

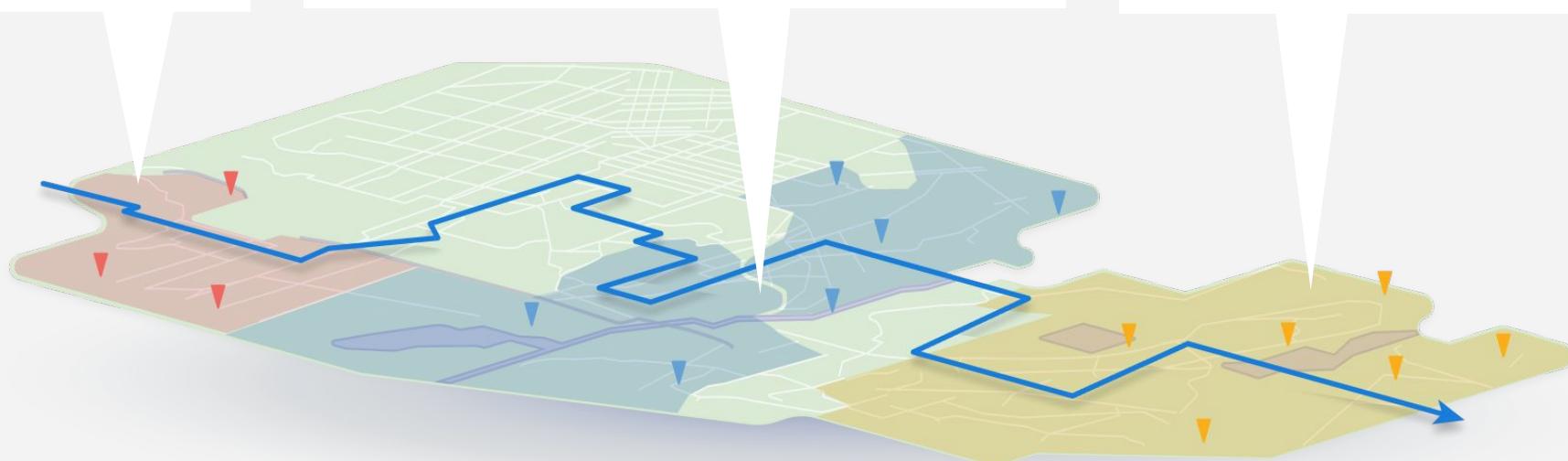


Exploring Your Visium Data: A Spot Deconvolution Story

CG000669 Rev A

Outline

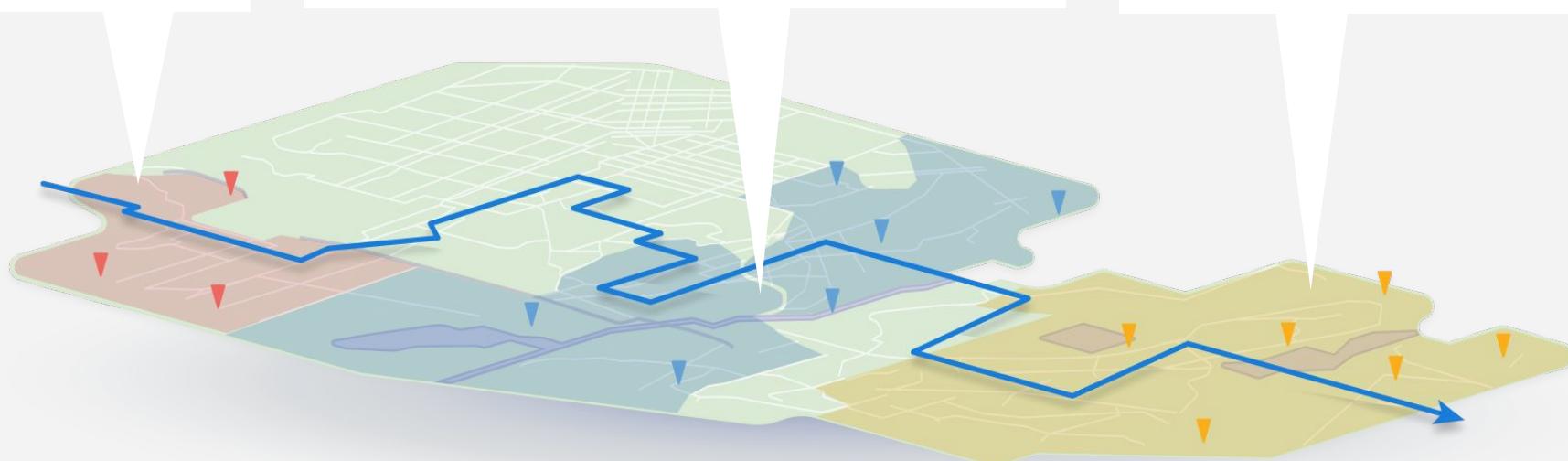
Experimental Design	Data Analysis	Data Exploration
<ul style="list-style-type: none">• Goal• Alzheimer's Disease• Question• Hypothesis	<ul style="list-style-type: none">• The Dataset• Data Analysis Plan• Spot Resolution• Spot Cell-type Deconvolution• Analyzing the Data	<ul style="list-style-type: none">• Exploring the Results• Participant Challenge• Exploring the Results Part 2• Paired scRNASeq data• What is Next



Experimental Design

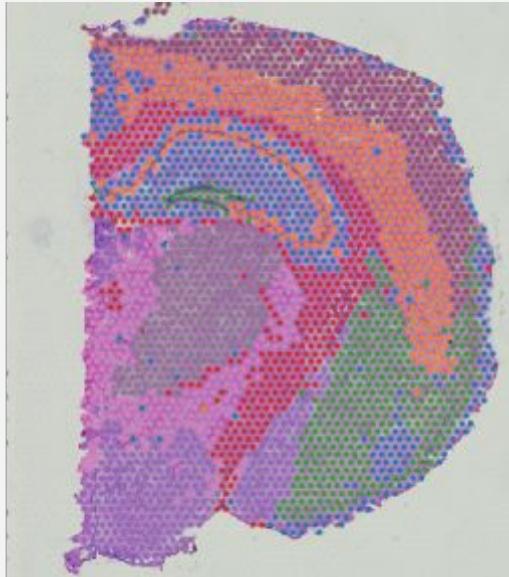
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Goal

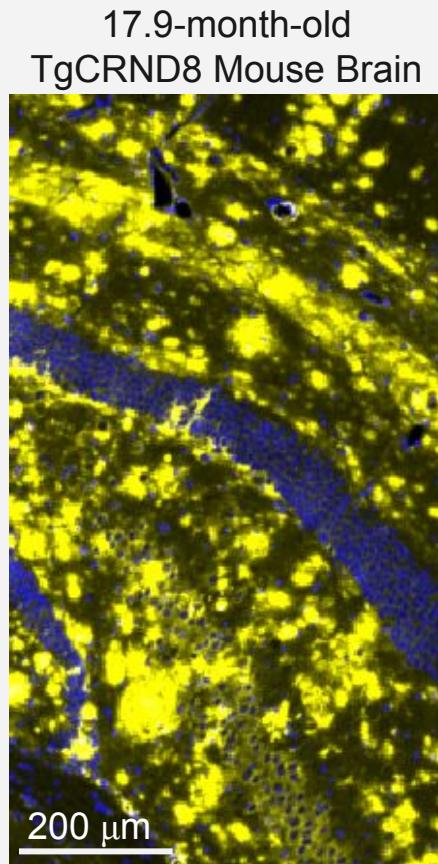
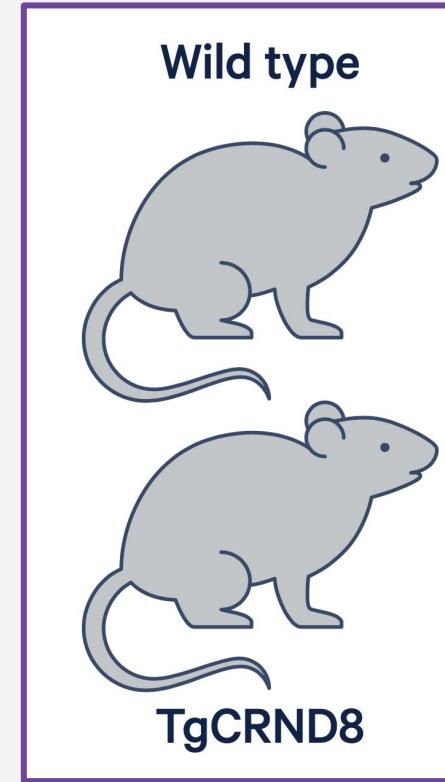
Study hippocampal gene expression in a Alzheimer's Disease model.



See our Application Note for more information ([“Single cell and spatial multiomics identifies Alzheimer’s disease markers”](#))

Alzheimer's Disease

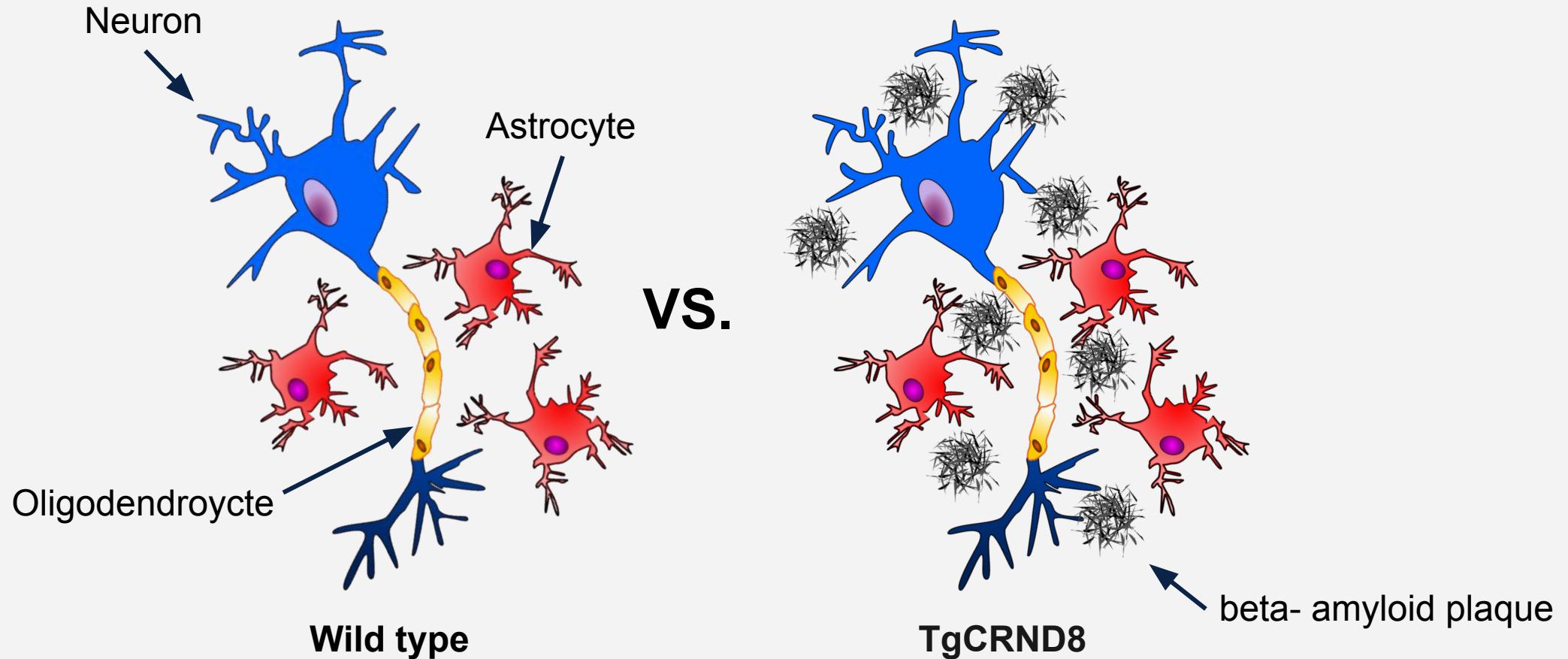
- Alzheimer's Disease is the most common cause of dementia
- Characterized by the accumulation of protein aggregates
- **Why:** disease pathogenesis is still poorly understood
- **Assay:** Visium FFPE
- **Model:** TgCRND8 AD-like mouse



TgCRND8 mice overexpress a mutant human amyloid precursor protein (APP) and develops amyloid beta-peptide deposits. (Blue is dapi nuclei, Yellow is beta-amyloid aggregates)

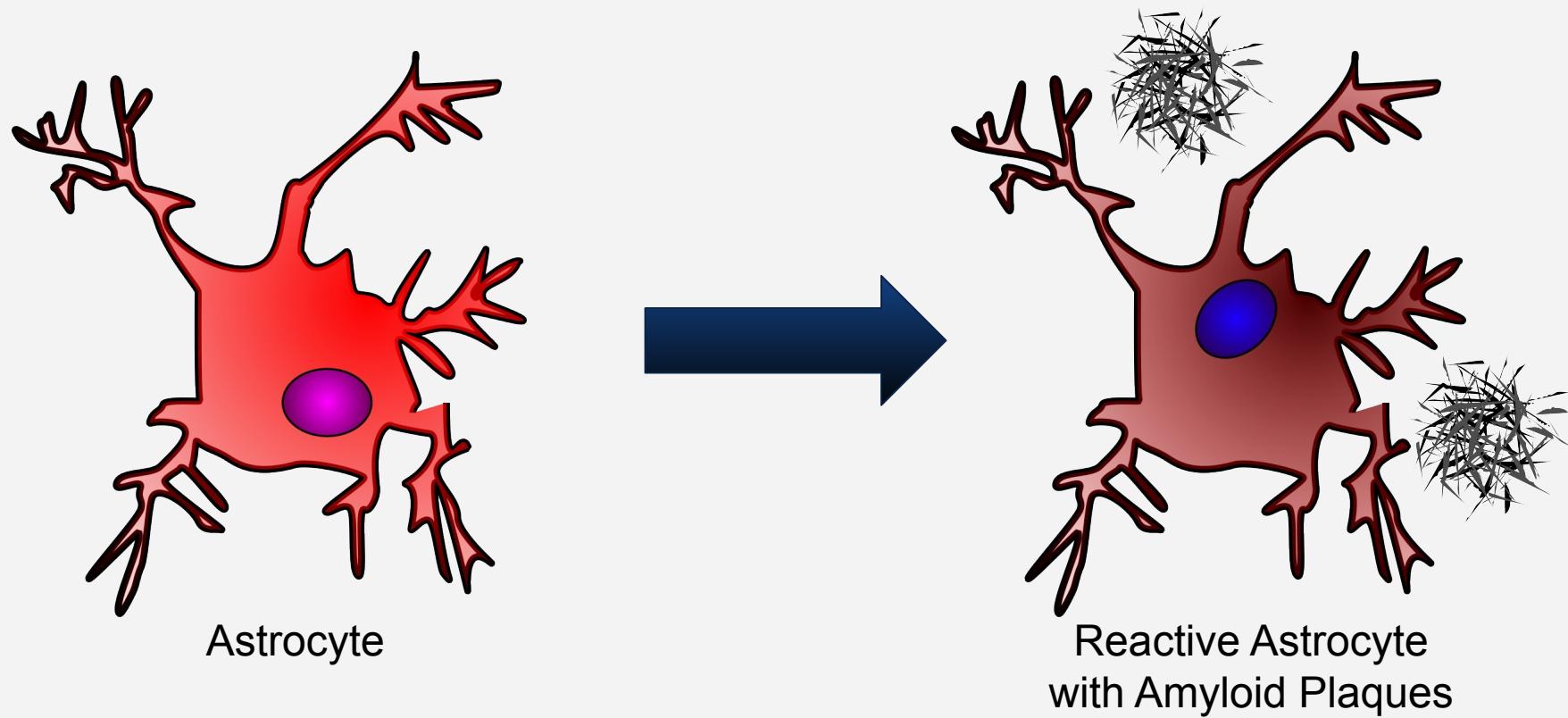
Question

Do amyloid plaque deposits in the hippocampus alter cell phenotype?



Hypothesis

Amyloid plaque deposits in the hippocampus are coincident with reactive astrocytes.





Data Analysis

Outline

Experimental Design

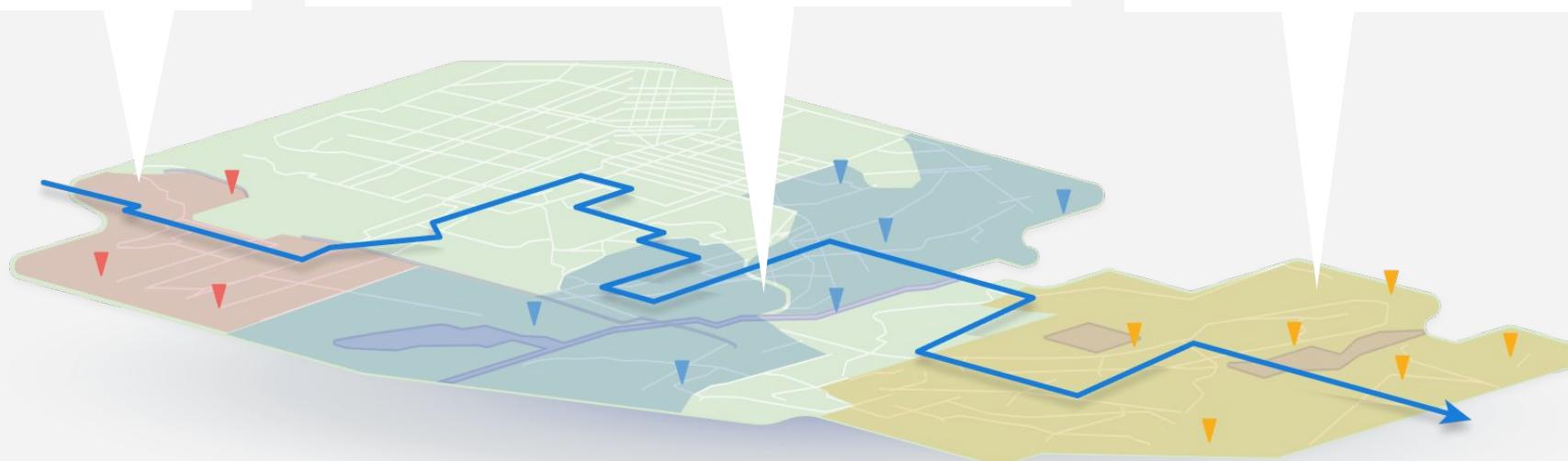
- Goal
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Data Analysis

- The Dataset
- Data Analysis Plan
- Spot Resolution
- Spot Cell-type Deconvolution
- Analyzing the Data

Data Exploration

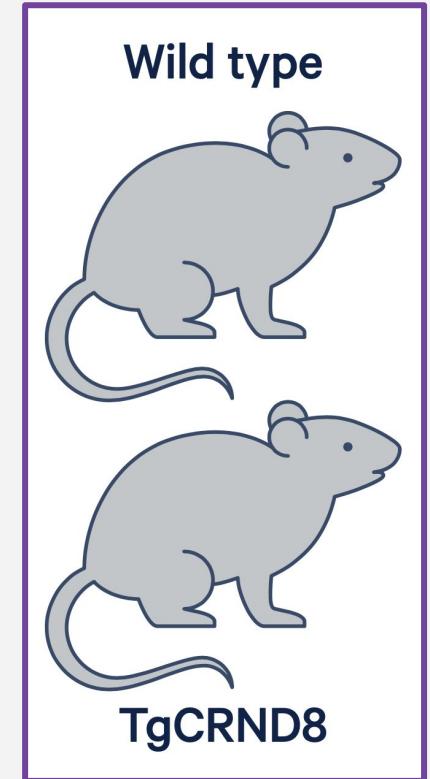
- Exploring the Results
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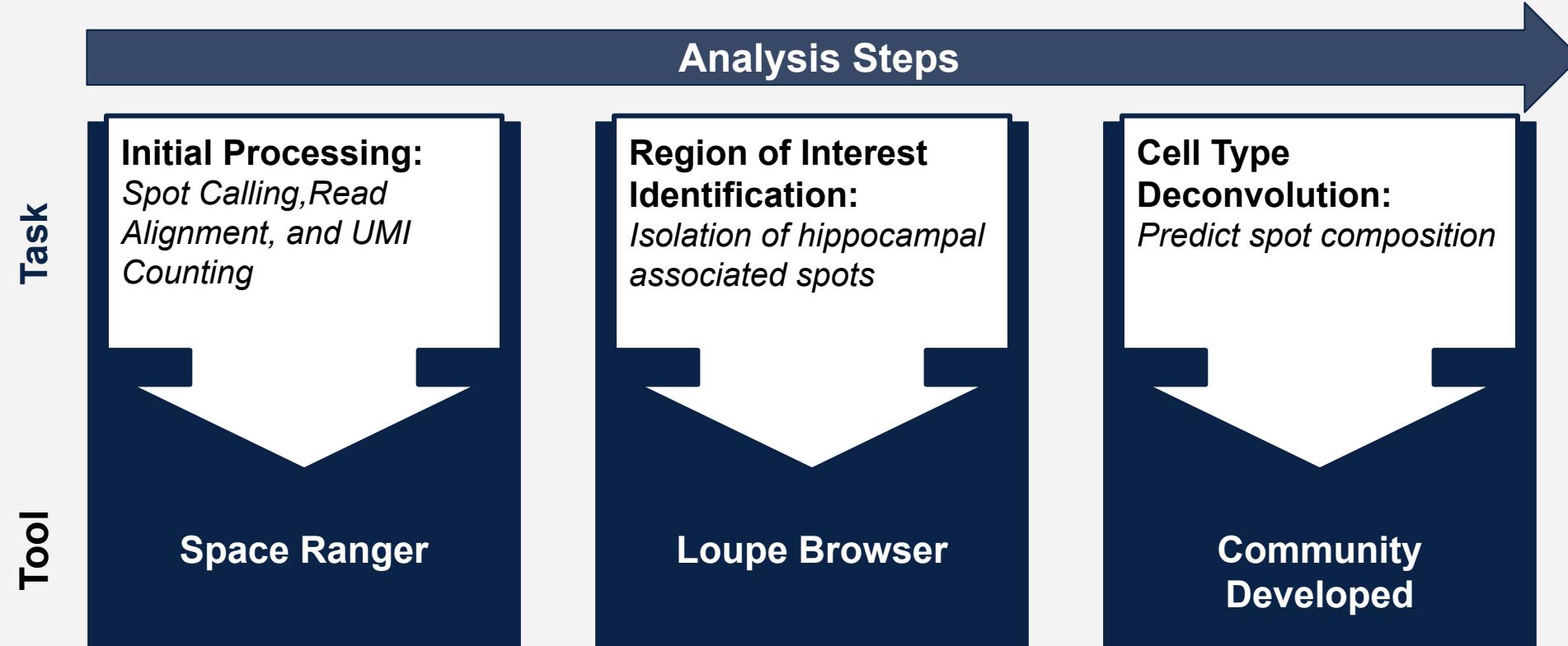
The Dataset

- **Design:** wild type and transgenic mice brains were collected (N=2 for each condition)
- **Paired data collected:** single cell ATAC, single cell RNAseq Gene expression, and Visium Spatial Gene Expression.
- We will start with **one 17.9-month-old** transgenic mouse brain

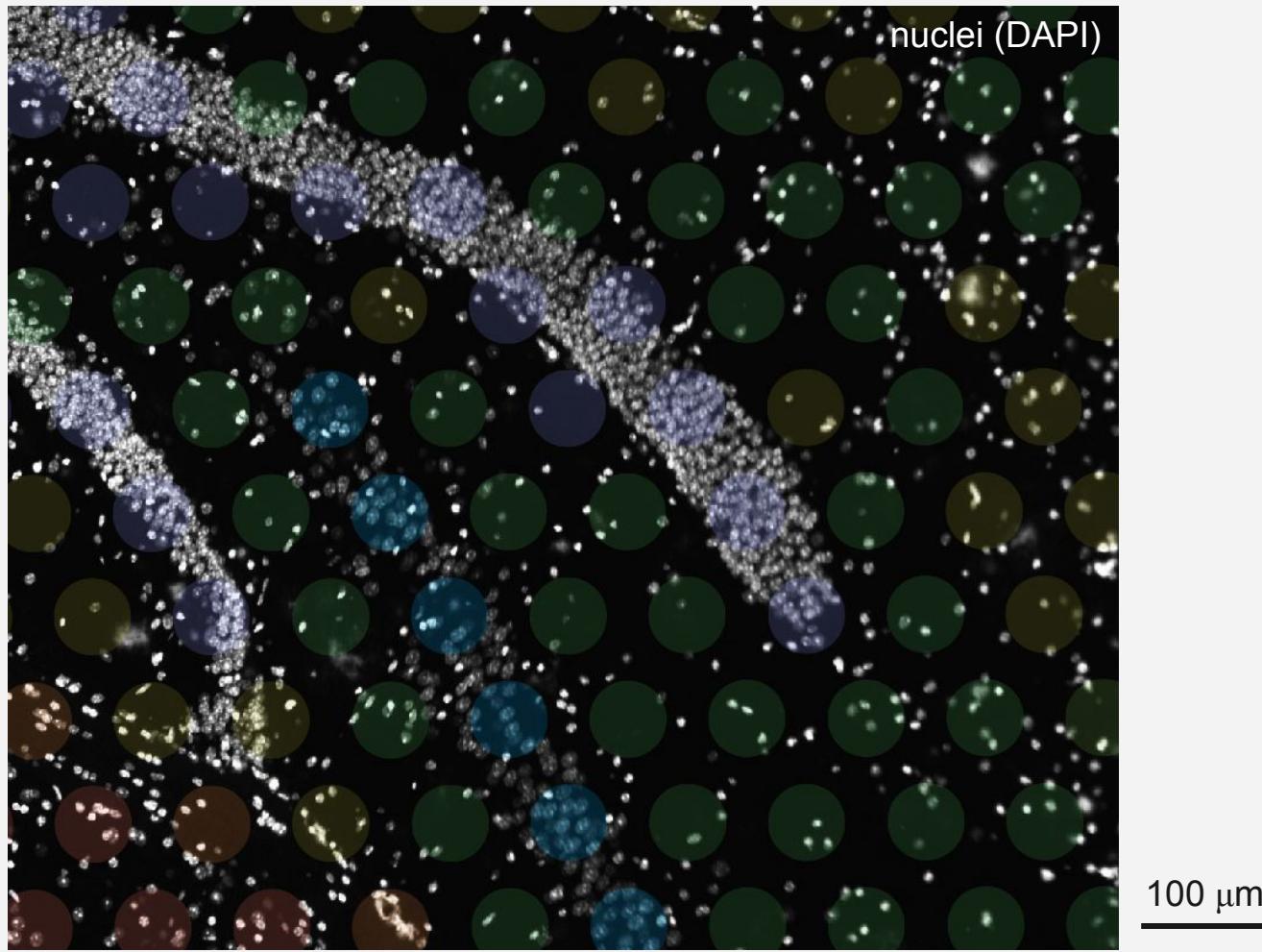
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Data Analysis Plan



Spot Resolution: Multiple Cells per Spot

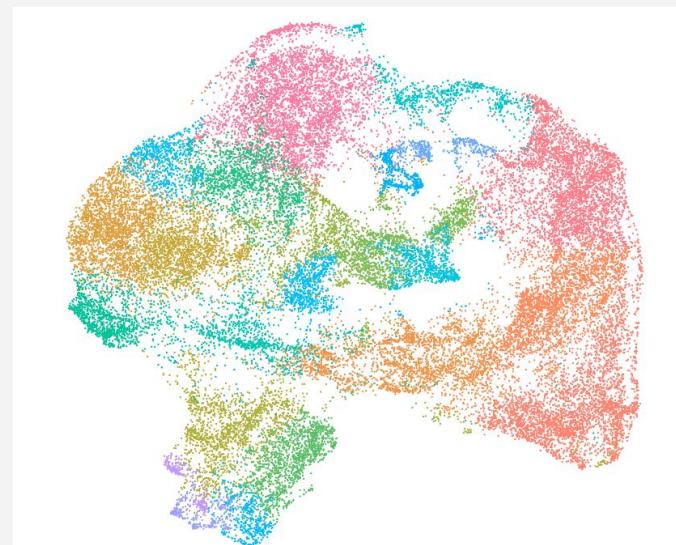


17.9 month old transgenic hippocampus

Spot Resolution: Deconvolution

Spot cell-type deconvolution is the computational prediction of a spot's cell-type composition.

Reference: Annotated bulk
or scRNAseq data



Reference-free:
List of genes

Gene 1
Gene 2
Gene 3
Gene 4
...
Gene N

Spot Cell-type Deconvolution: Community Developed Software

Reference



SpatialDWLS



Spacexr
(RCTD)

Reference-free

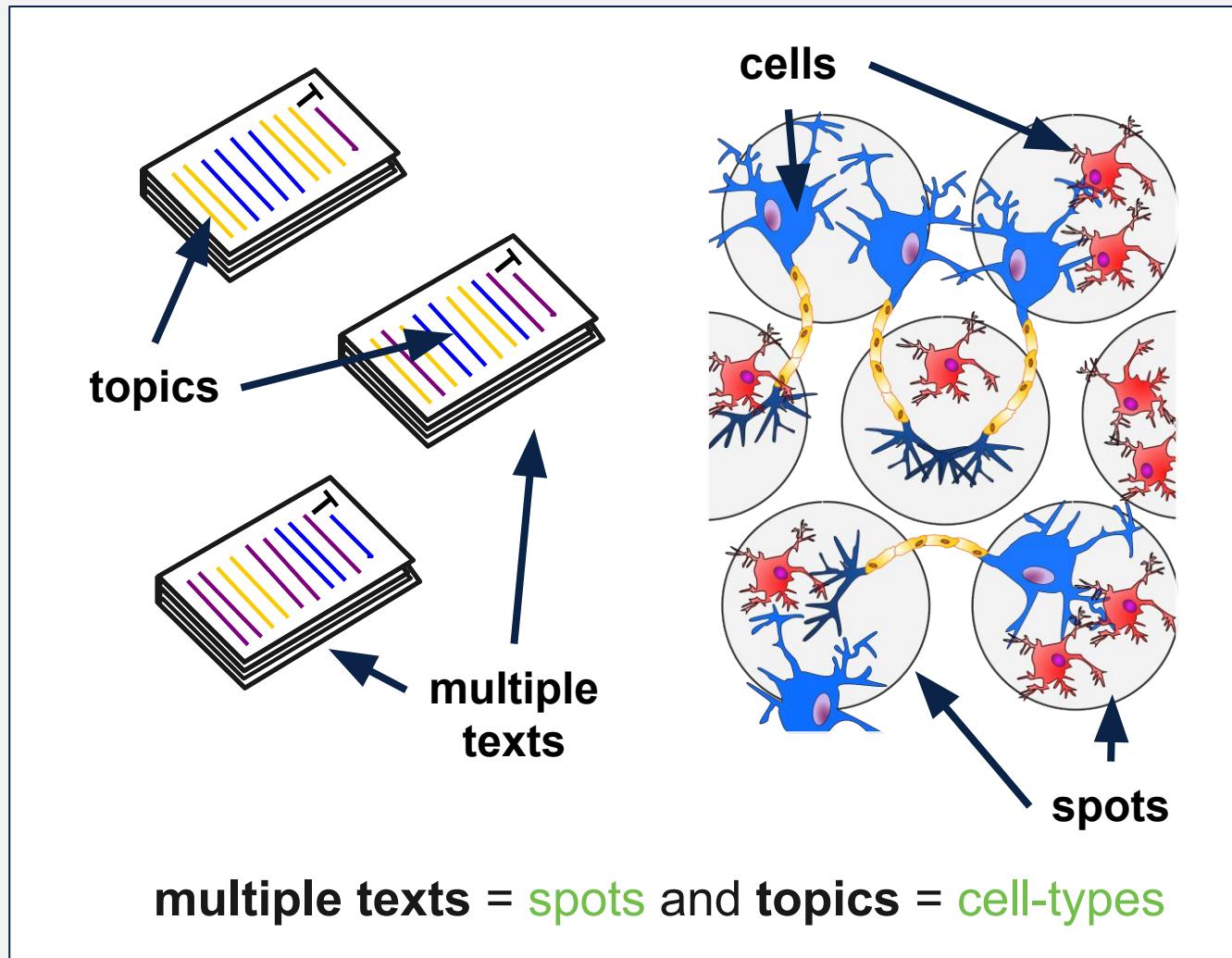


STdeconvolve

See our Analysis Guides to learn more about deconvolution:

- [Publication Highlight: Benchmarking Methods to Integrate Spatial and Single-cell Transcriptomics Data](#)
- [Integrating 10x Visium and Chromium data with R](#)
- [Integrating Single Cell and Visium Spatial Gene Expression Data](#)

Spot Cell-Type Deconvolution: STdeconvolve



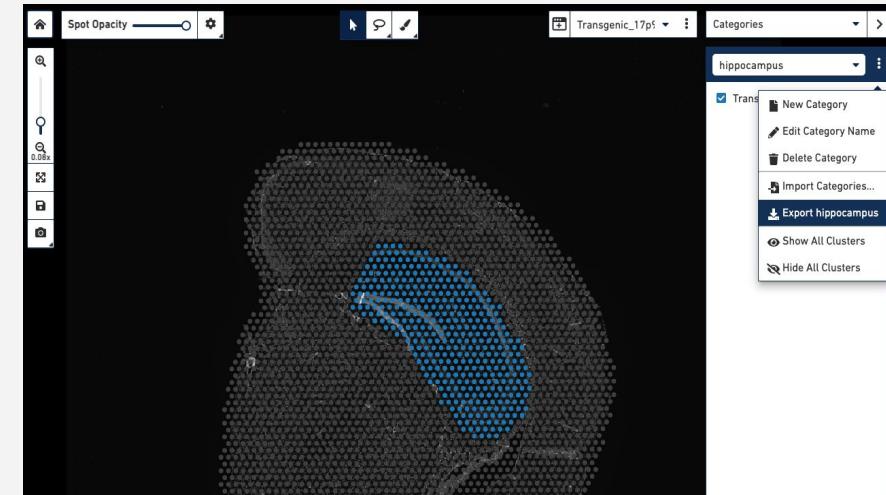
Analyzing the Data

General Steps Post Space Ranger

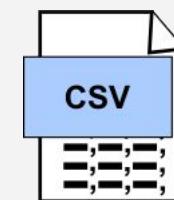
Analyzing the Data

General Steps Post Space Ranger

1. (optional) Identify regions of interest
 - o **Reason:** Isolate spots in the hippocampus to focus on hippocampal cells



Loupe Browser



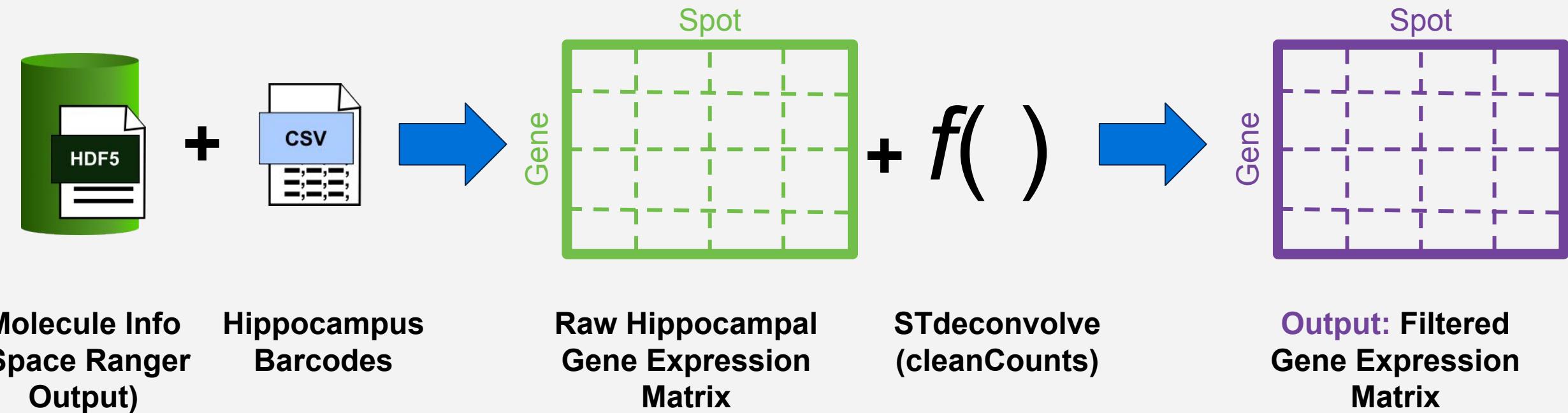
Barcode,hippocampus
AAACGAGACGGTTGAT-1,Trans_17p9_rep1
AAACTGCTGGCTCAA-1,Trans_17p9_rep1
AAAGGCTACGGACCAT-1,Trans_17p9_rep1
AAAGGCTCTCGCGCCG-1,Trans_17p9_rep1
...

Output: Exported Barcodes

Analyzing the Data

General Steps Post Space Ranger

1. (optional) Identify regions of interest
2. Filter gene expression matrix
 - Reason: Remove poorly expressed genes and low quality spots



Analyzing the Data

General Steps Post Space Ranger

1. **(optional)** Identify regions of interest
2. Filter gene expression matrix
3. Generate a corpus (spots) using the gene list
 - **Reason:** We need to identify genes that can define topics (cell-types)

Analyzing the Data

Where does the gene list come from?

Prior knowledge

- Canonical cell-type markers

Experimentally determined

- Overdispersed genes (genes with greater than expected variation)
- ...

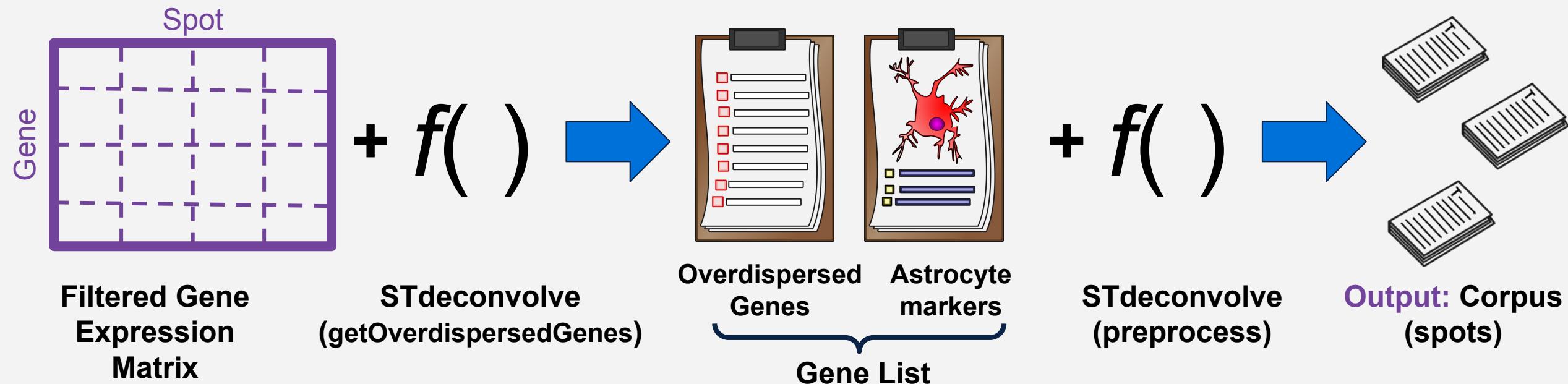
Hybrid

- Combination of experimentally determined and canonical cell-type markers

Analyzing the Data

General Steps Post Space Ranger

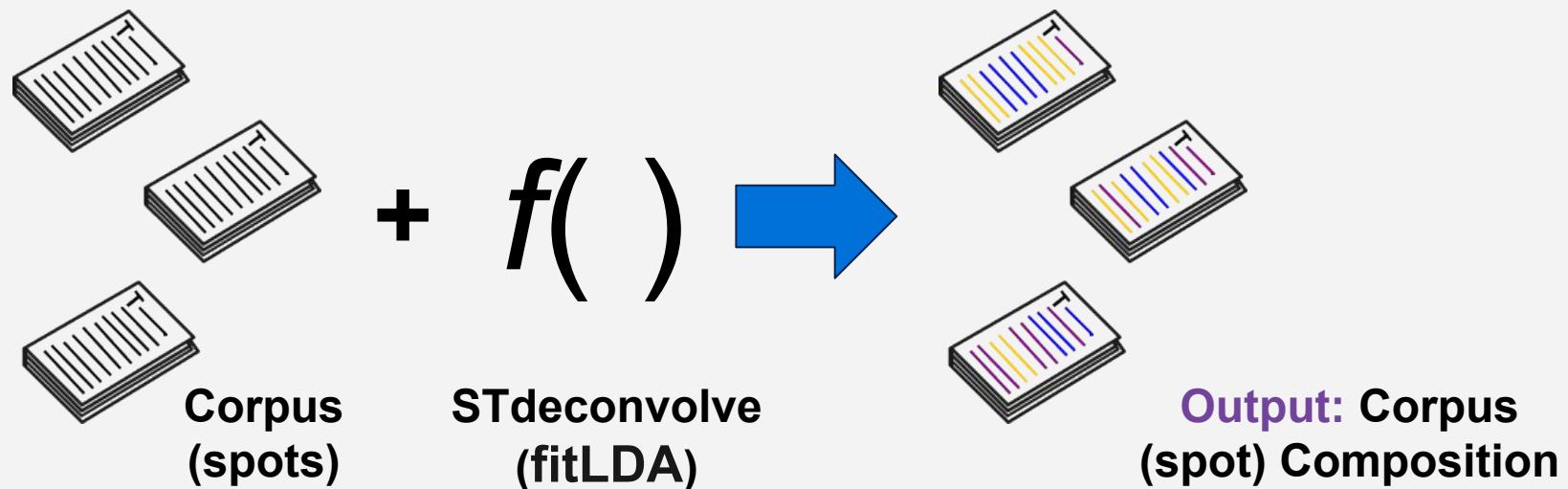
1. (optional) Identify regions of interest
2. Filter gene expression matrix
3. Generate a corpus (spots) using the gene list
 - Reason: We need to identify genes that can define topics (cell-types)



Analyzing the Data

General Steps Post Space Ranger

1. (optional) Identify regions of interest
2. Filter gene expression matrix
3. Generate a corpus (spots) using the gene list
4. Run the analysis
 - **Reason:** predict cell-type composition





Data Exploration

Outline

Experimental Design

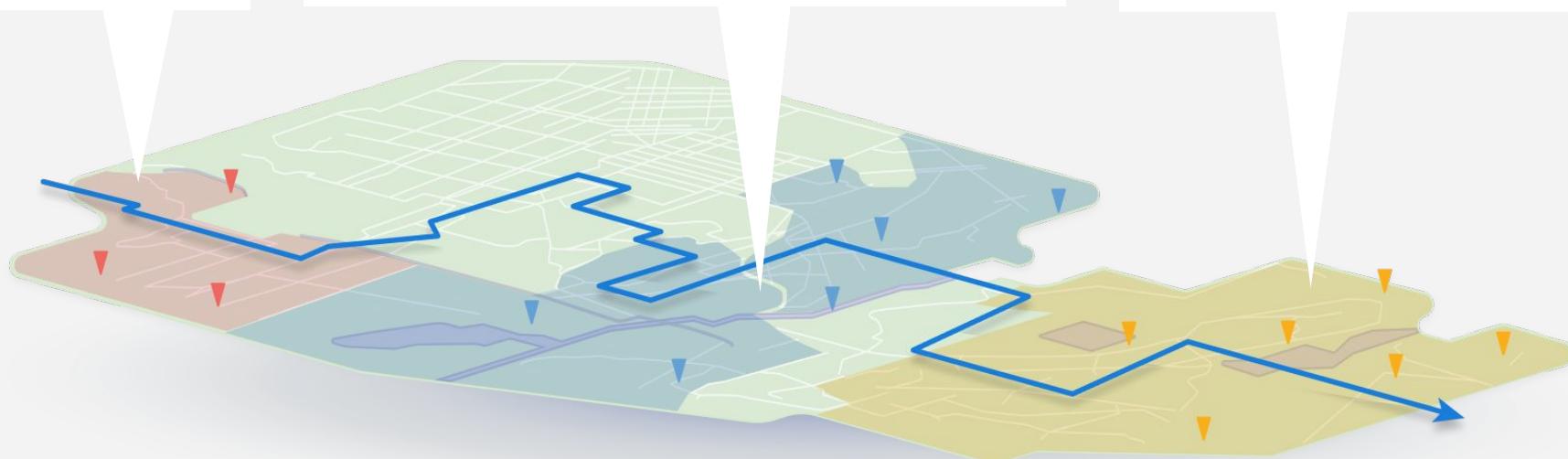
- Goal
- Alzheimer's Disease
- Question
- Hypothesis

Data Analysis

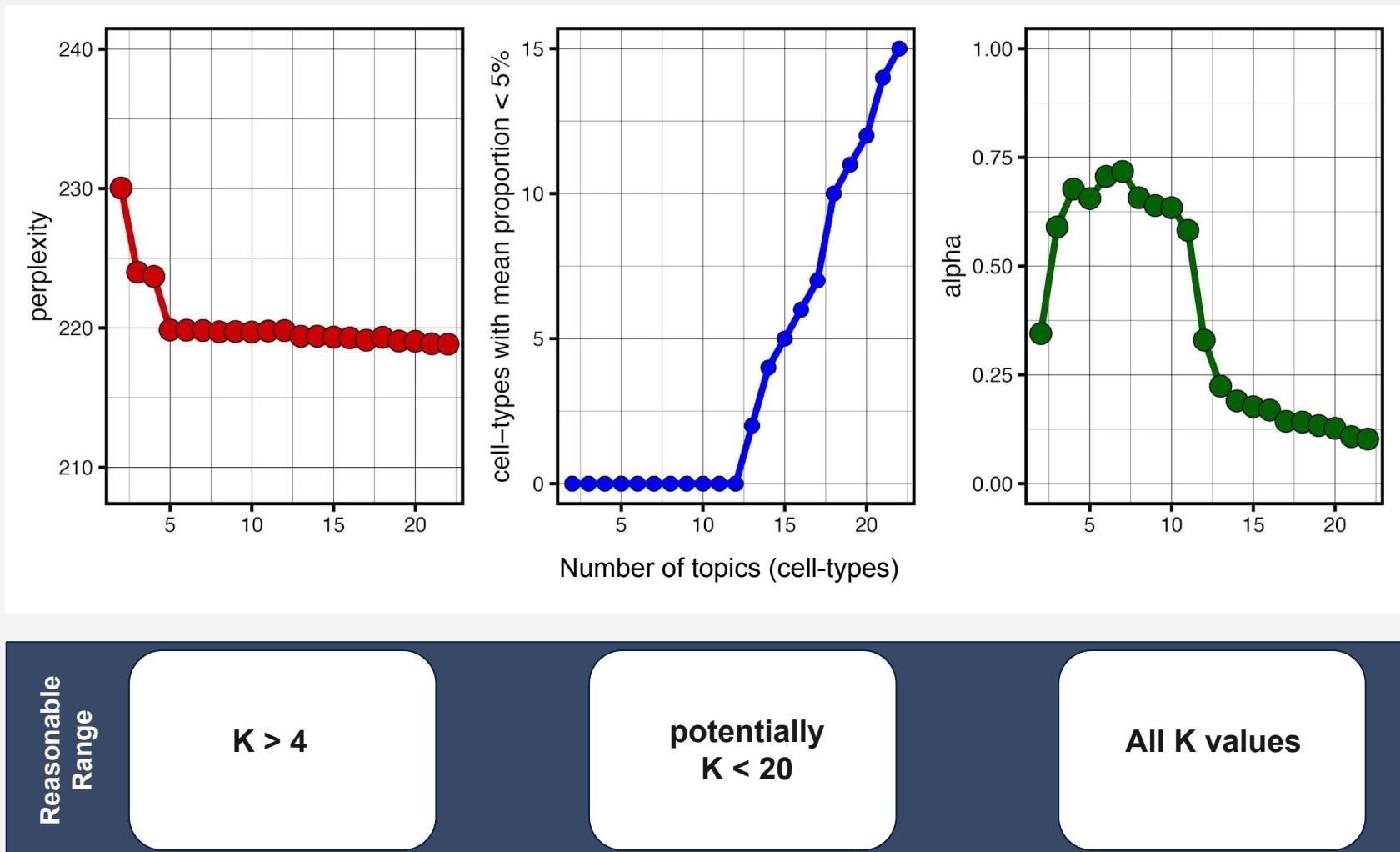
- The Dataset
- Data Analysis Plan
- Spot Resolution
- Spot Cell-type Deconvolution
- Analyzing the Data

Data Exploration

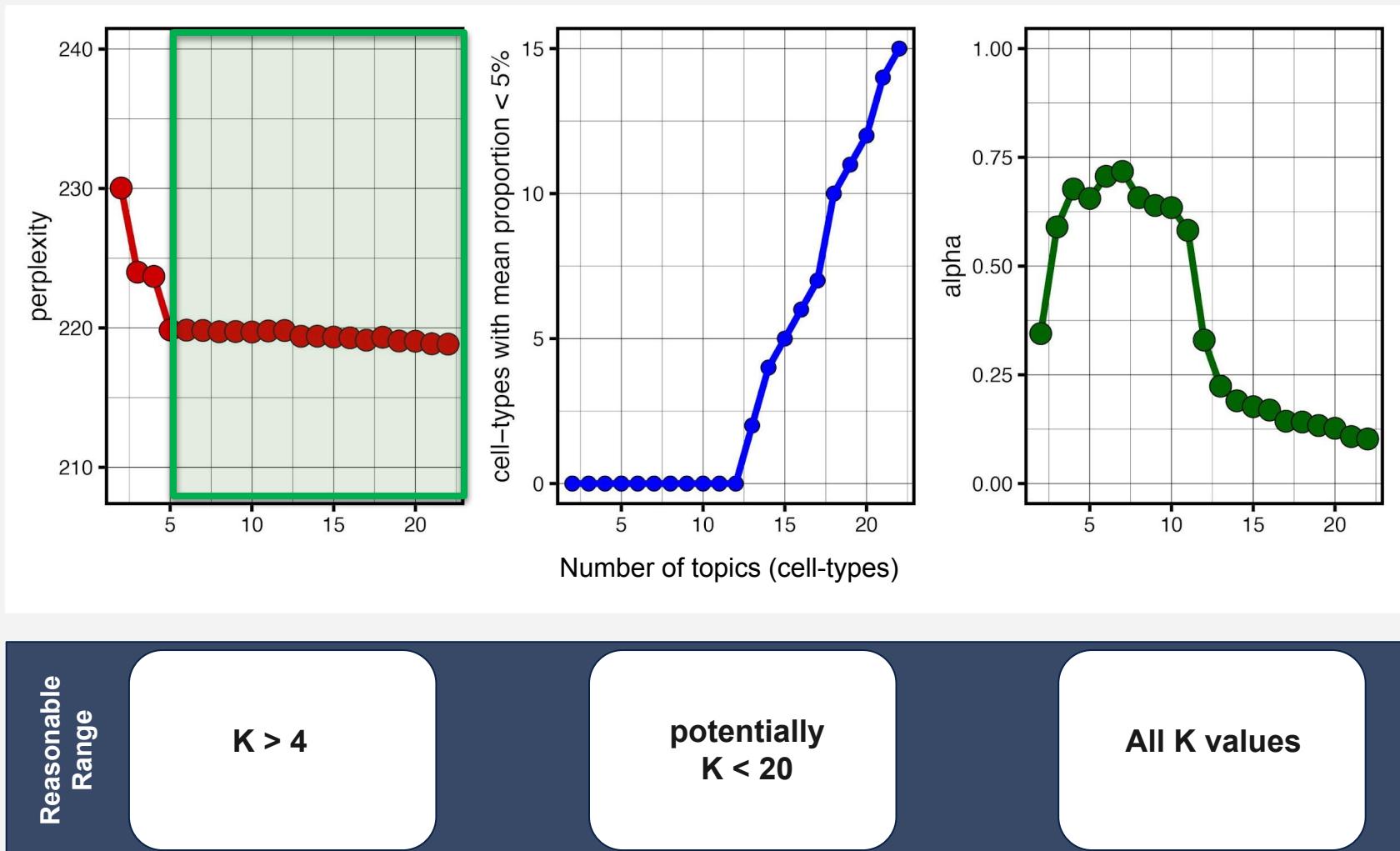
- Exploring the Results
- Participant Challenge
- Exploring the Results Part 2
- Paired scRNASeq data
- What is Next



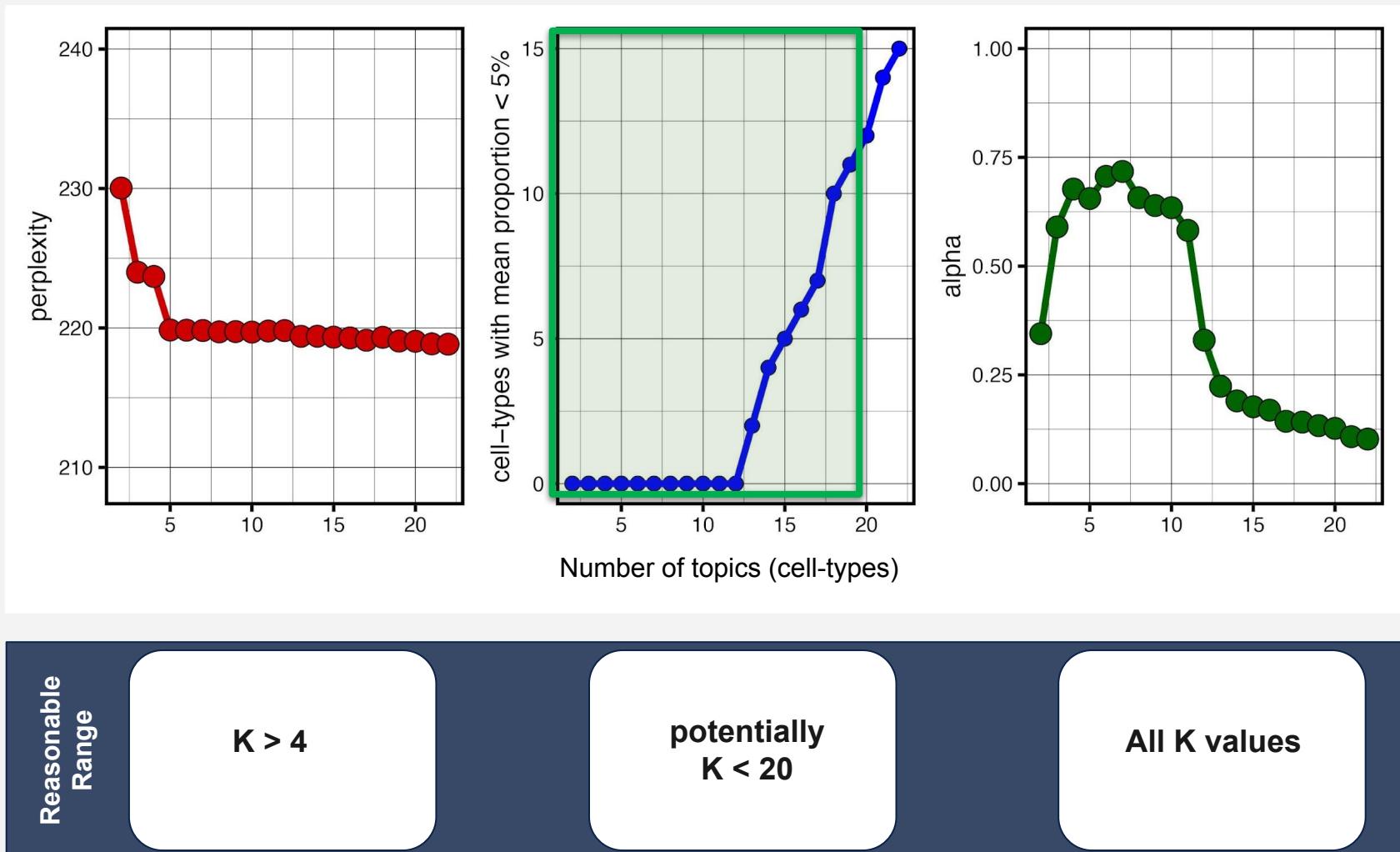
Exploring the Results: Selecting K



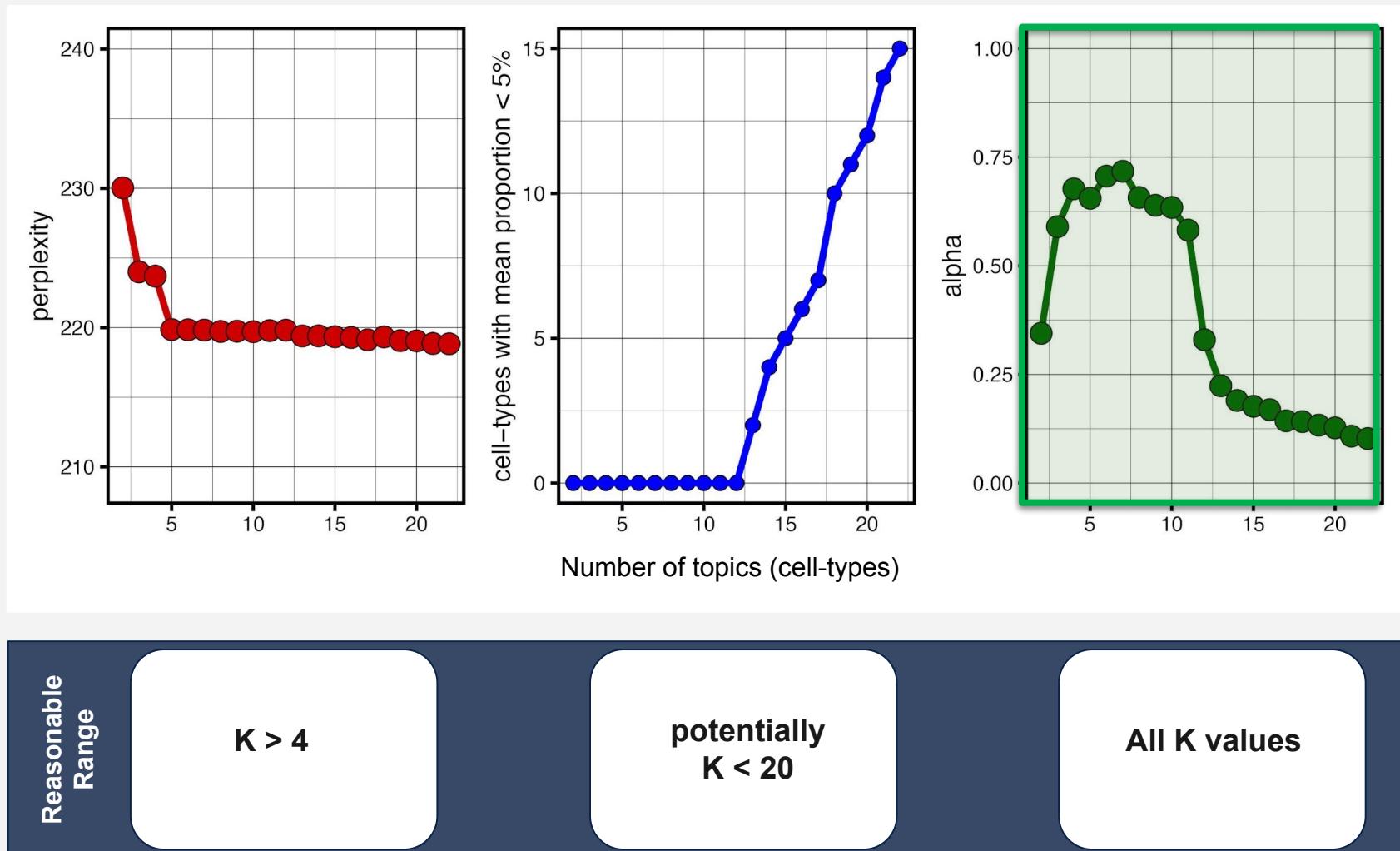
Exploring the Results: Selecting K



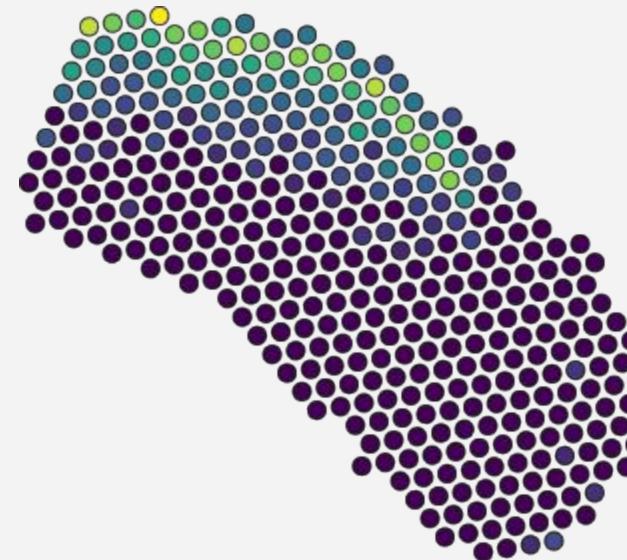
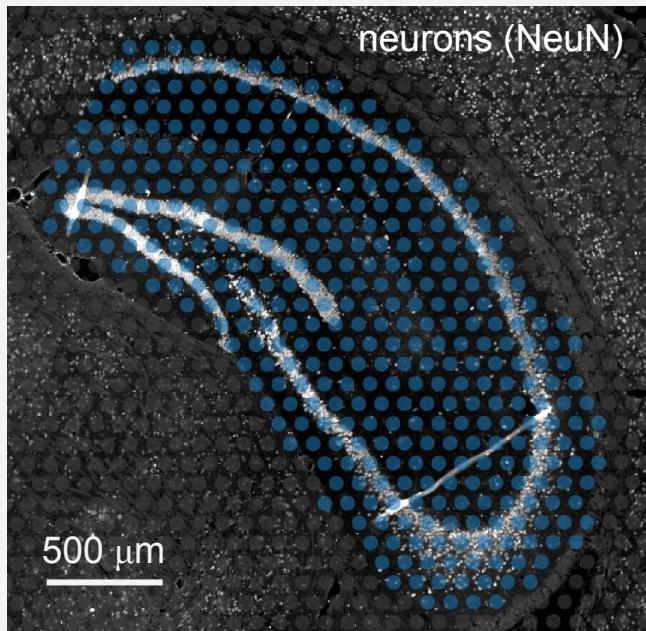
Exploring the Results: Selecting K



Exploring the Results: Selecting K



Exploring the Results: Topic 12



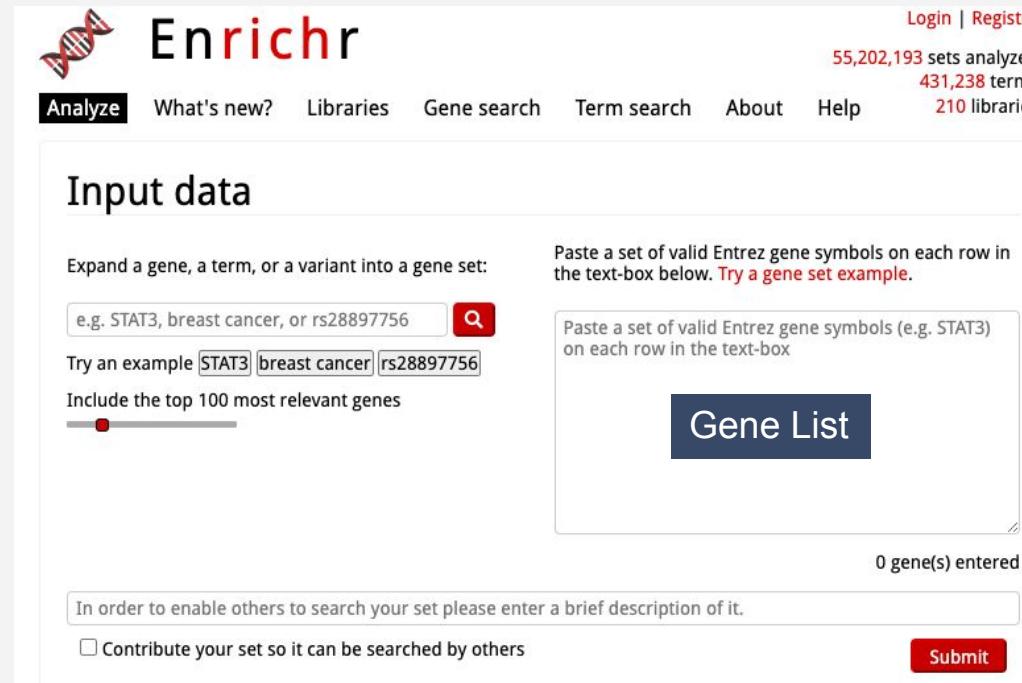
Gene	log2 FC
Fibcd1	6.1
Man1a	3.8
Ccn3	3.6
Kcnh7	3.5

Results: CA1 pyramidal neurons

See our Analysis Guides to learn more about cell-type annotation ([“Web resources for cell type annotation”](#)).

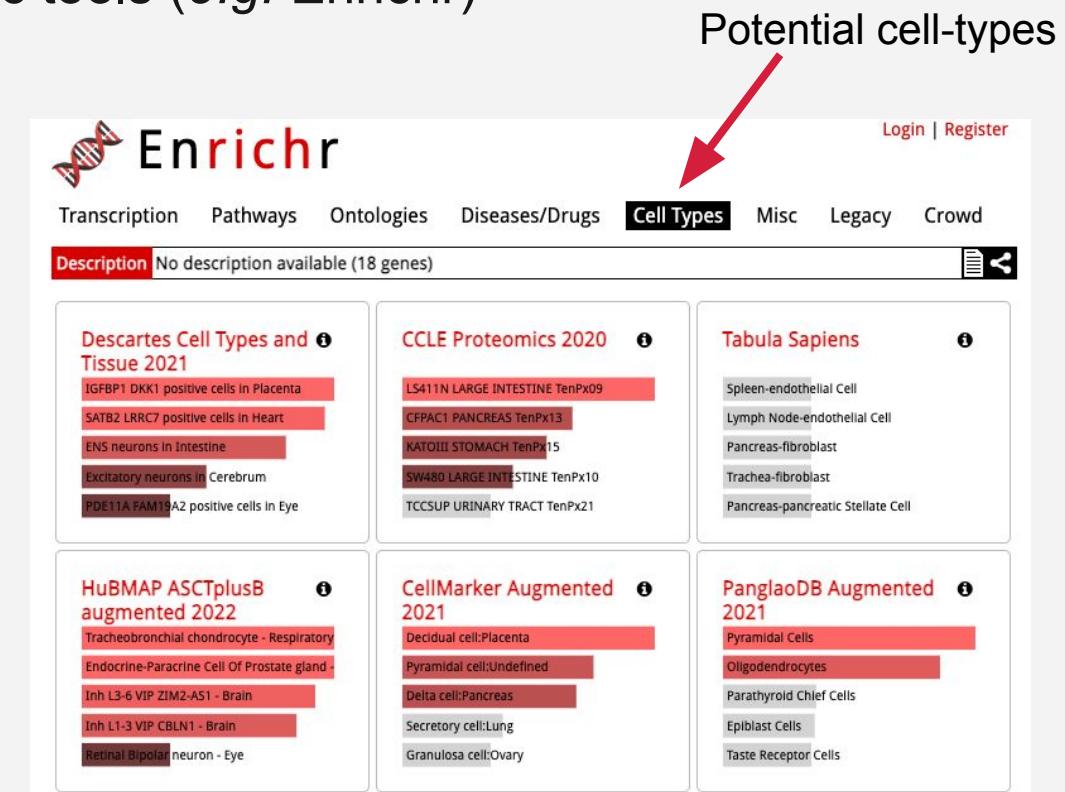
Participant Challenge: Identify the Topics

Resources: Neighbor, Histology, Google, bioinformatic tools (e.g. Enrichr)

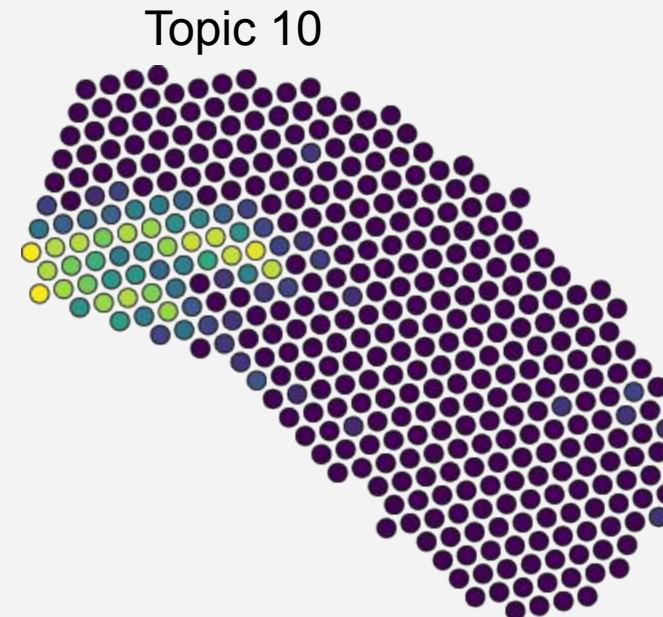
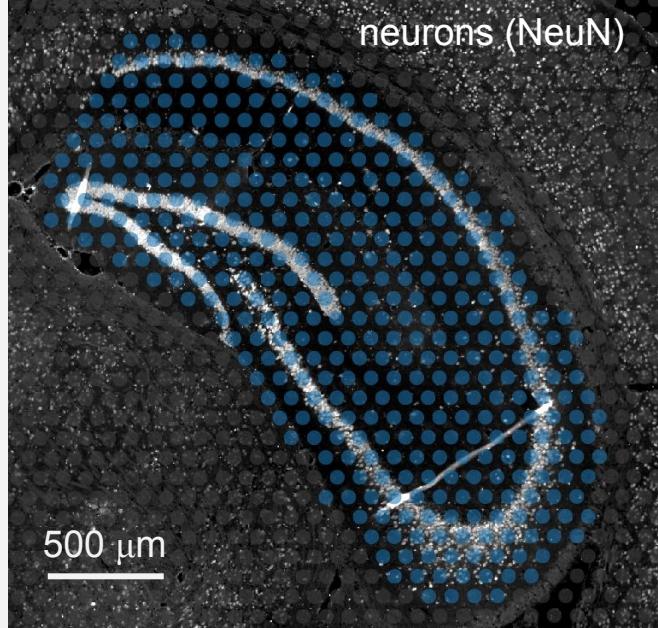


The screenshot shows the Enrichr homepage. At the top, there's a navigation bar with links for Analyze, What's new?, Libraries, Gene search, Term search, About, Help, Login | Register, and statistics: 55,202,193 sets analyzed, 431,238 terms, and 210 libraries. Below the navigation is a section titled "Input data". It contains two text boxes: one for entering gene symbols separated by commas or spaces, and another for pasting a gene set example. A "Gene List" button is located below these boxes. A progress bar indicates "Include the top 100 most relevant genes". At the bottom, there's a description field, a "Submit" button, and a checkbox for contributing the set.

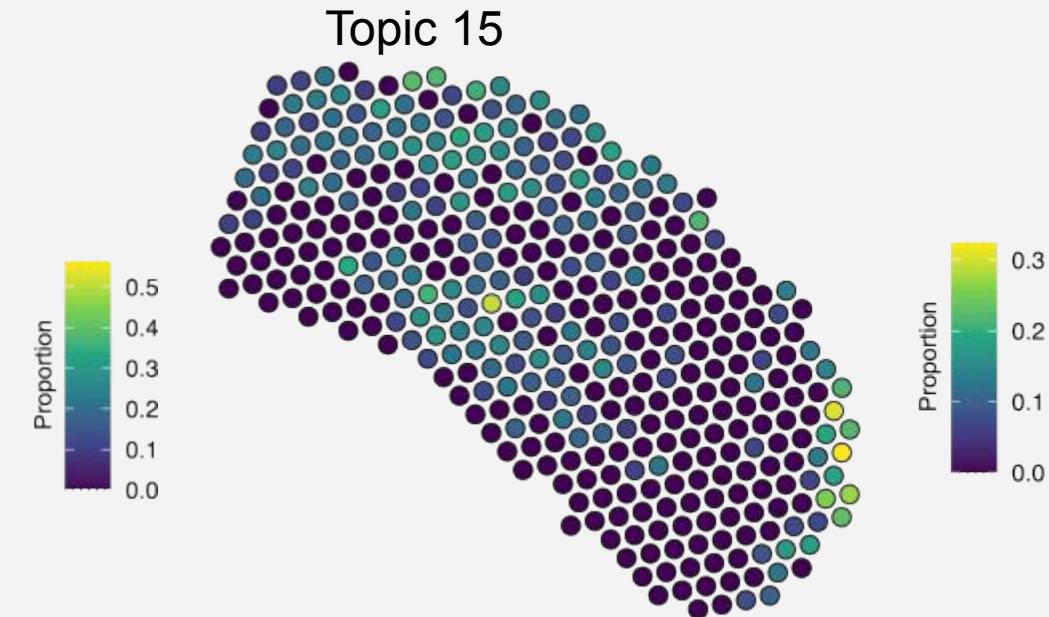
(<https://maayanlab.cloud/Enrichr/>)



Participant Challenge

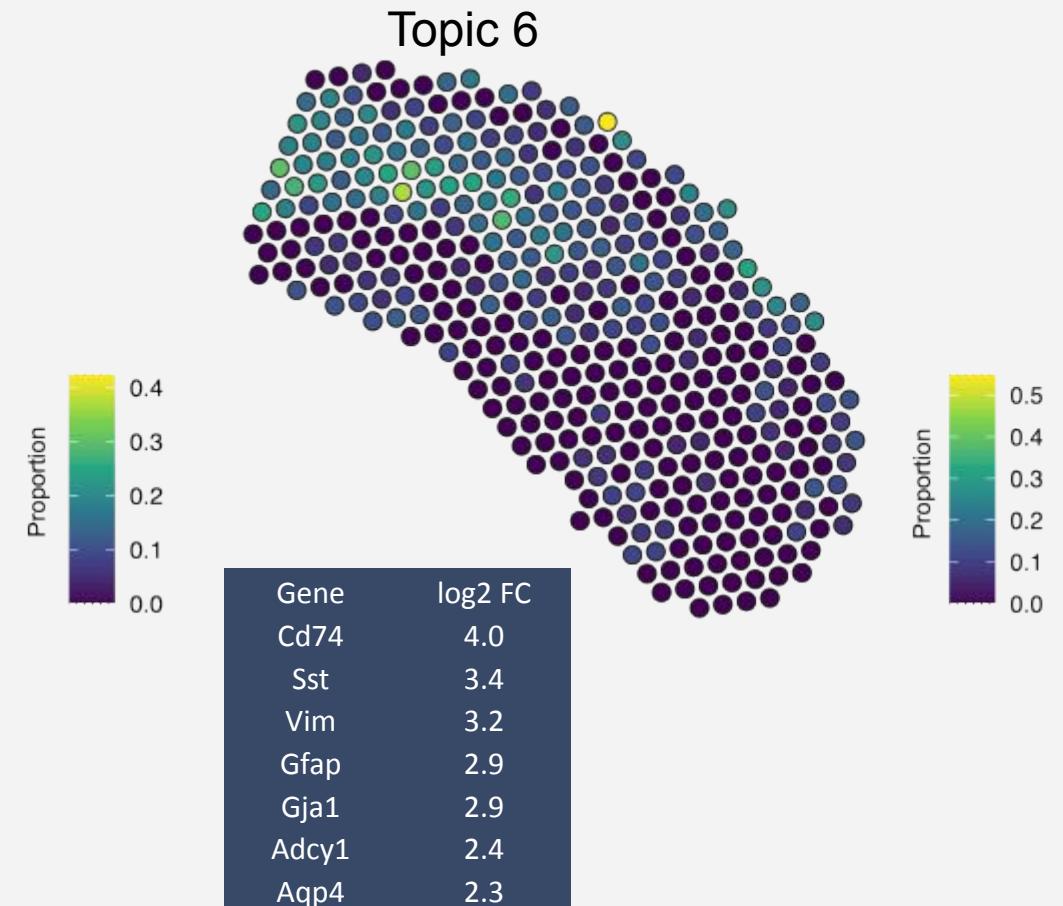
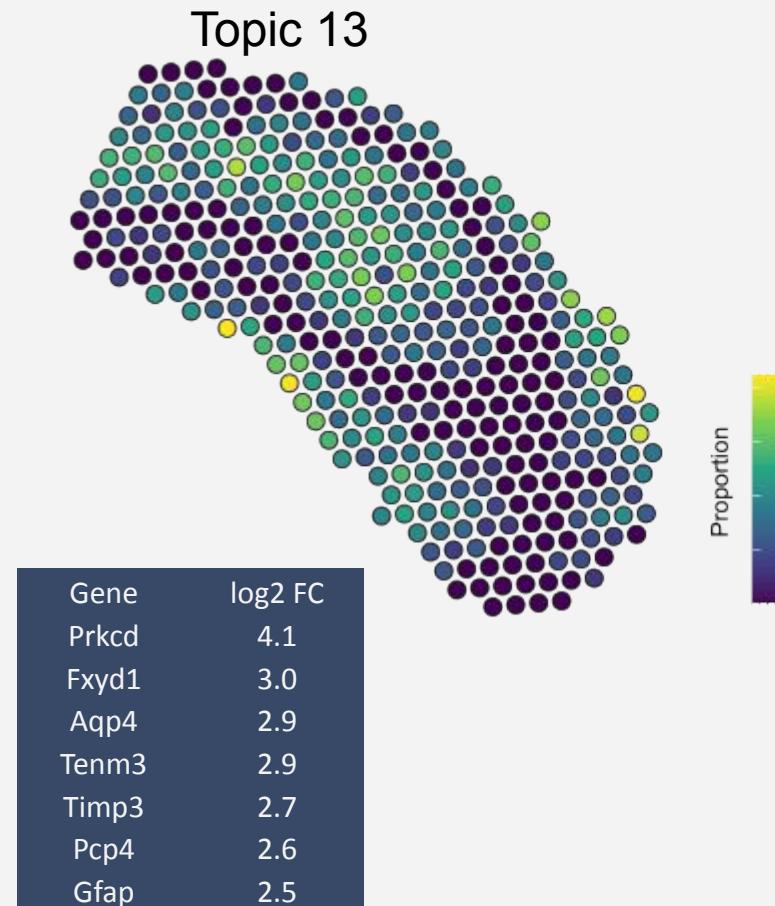
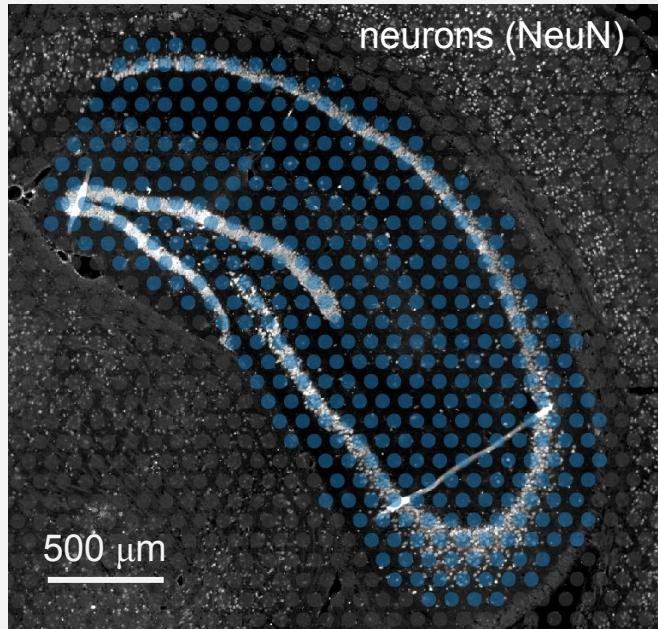


Gene	log2 FC
C1ql2	4.0
Prox1	3.5
Sema5a	3.1
Glis3	3.1
Lrrtm4	3.0
Rasl10a	2.8
Smoc2	2.7



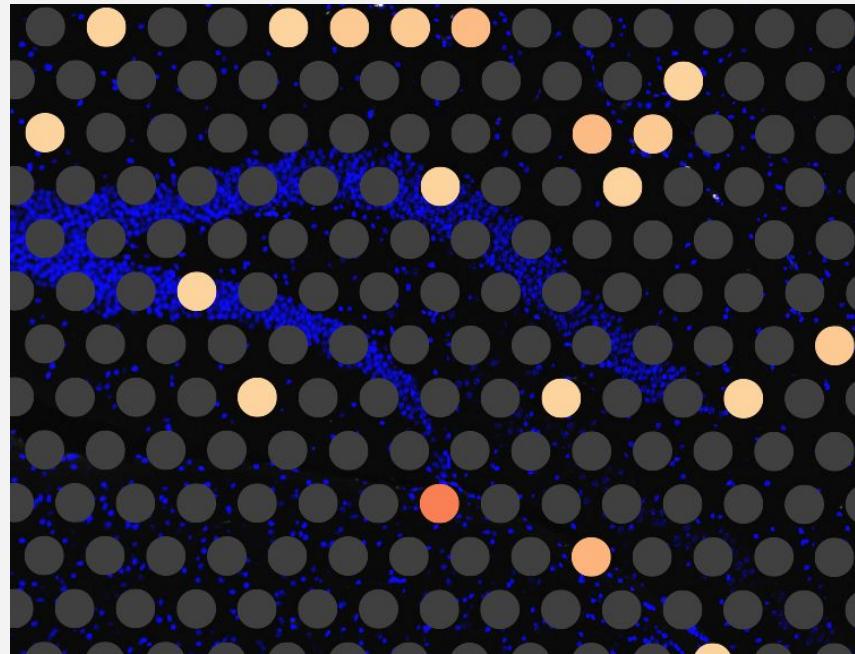
Gene	log2 FC
Camkk1	2.0
Sst	2.0
Diras2	1.9
Cacna2d1	1.9
Syn1	1.8
Atp6v1c1	1.8
Cck	1.8

Exploring the Results Part 2

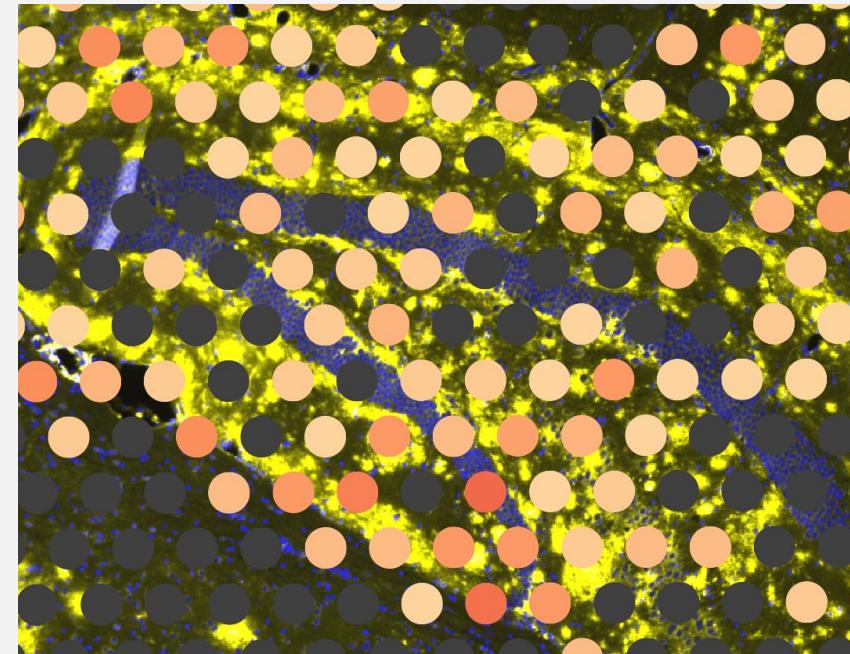


Results: Astrocytes (Topic 13) and Reactive Astrocytes (Topic 6)

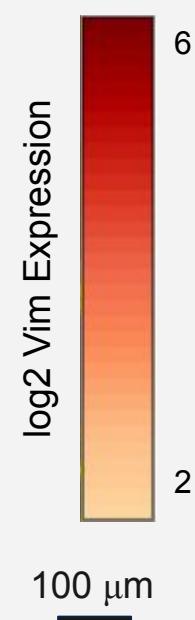
Comparison: Transgenic to Wild Type Mice



13.2-month-old
Mouse Brain



17.9-month-old
TgCRND8 Mouse Brain



Blue is dapi nuclei and Yellow is beta-amyloid aggregates

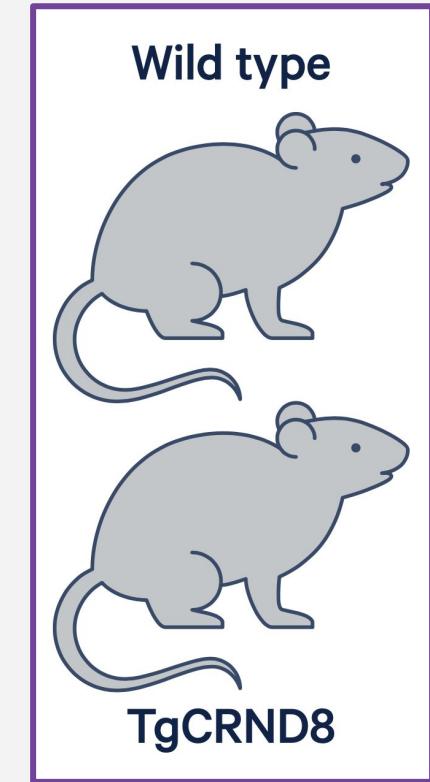
Paired scRNAseq data

Recall

- **Design:** wild type and transgenic mice brains were collected (N=2 for each condition)
- **Paired data collected:** single cell ATAC, single cell RNAseq Gene expression, and Visium Spatial Gene Expression.

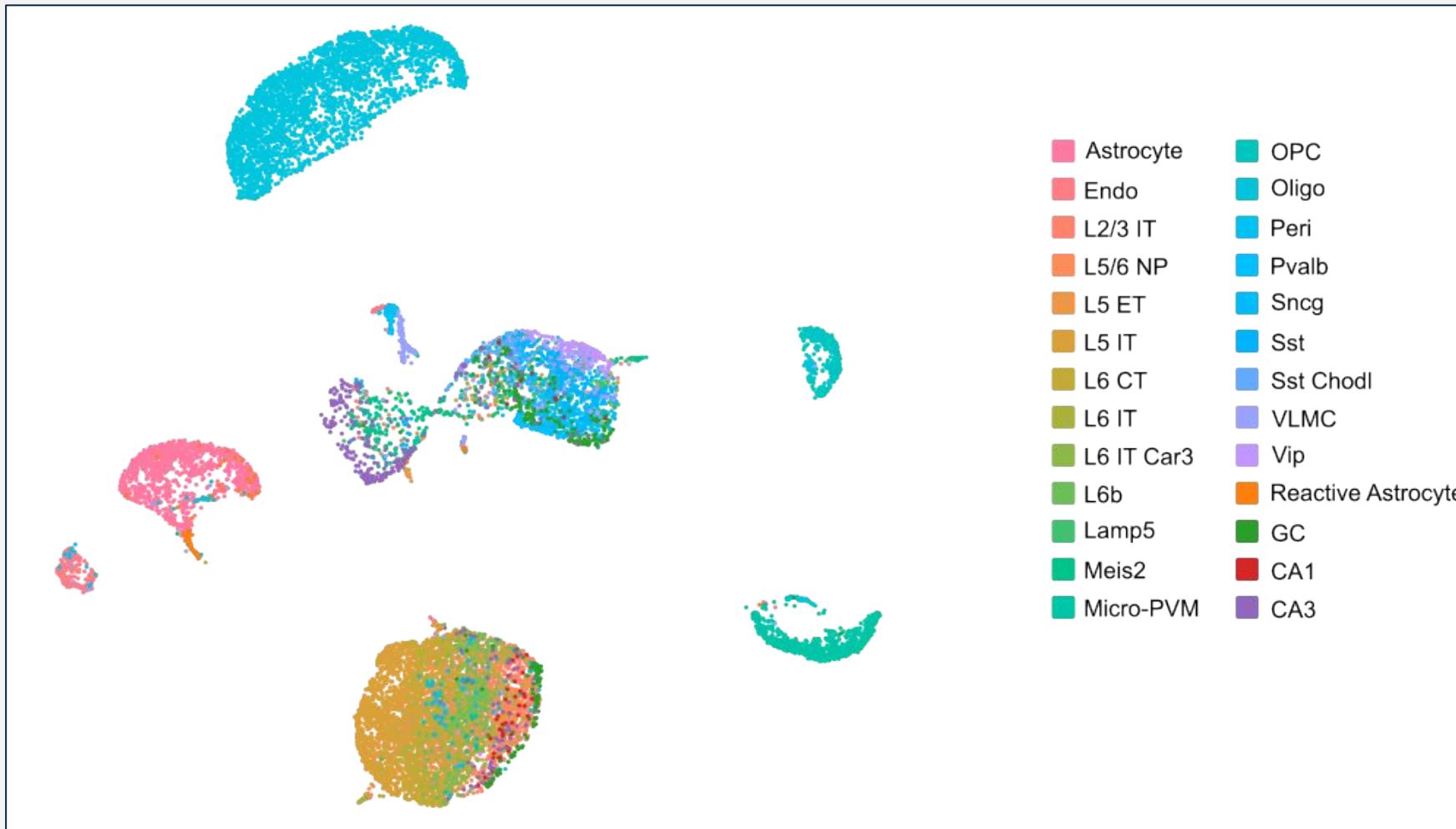
Single cell RNAseq data added benefits

- Single cell resolution
- Gene expression information
- Potential for more analyses ...



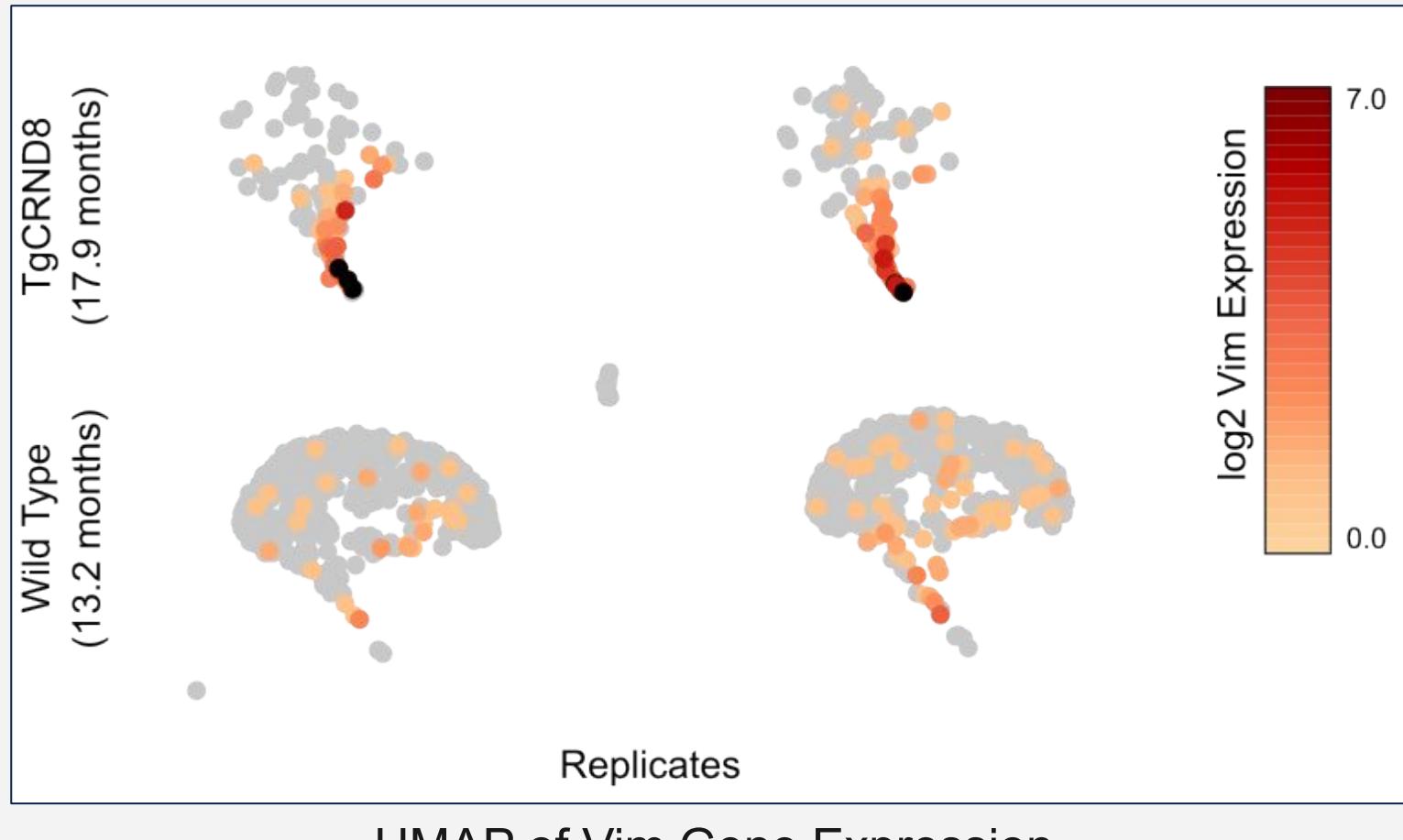
See our Application Note for more information ([“Single cell and spatial multiomics identifies Alzheimer’s disease markers”](#))

Paired scRNAseq data



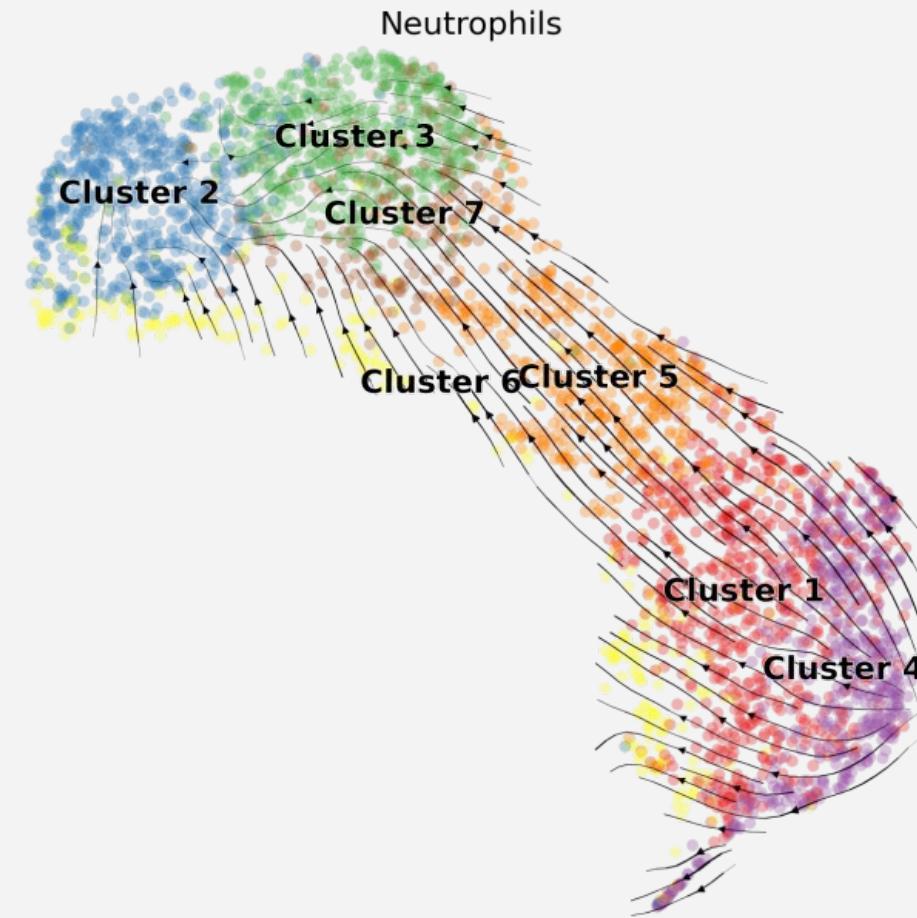
UMAP of Wildtype (13.2 months) and TgCRND8 (17.9 months) (N=2 per condition) clustering

Paired scRNAseq data



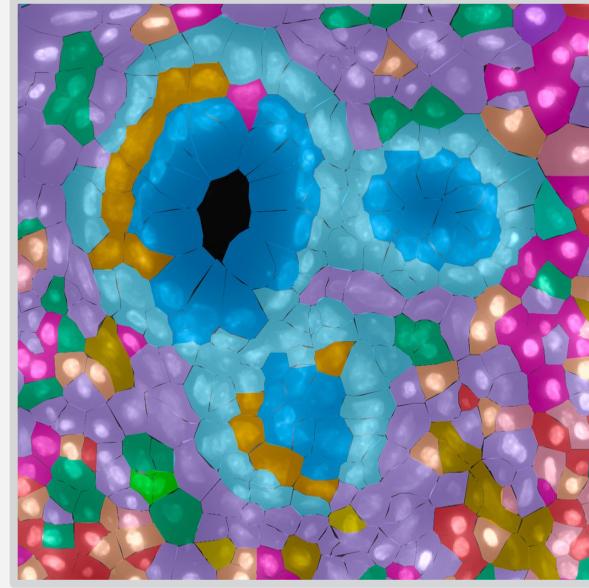
Paired scRNASeq data: Mechanistic Studies

- **Pathway Analysis**
- **Trajectory Analysis**



- See our Analysis Guide to learn more about trajectory analysis (["Trajectory Analysis using 10x Genomics Single Cell Gene Expression Data"](#))

What is Next: Xenium In Situ



- Xenium V1 FF Mouse Brain Probe Panel
 - 3 out of 46 astrocyte markers used in deconvolution are in the probe panel (Gfap, Aqp4, and Cd44)
- Custom add on panel to capture reactive astrocytes (e.g. Vim, S100a10, Ctnnb1 ...)

Summary

- Discussed spot cell-type decomposition
- Introduced the concept of reference and reference-free deconvolution
- Demonstrated reference-free deconvolution
- Assigned cell-types to topics
- Discussed expanding the analysis using:
 - scRNAseq
 - 10x Xenium Insitu

Thank you

10xgenomics.com

Resources:

Application Notes

- “[Single cell and spatial multiomics identifies Alzheimer’s disease markers](#)”

Analysis Guides

- “[Publication Highlight: Benchmarking Methods to Integrate Spatial and Single-cell Transcriptomics Data](#)”
- “[Integrating Single Cell and Visium Spatial Gene Expression Data](#)”
- “[Integrating 10x Visium and Chromium data with R](#)”
- “[Trajectory Analysis using 10x Genomics Single Cell Gene Expression Data](#)”
- “[Web resources for cell type annotation](#)”

Google Colab

- https://colab.research.google.com/github/10XGenomics/analysis_guides/blob/main/reference_17p9_rep1_with_strocytes.v2.GitHub.ipynb