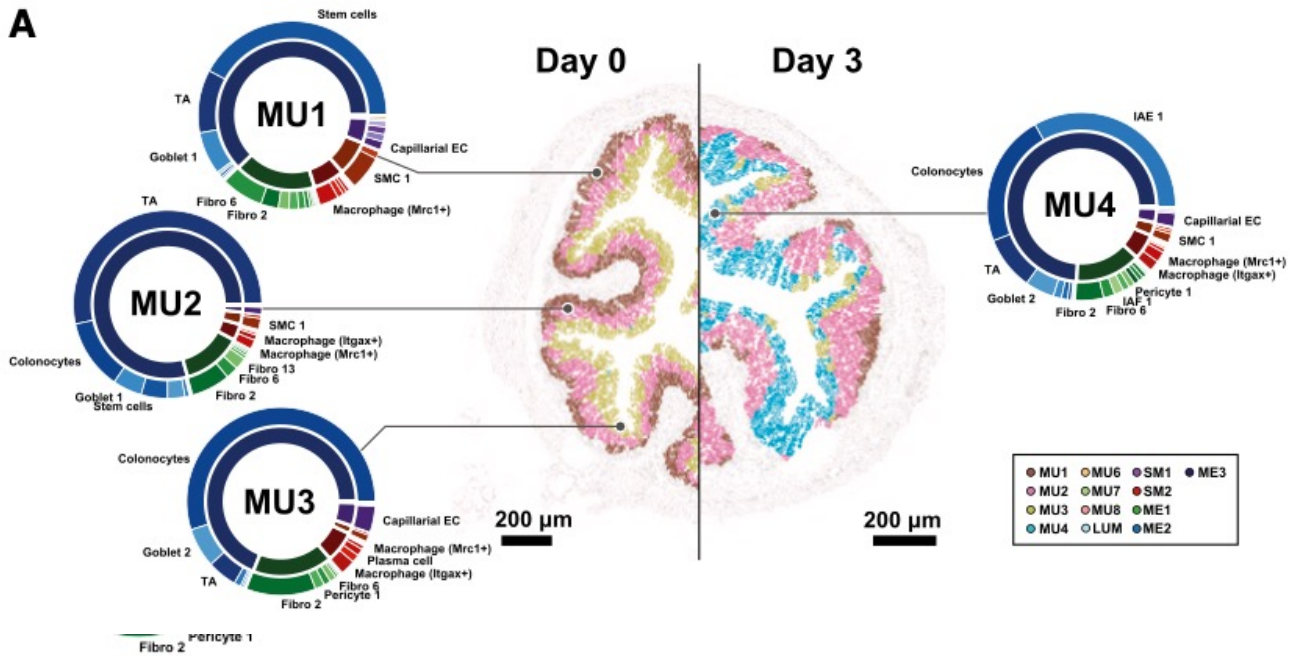
Contiguous crypts spanning lengths of multiple crypts

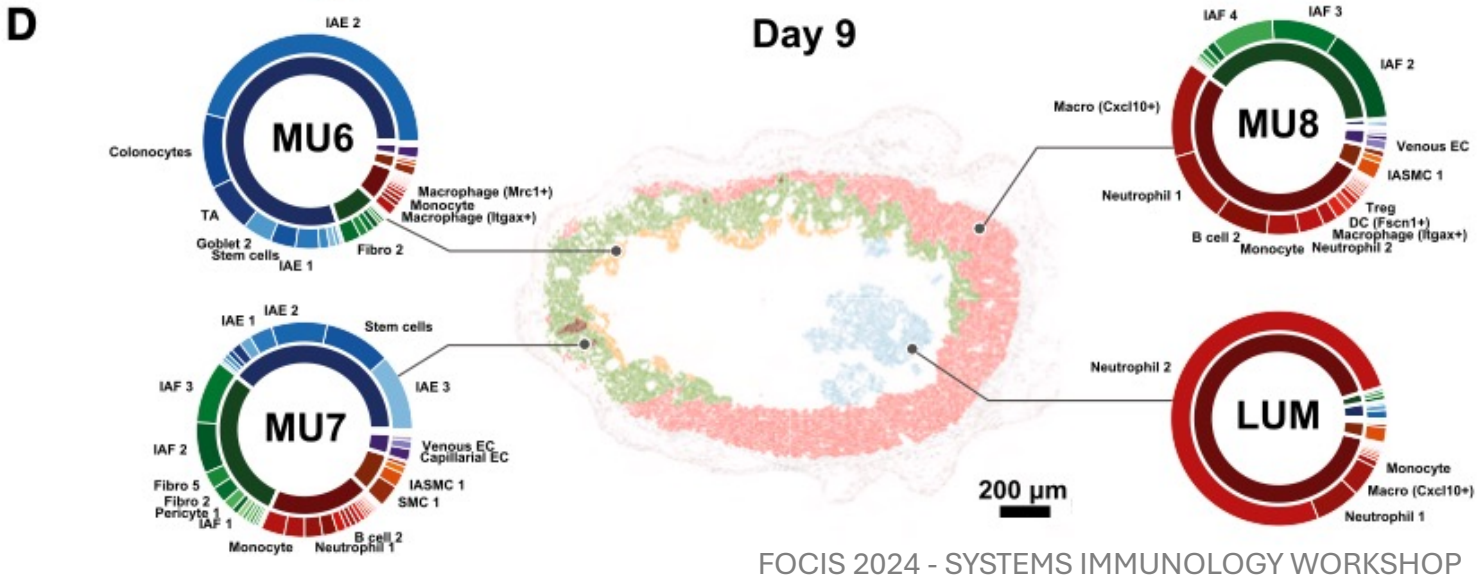
Entire gut substantial inter-slice and inter-mouse heterogeneity

Day 21

In repair phase see substantial variation still persisting from disease phase

Mucosal Cellular neighborhoods driven by polarization and recruitment of distinct cell populations

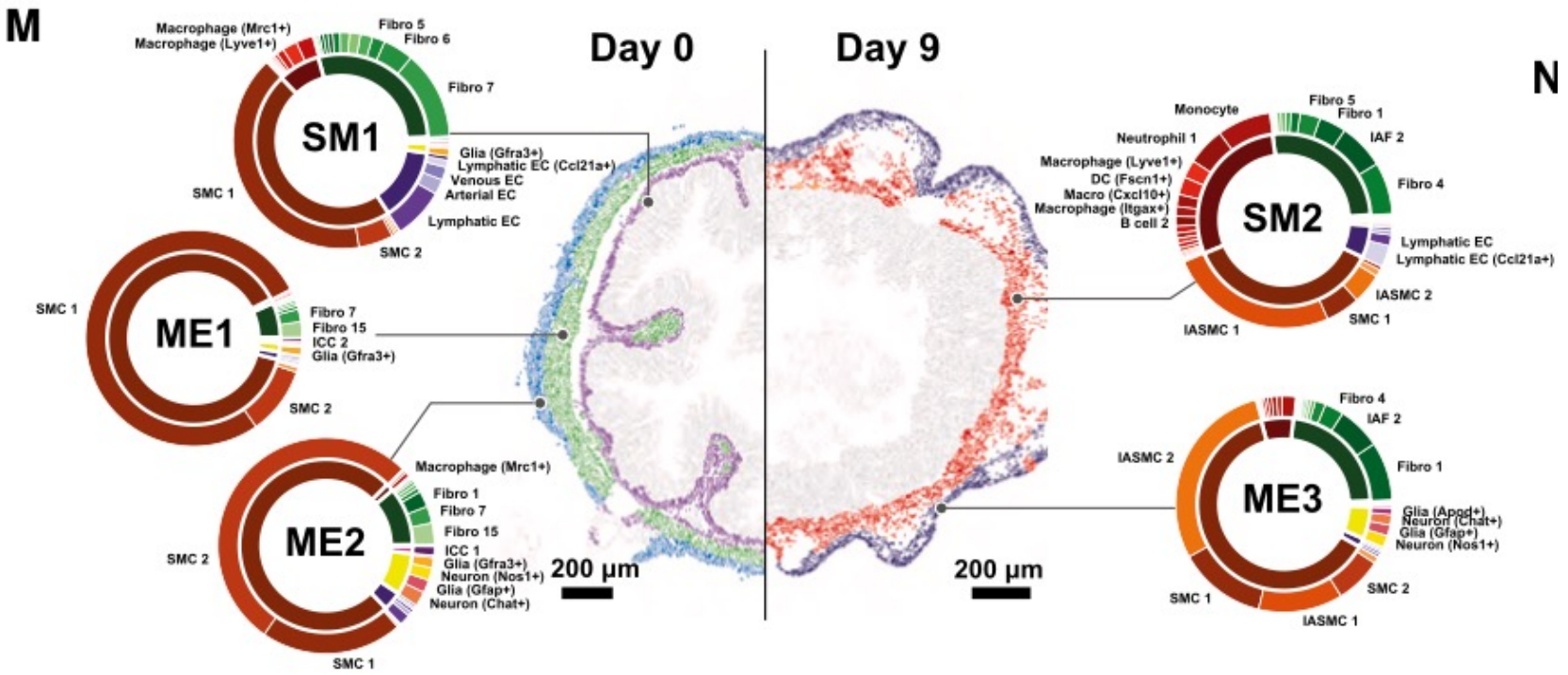




- Emergence of MU4 at Day 3: inflammation associated populations: IAE1, IAF1 and IAF2
- IAE1 and IAF1 Polarization spatially coordinated

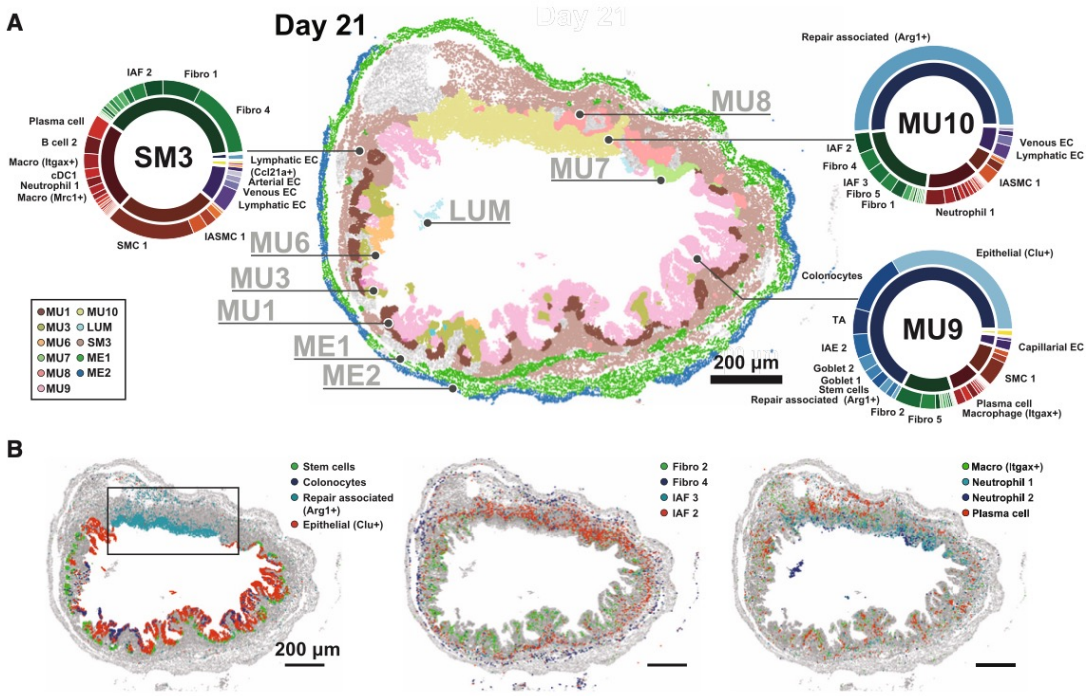
- Mucosa completely remodeled and crypt base to tip abolished;
- New Populations emerge in Mucosa
  - MUC6 proinflammatory version of MUC3, IAE1
  - MUC7: IAF1, IAF2, IAF3
  - MUC8: IAF2, IAF3, IAF4, greater diversity of immune populations
  - LUM: neutrophil 2, polarized form of neutrophil 1

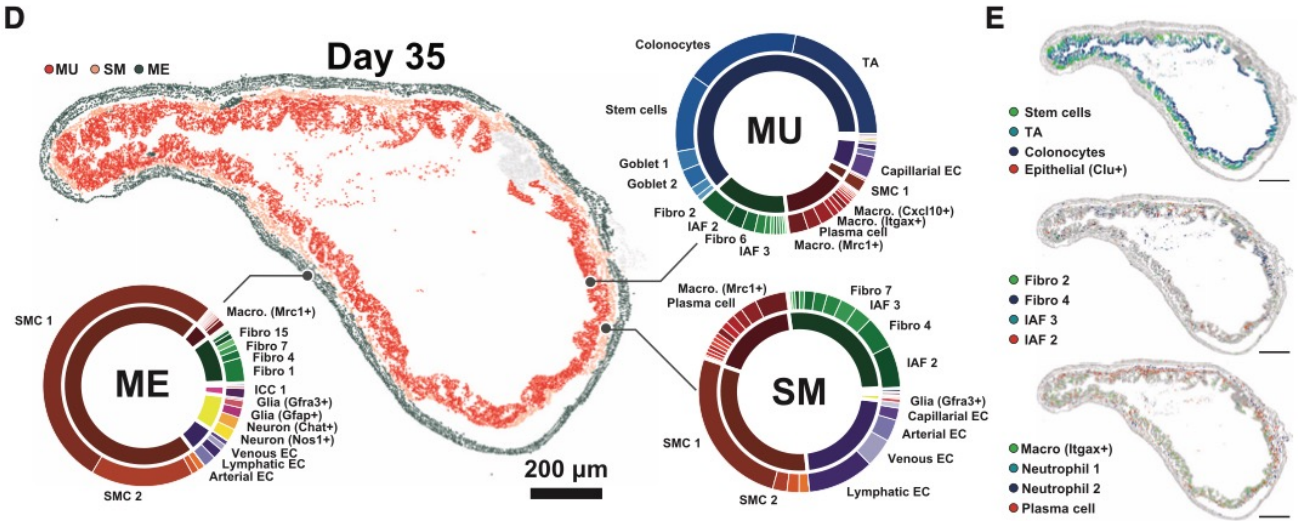
Non-Mucosa Cellular Remodeling after DSS Treatment



- ❑ Observed expected thickening of non-mucosal neighborhoods.
- ❑ Sub-mucosa neighborhood SM1 replaced by SM2.
  - ❑ Fibro 7 to Fibro 4
  - ❑ Lymphatic EC to Ccl21+ lymphatic EC
  - ❑ SMC1 to IASCM1
  - ❑ Increased monocyte, neutrophil, DC (Fscn1+), Bcell 2 abundance.
- ❑ Emergence of multiple IASMC replaced ME1 and ME2 with ME3.

Day 21 and Day 35 after DSS Treatment: Repair Phase

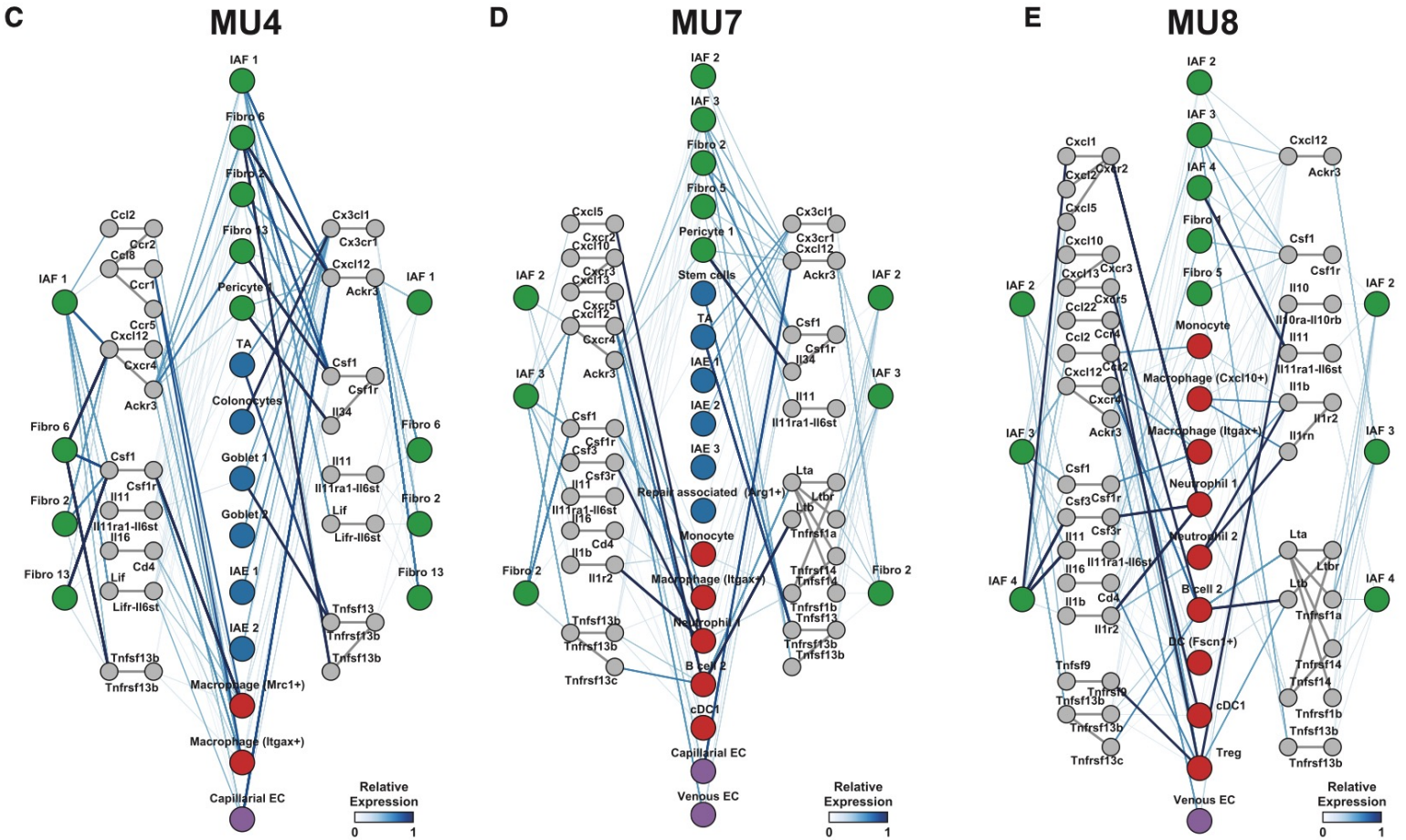




- Mucosal morphology not restored at Day 21
- See presence of MU1, MU2, MU3 (mucosa), ME1 and ME2 (muscularis externa); shifts in cell abundance (i.e. adaptive immunity).
- Emergence of MU9, MU10, MU11.
- MU9 associated with partial restoration of Crypt structure, absence of IAFs, Clu+ epithelial cells.
- MU10 thought of as the repairing ulcer, repair associated epithelia, immune enrichment, some IAFs.
- SM3 healthy SM1 + immune/fibroblasts of SM2

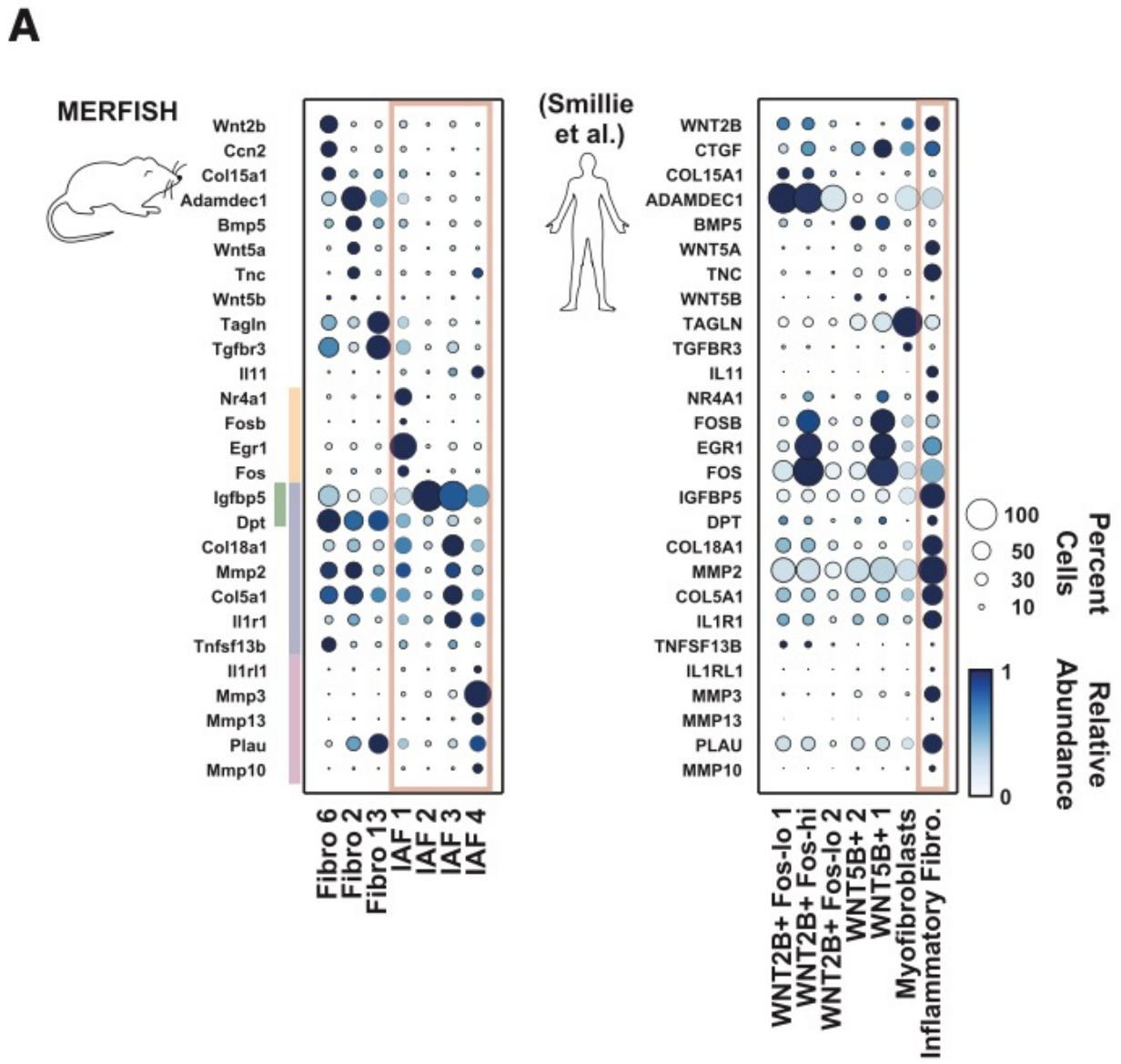
- Mucosal morphology almost completely recovered.
- Residual presence of inflammation from IAF2 and IAF3 and increased plasma cells.
- Submucosa and musclaris externa also has residual signs of inflammation.

IAFs Orchestrate Unique Cell-Cell Interactions with Cytokines and Chemokines



- IAF2 and IAF3 display unique interactions with Tregs via the *Tnfsf9-Tnfrsf9* and *Cxcl13-Cxcr3/Cxcr5* axis.
- IAF4 could reinforce the recruitment and activation of neutrophils to MU8 via the expression of *Cxcl1*, *Cxcl2*, *Cxcl5*, and *Csf3*

Comparison of Merfish Colitis Model to Smillie et al scRNASeq



- IAF populations observed in colitis model similar to the Il11+ IAF population in Smillie et al.
- Mouse neighborhoods share molecular features in human UC?
  - Genes associated with UC disease severity, enriched in human ulcers.
  - Mouse homologs enriched into two neighborhoods and produced by a variety of immune populations and IAF3 abd IAF4.

Summary of Main Learnings

- 940 gene panel resolved expected gut structures (mucosa, sub-mucosa, musclaris externa, submucosal plexus, and myenteric plexus, occasionally lymphoid follicle and stray serosa)
- Emergence of Immune Associated Endothelial cells (IAE1, IAE2, IAE3) and Immune Associated Fibroblasts at Day 3-Day 9 (IAF1, IAF2, IAF3, IAF4).
- Massive upregulation of Immune Population into lamina propia and other locations
  - (Monocytes and Macrophage CXCL10+ (LP), Dendritic cells (cDC1, Fscn1+, Ccl22+, mucosa),
  - Neutrophil 1 (LP) and Neutrophil 2 (lumen),
  - Tcells (cytotoxic Tcells,CD4+ Ccr7+ helper cells, Tregs, LP), T follicular helper cells (follicle),
  - NK cells (LP), ILC2 and ILC3 (LP), Bcells1 and Bcell 2 (LP and lymphoid follicles
- Neighborhood analysis predicted 25 clusters based on anatomical location and stage of disease, Mucosa (1-11), Lumen, Submucosa (SM1-3), Musclaris externa (ME1-4), Follicles (FOL1-3), Mesentery (MES1-3).
- Spatially resolved ligand-receptor interactions show intricate network of cytokines and chemokines interacting with immune associated fibroblast states.
- Progression of disease in DSS model

Key Take Spatial Home Messages

- ❑Spatial Transcriptomics provides the ability to simultaneously chart the cellular and spatial organization of tissues.
- ❑In situ can define populations not easily seen in scRNA- seq due to increased molecular sensitivity, higher cellular throughput, and lack of dissociation-induced perturbations .
- ❑Cell Segmentation free methods worked well to visualize distribution of RNAs in different layers of colon.
- ❑Cell Neighborhoods analysis can be used to understand progression of disease in terms of spatial remodeling of tissue structures.
- ❑Spatially resolved CellphoneDB method developed to identify ligand-receptor pairs for in situ data.

Thank you to organizers