



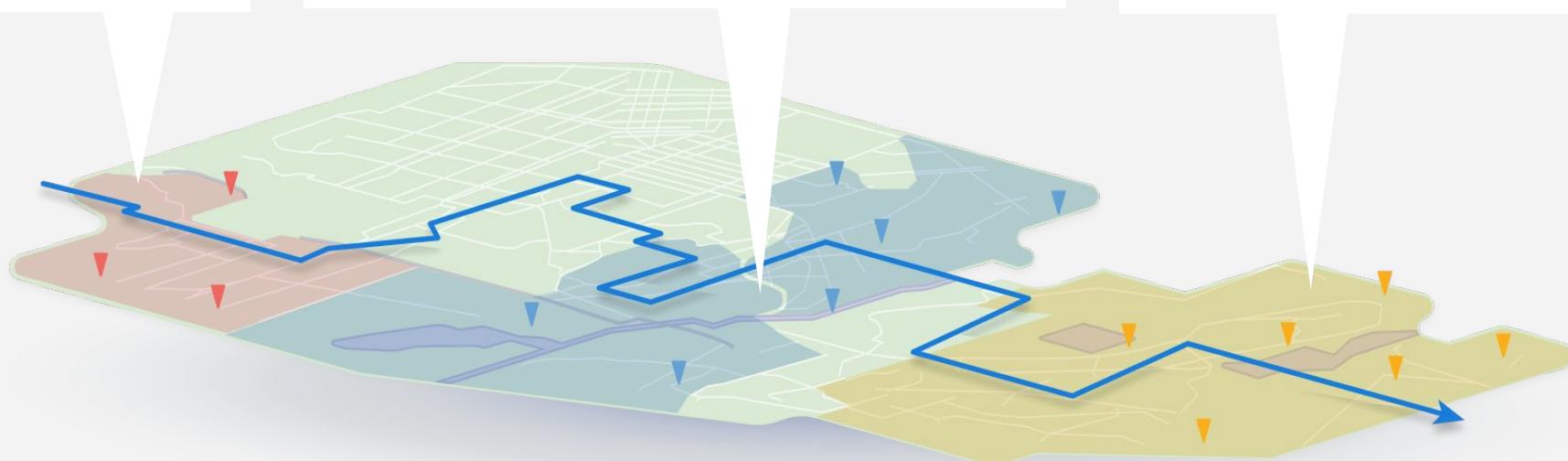
# Exploring Your Visium Data: A Spot Deconvolution Story

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CG000669 Rev A

# Outline

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

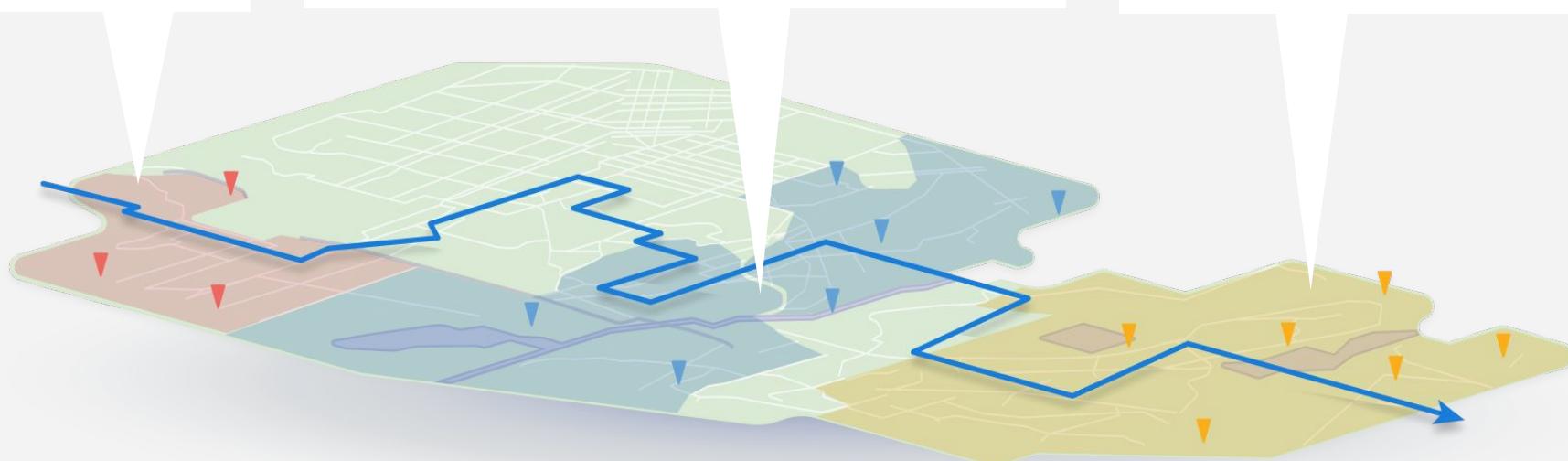


# Experimental Design

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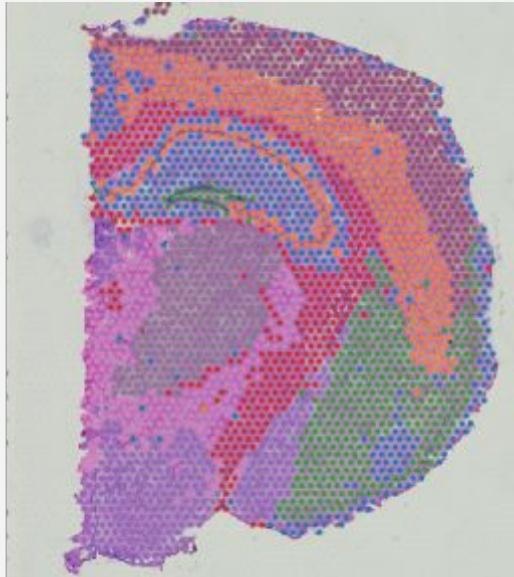
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# Goal

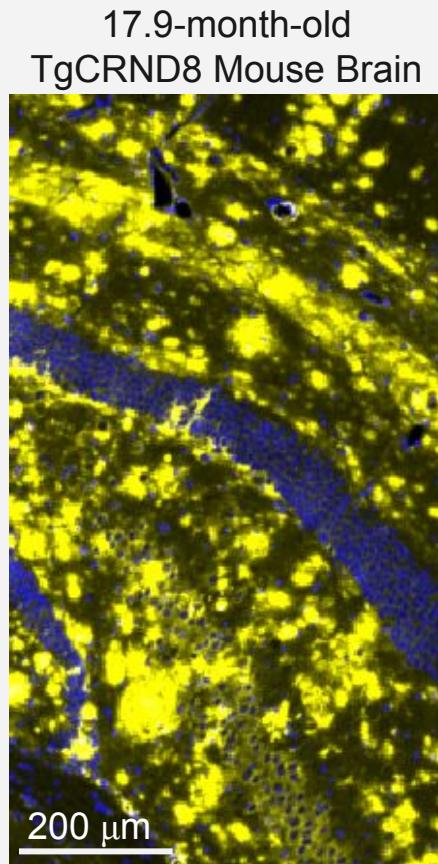
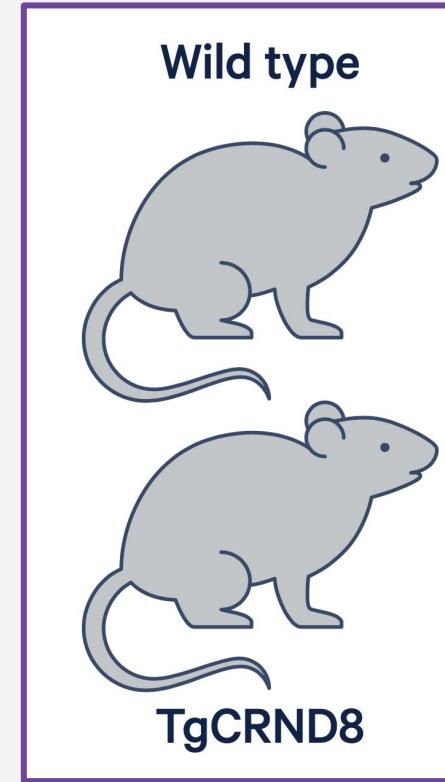
Study hippocampal gene expression in a Alzheimer's Disease model.



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

# Alzheimer's Disease

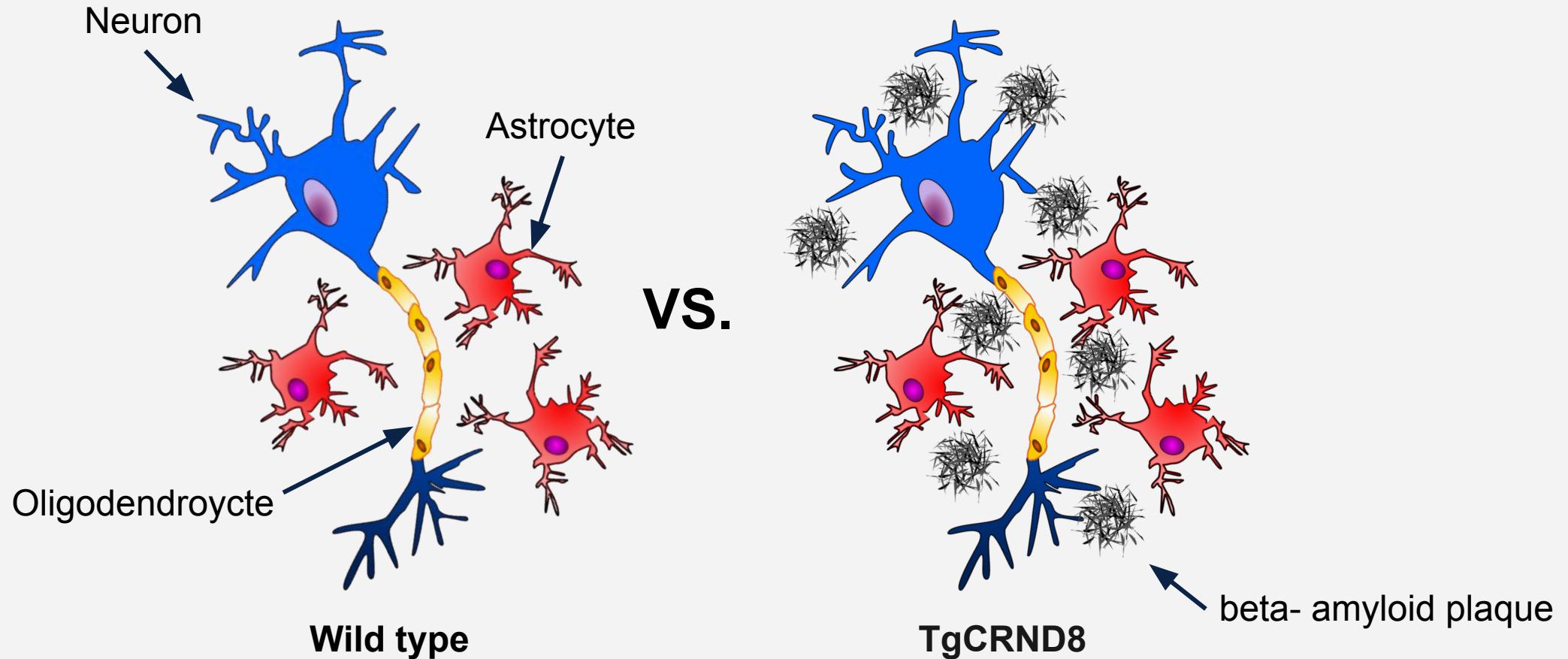
- Alzheimer's Disease is the most common cause of dementia
- Characterized by the accumulation of protein aggregates
- **Why:** disease pathogenesis is still poorly understood
- **Assay:** Visium FFPE
- **Model:** TgCRND8 AD-like mouse



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)

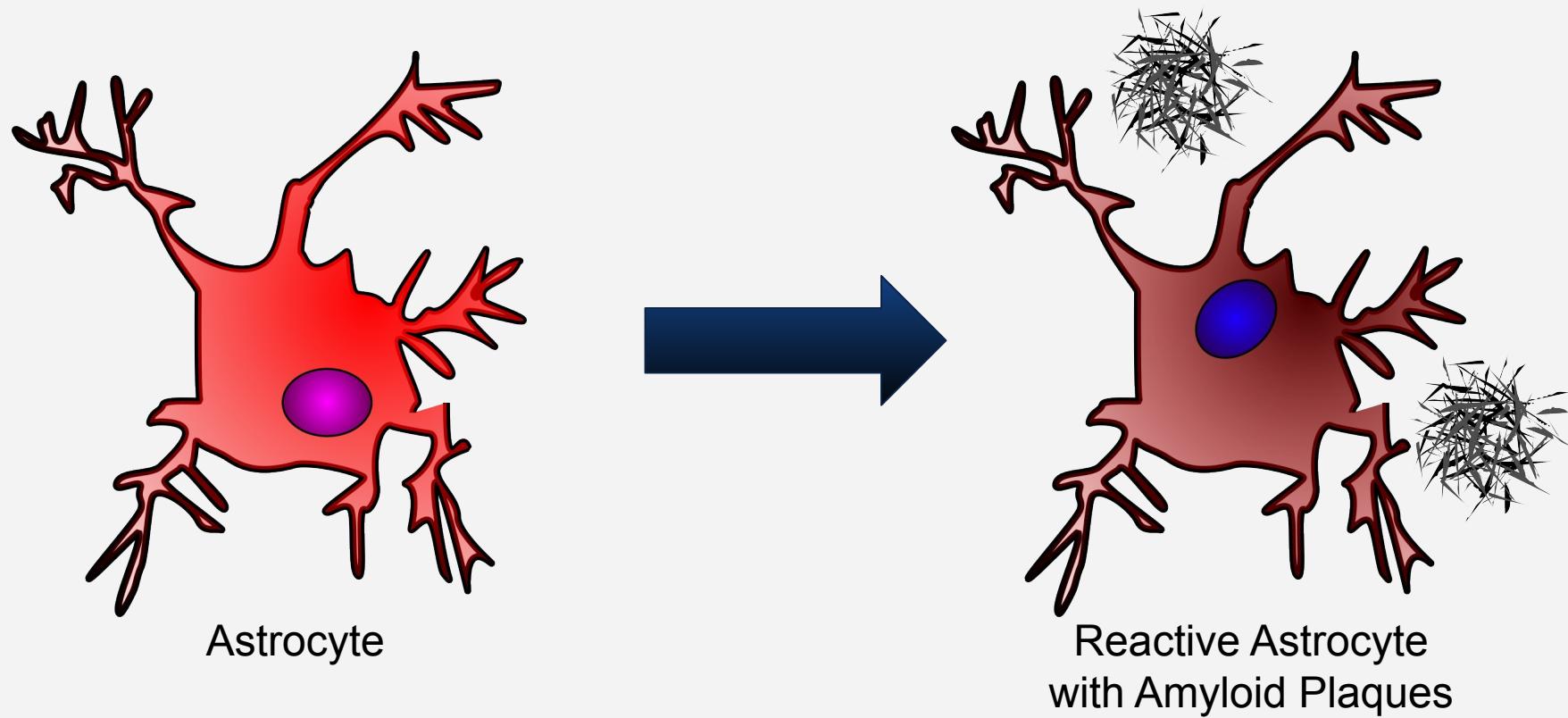
# Question

Do amyloid plaque deposits in the hippocampus alter cell phenotype?



# Hypothesis

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





# Data Analysis

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# Outline

## Experimental Design

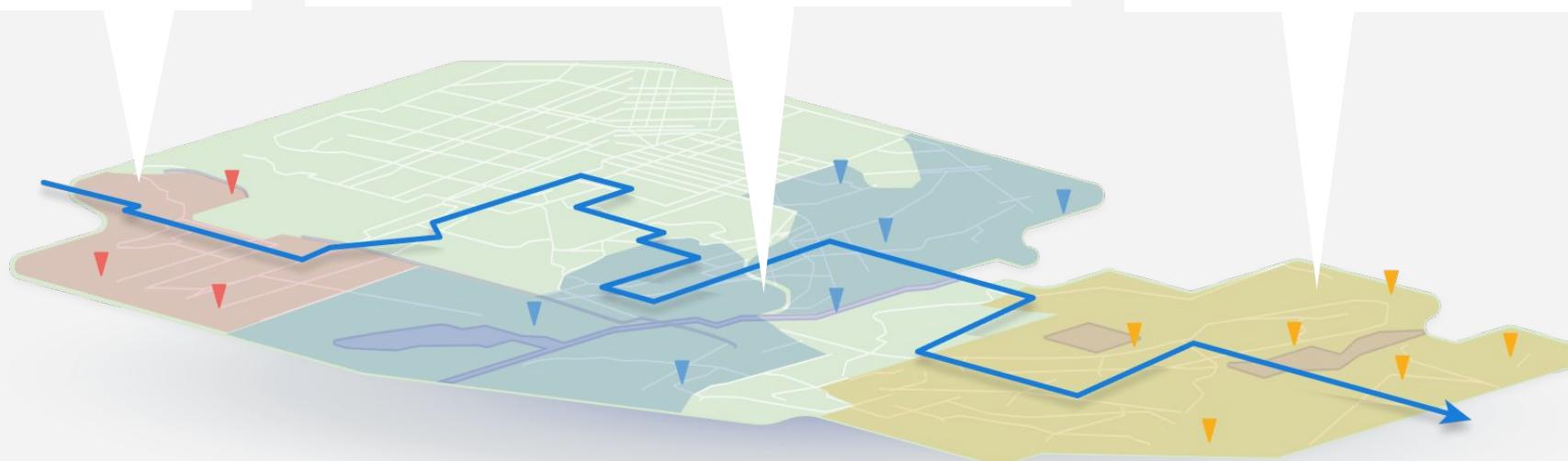
- Goal
- Alzheimer's Disease
- Question
- Hypothesis

## Data Analysis

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

## Data Exploration

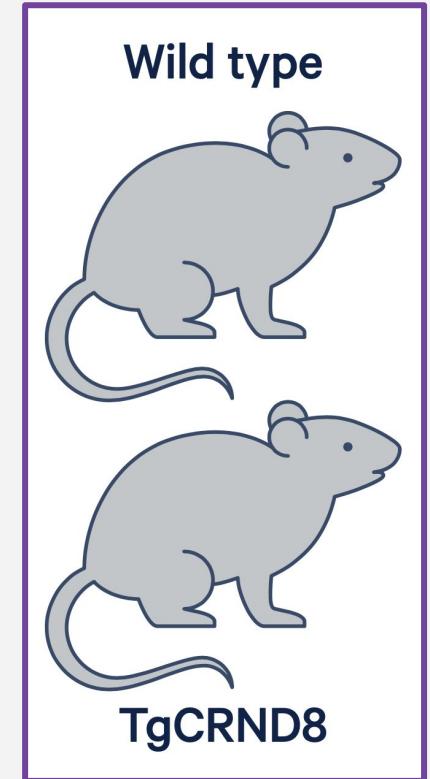
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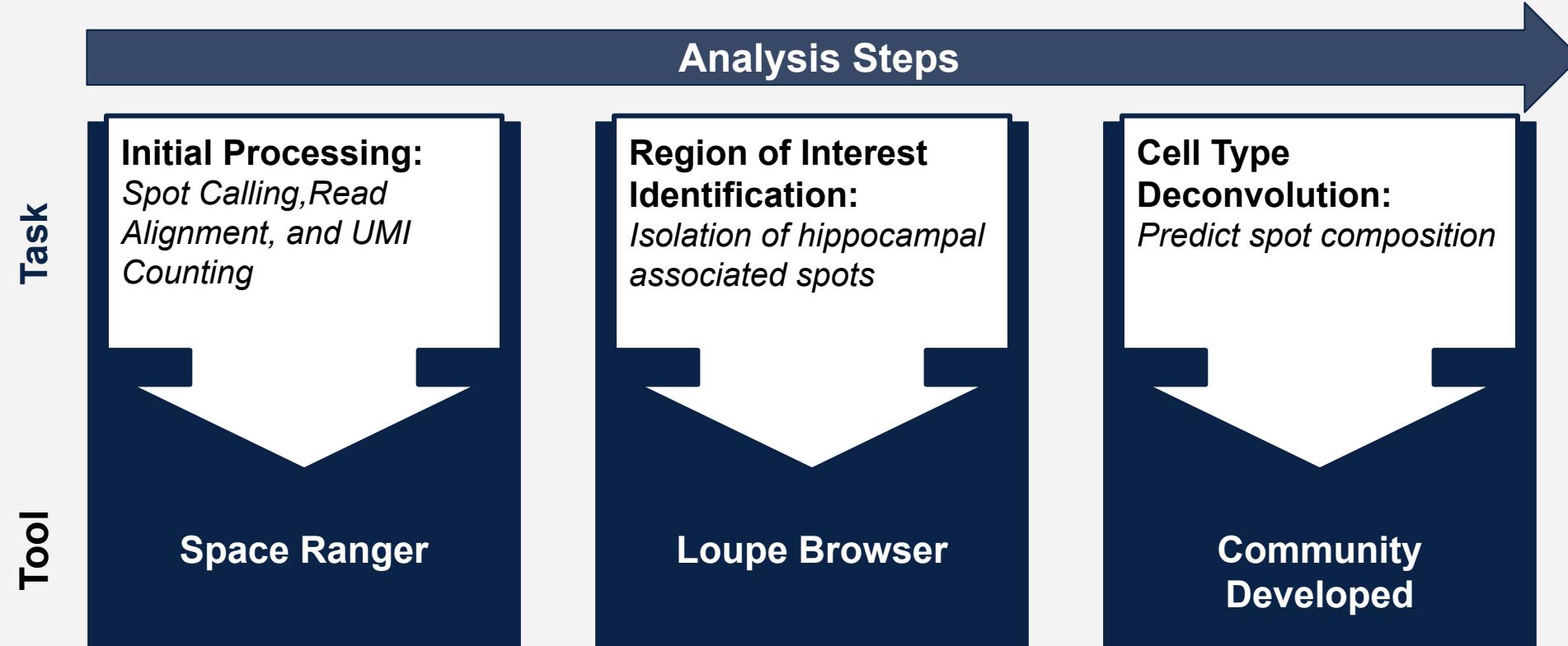
# The Dataset

- **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

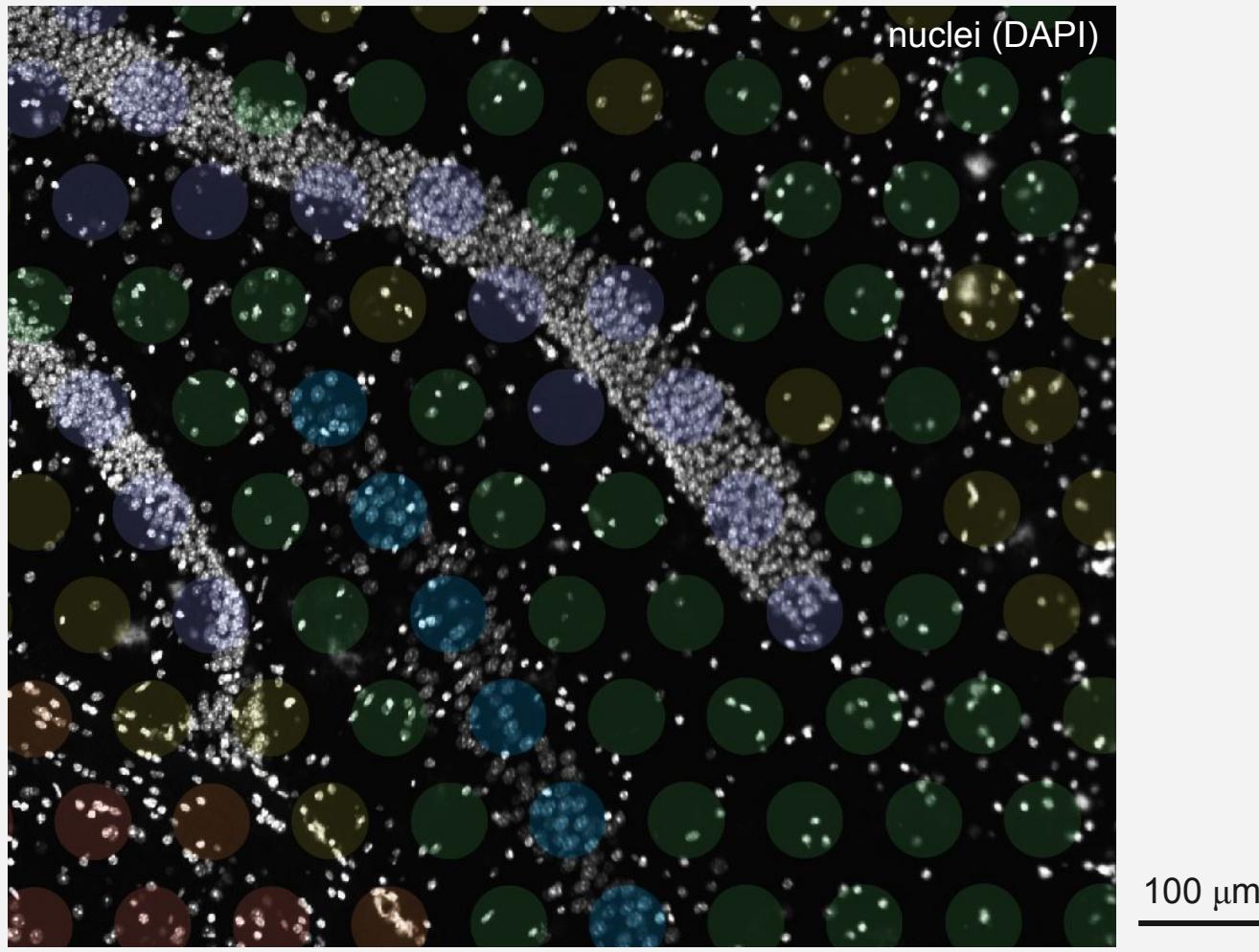
See our Application Note for more information ([“Single cell and spatial multiomics identifies Alzheimer’s disease markers”](#))



# Data Analysis Plan



# Spot Resolution: Multiple Cells per Spot

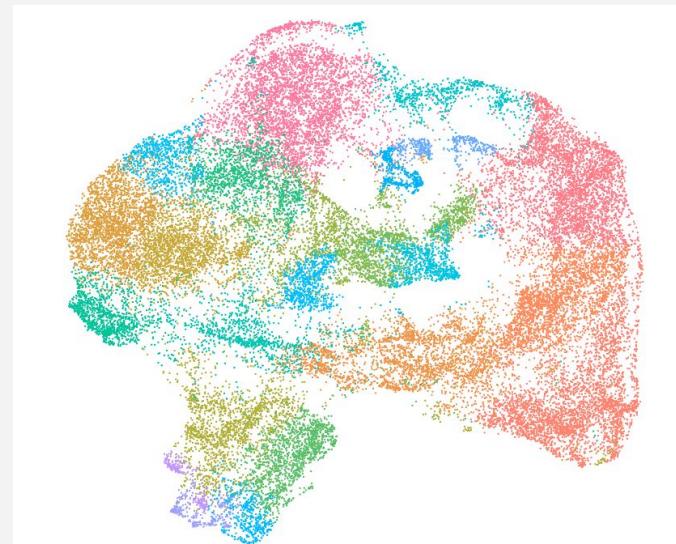


17.9 month old transgenic hippocampus

# 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

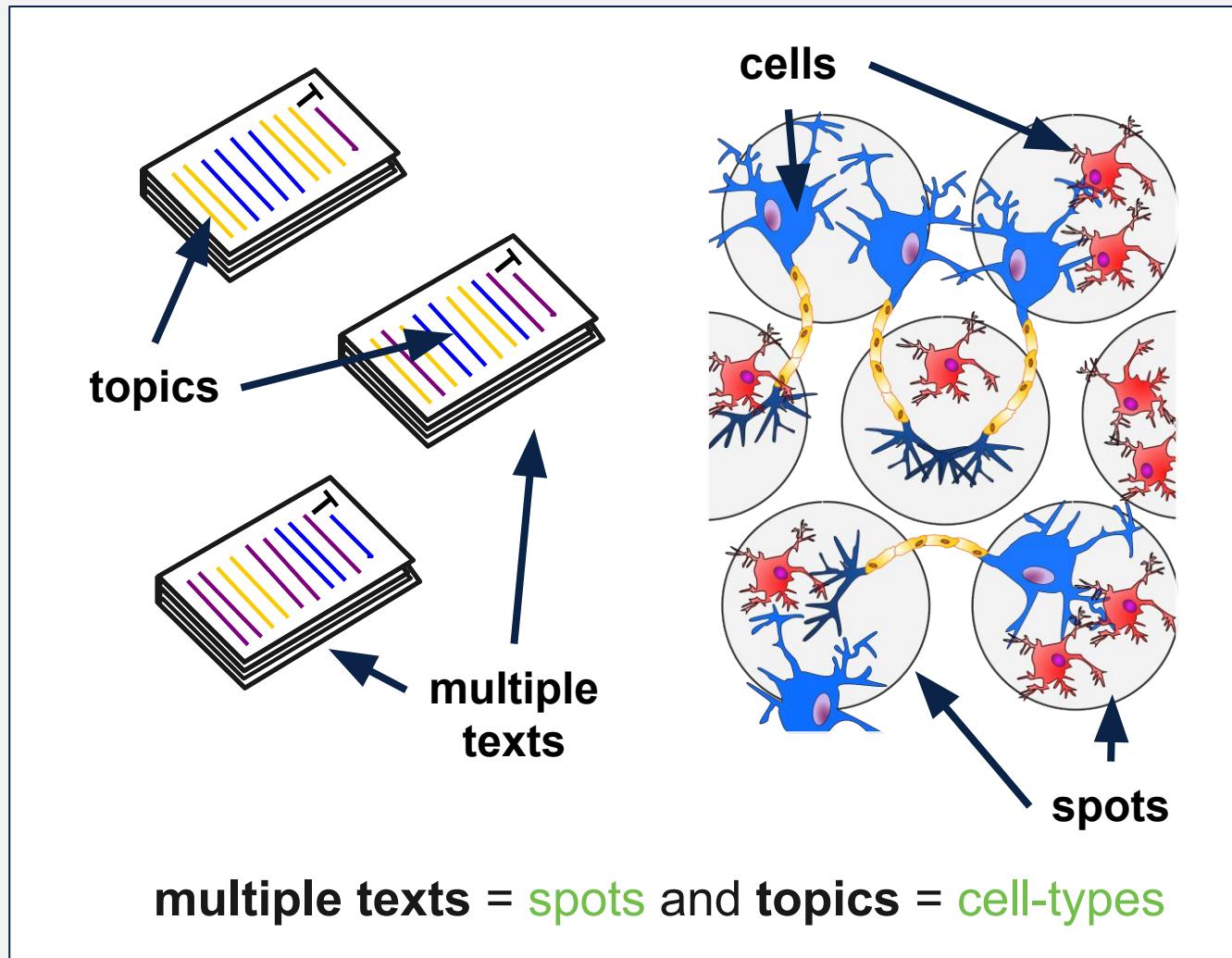


STdeconvolve

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



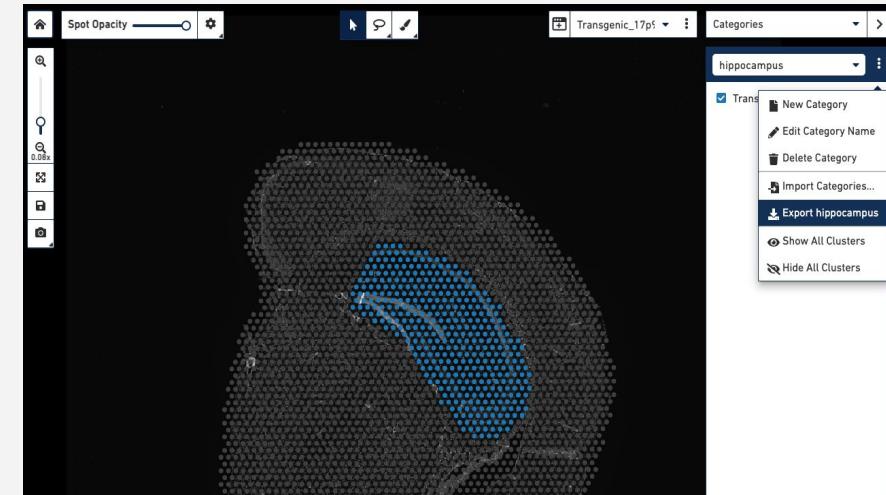
# Analyzing the Data

General Steps Post Space Ranger

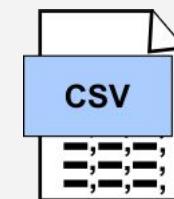
# Analyzing the Data

## 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



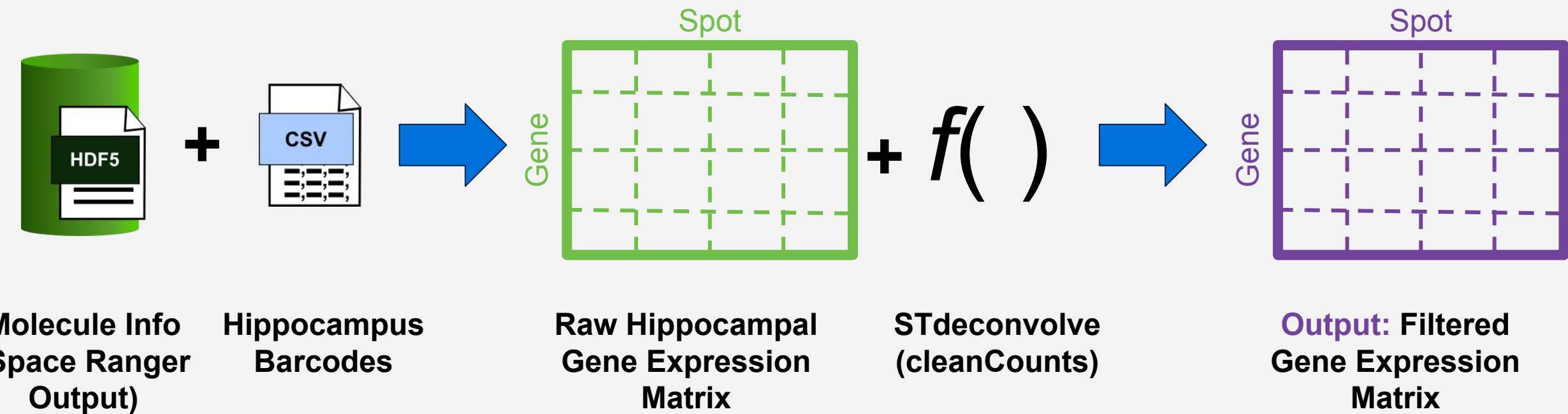
Barcode,hippocampus  
AAACGAGACGGTTGAT-1,Trans\_17p9\_rep1  
AAACTGCTGGCTCAA-1,Trans\_17p9\_rep1  
AAAGGCTACGGACCAT-1,Trans\_17p9\_rep1  
AAAGGCTCTCGCGCCG-1,Trans\_17p9\_rep1  
...

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)
- ...

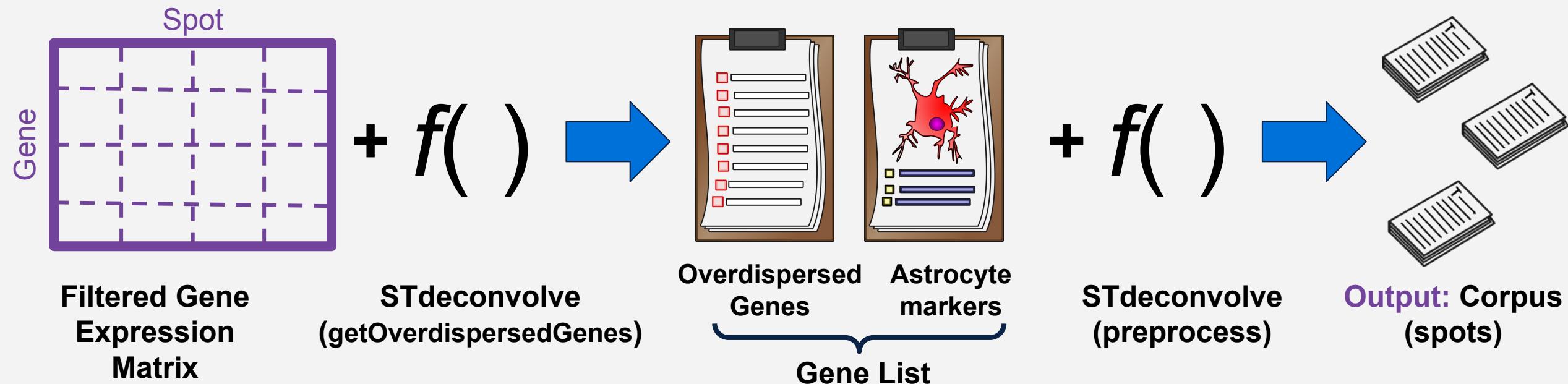
## Hybrid

- Combination of experimentally determined and canonical cell-type markers

# Analyzing the Data

## General Steps Post Space Ranger

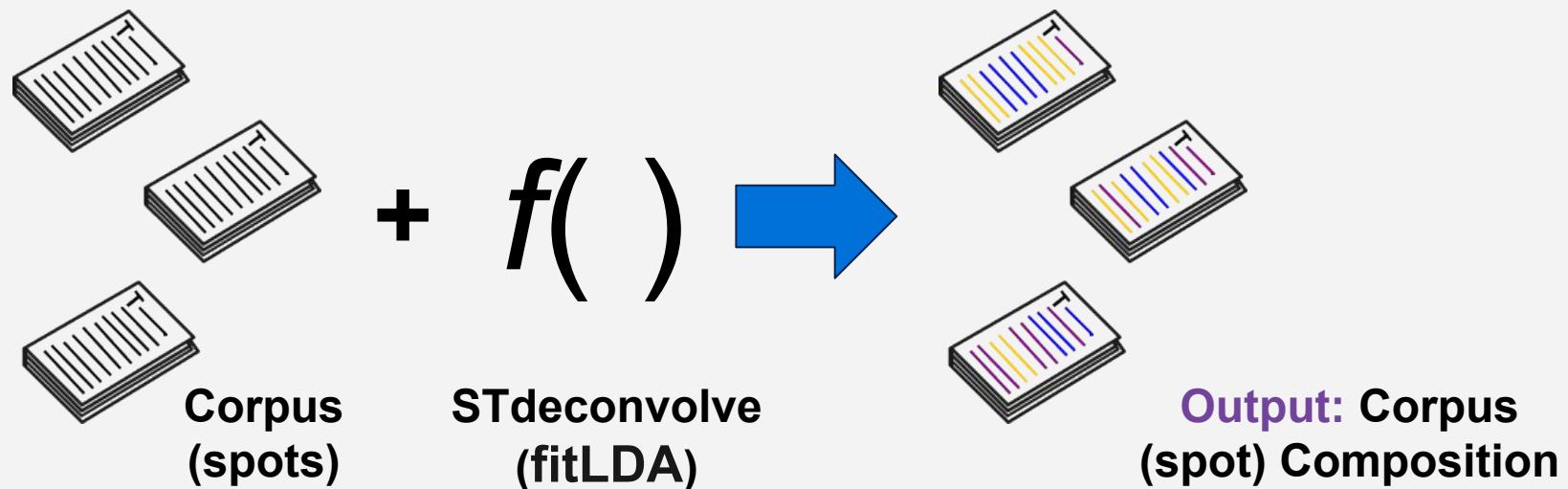
1. (optional) Identify regions of interest
2. Filter gene expression matrix
3. Generate a corpus (spots) using the gene list
  - Reason: We need to identify genes that can define topics (cell-types)



# 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

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# Outline

## Experimental Design

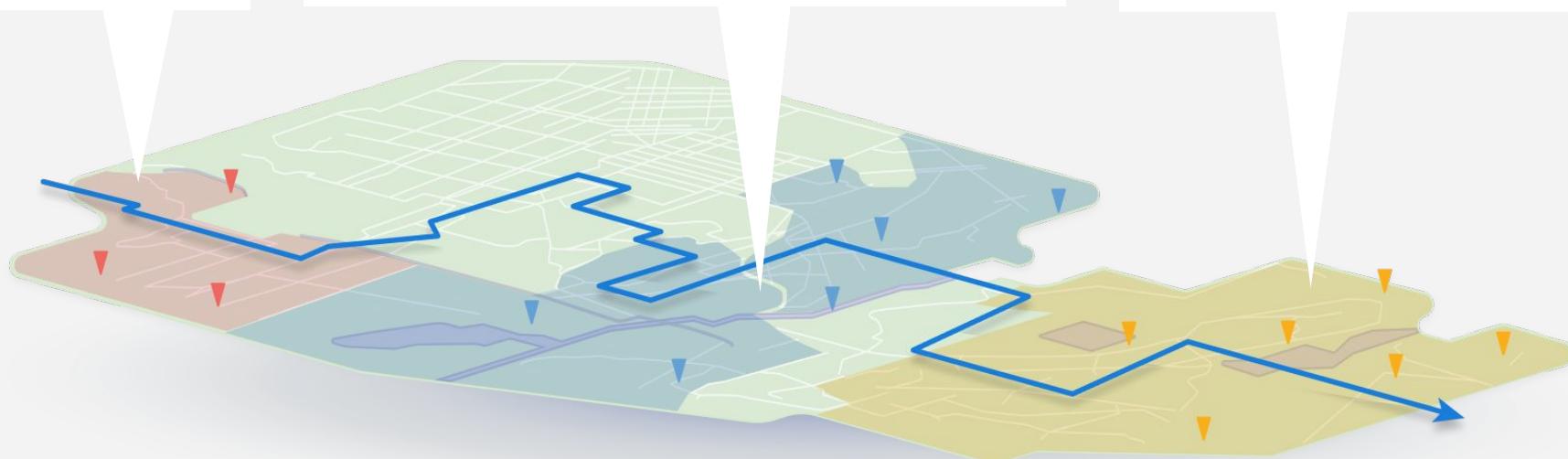
- Goal
- Alzheimer's Disease
- Question
- Hypothesis

## Data Analysis

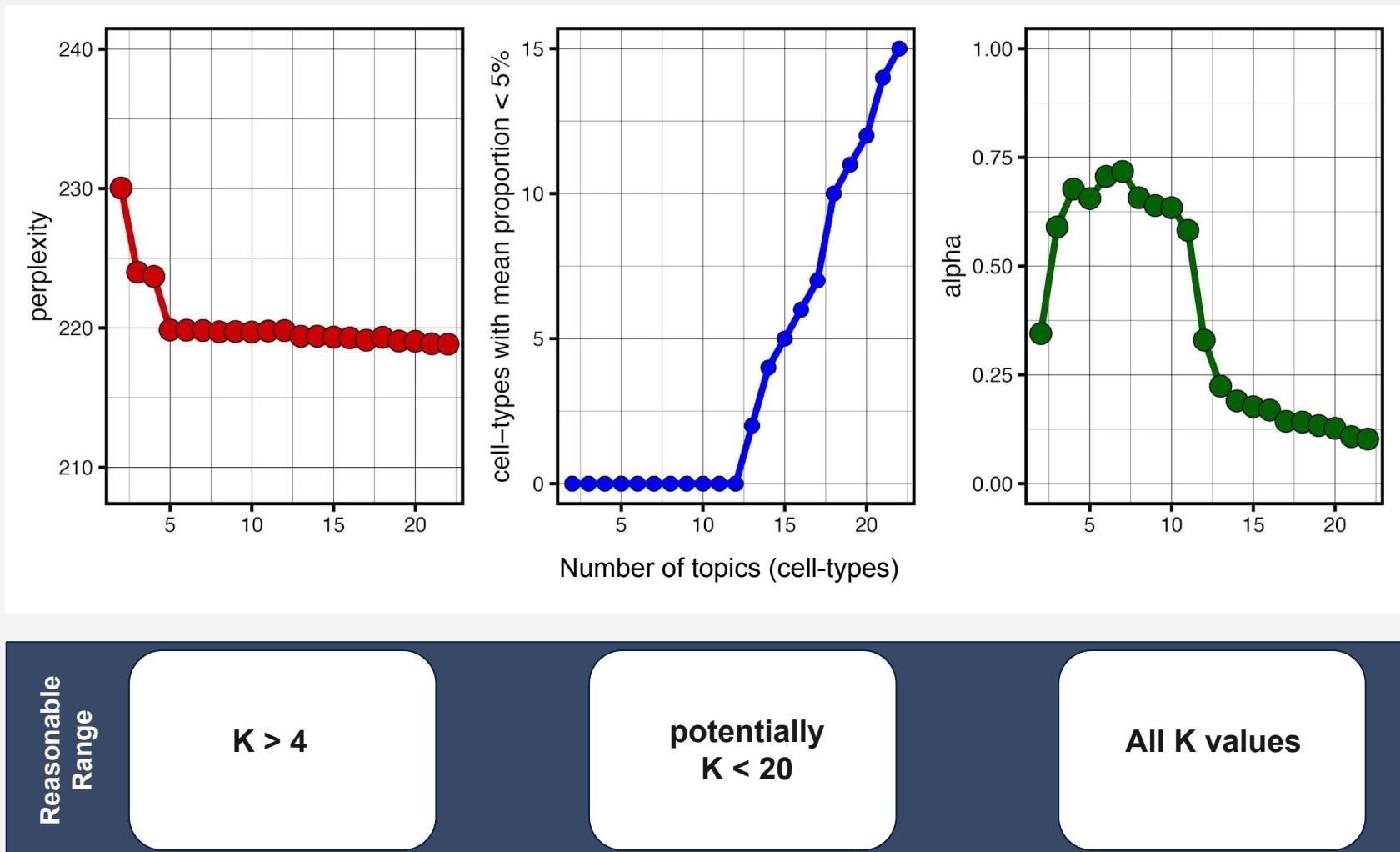
- The Dataset
- Data Analysis Plan
- Spot Resolution
- Spot Cell-type Deconvolution
- Analyzing the Data

## Data Exploration

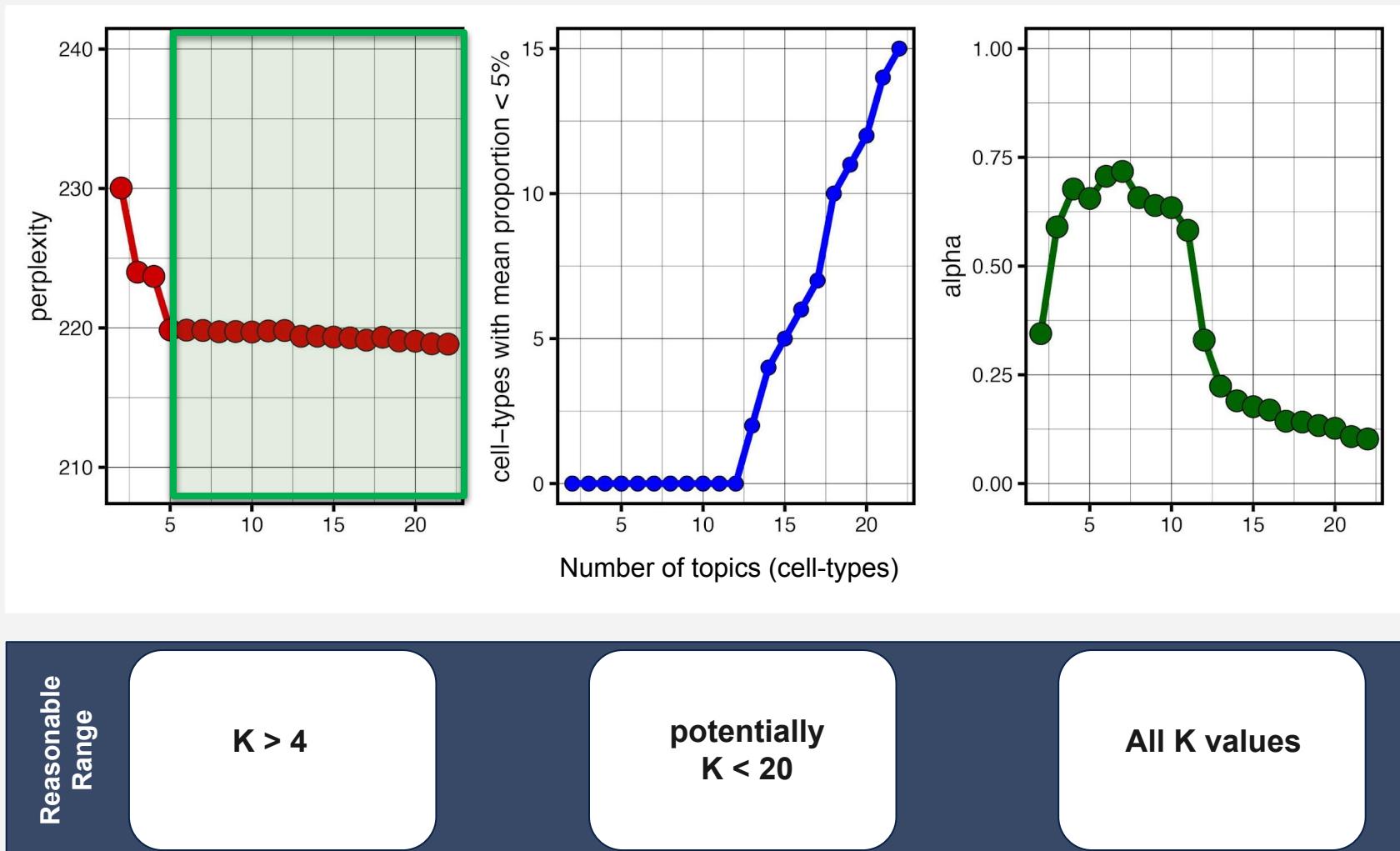
- Exploring the Results
- Participant Challenge
- Exploring the Results Part 2
- Paired scRNASeq data
- What is Next



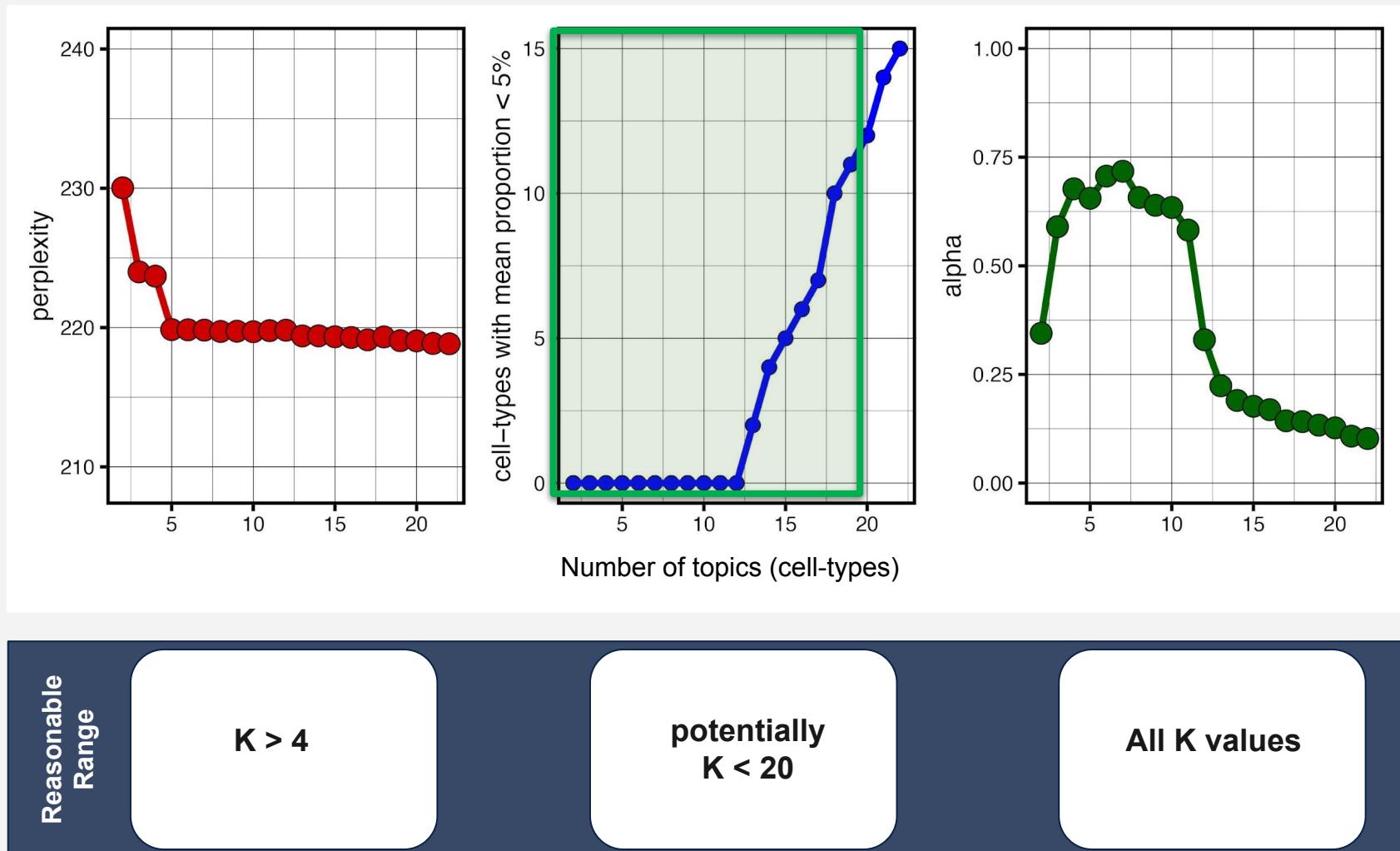
# Exploring the Results: Selecting K



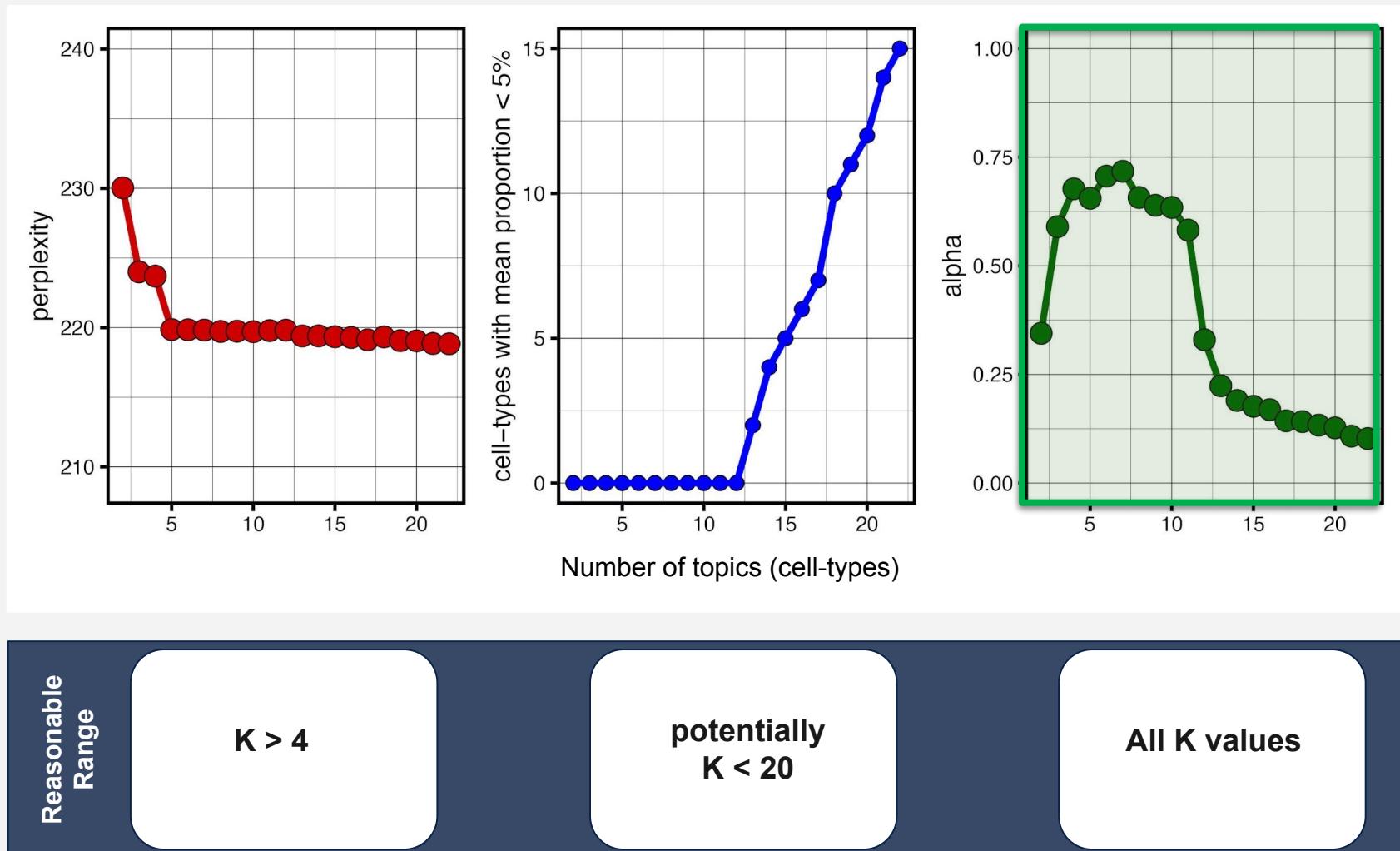
# Exploring the Results: Selecting K



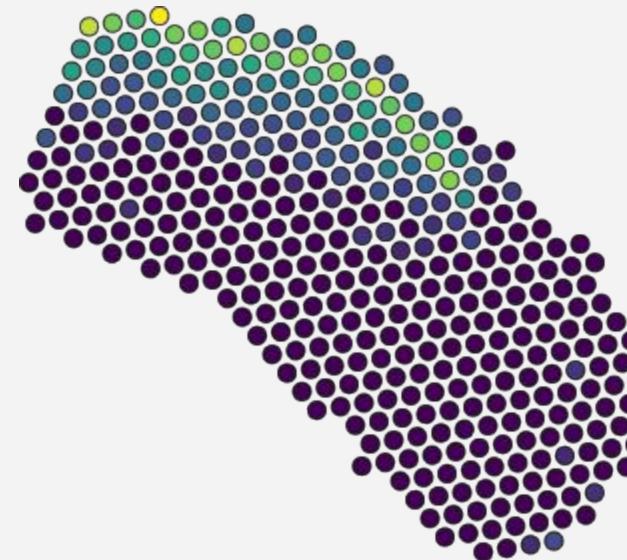
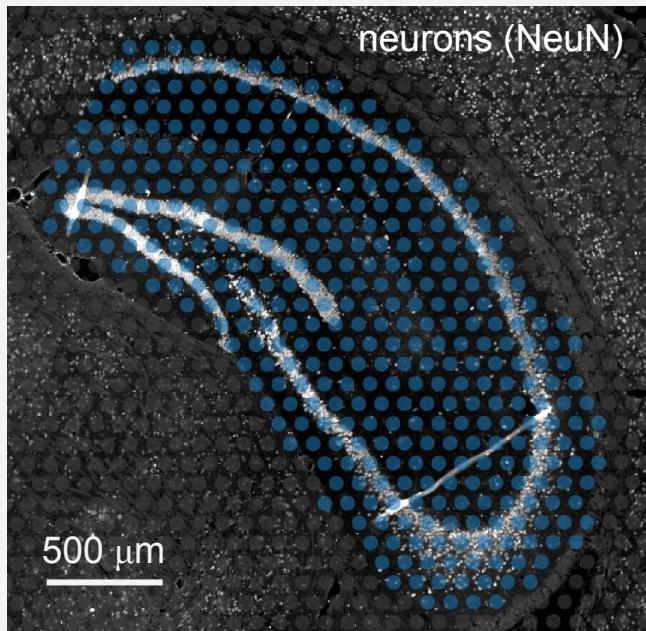
# Exploring the Results: Selecting K



# 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)

 **Enrichr**

Login | Register  
55,202,193 sets analyzed  
431,238 terms  
210 libraries

Analyze What's new? Libraries Gene search Term search About Help

### Input data

Expand a gene, a term, or a variant into a gene set:  
e.g. STAT3, breast cancer, or rs28897756  

Paste a set of valid Entrez gene symbols on each row in the text-box below. Try a gene set example.  
Paste a set of valid Entrez gene symbols (e.g. STAT3) on each row in the text-box

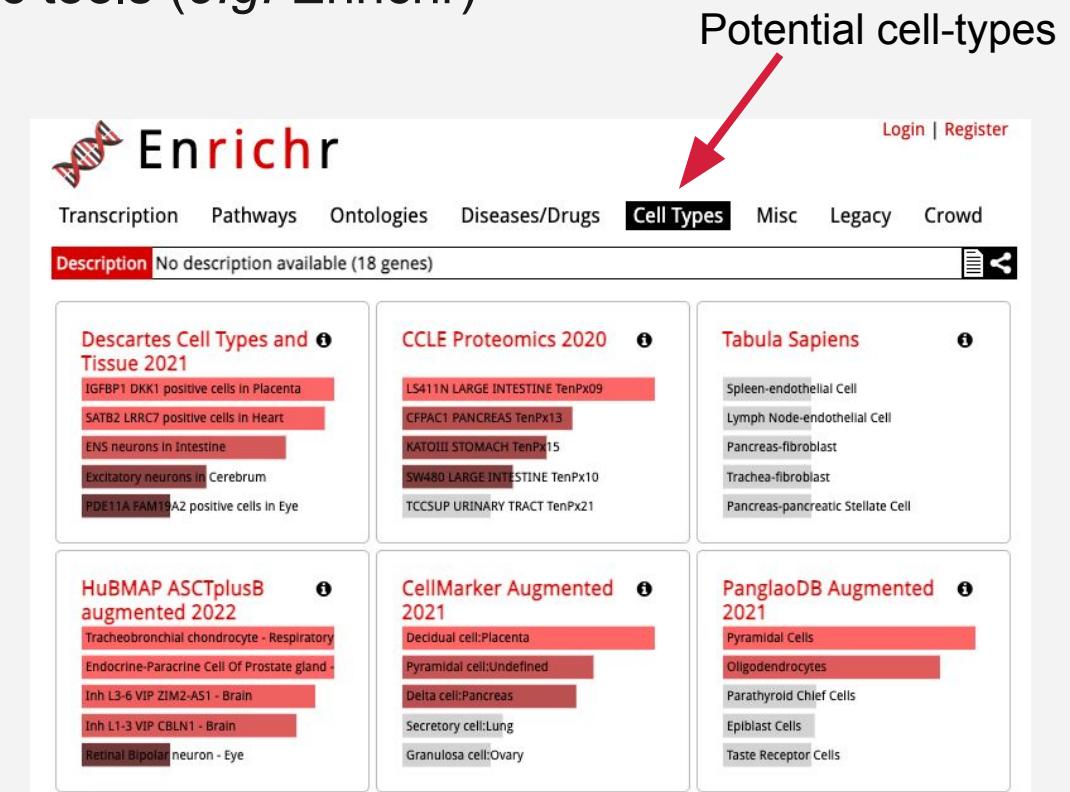
**Gene List**

0 gene(s) entered

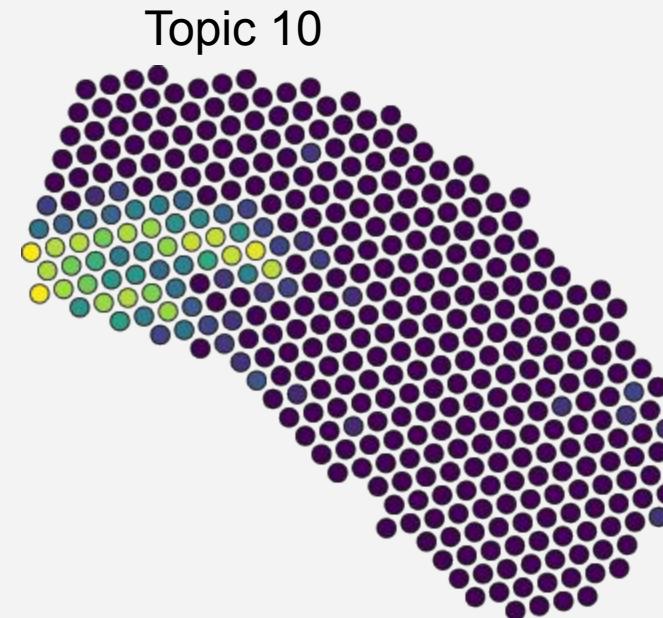
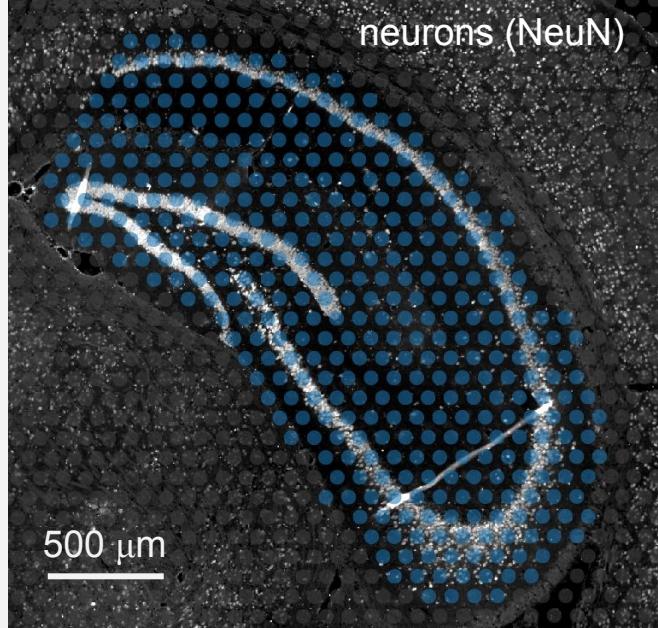
In order to enable others to search your set please enter a brief description of it.

Contribute your set so it can be searched by others **Submit**

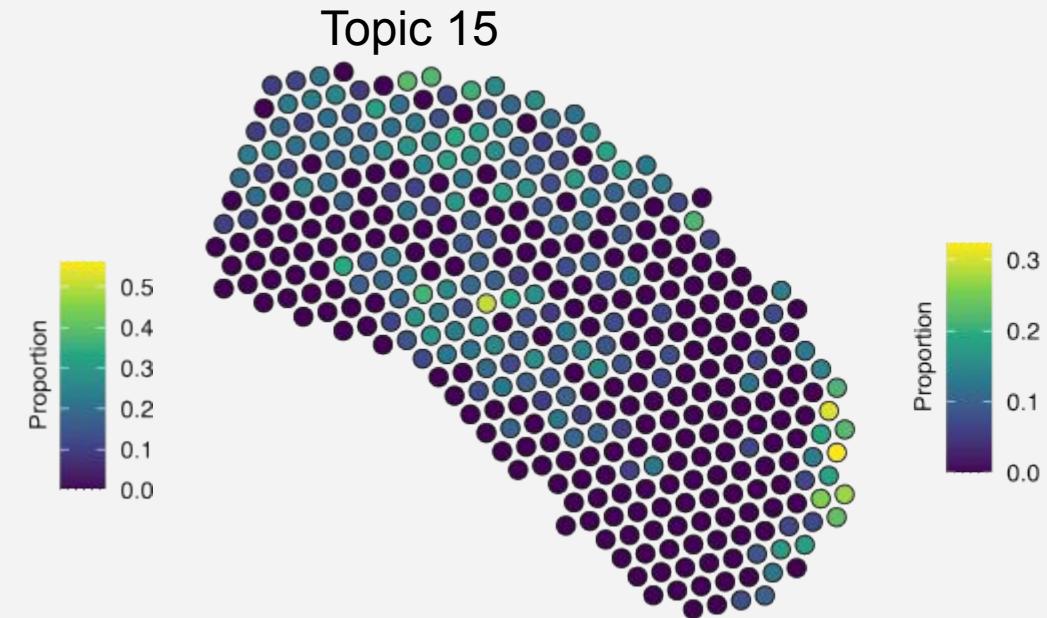
(<https://maayanlab.cloud/Enrichr/>)



# Participant Challenge

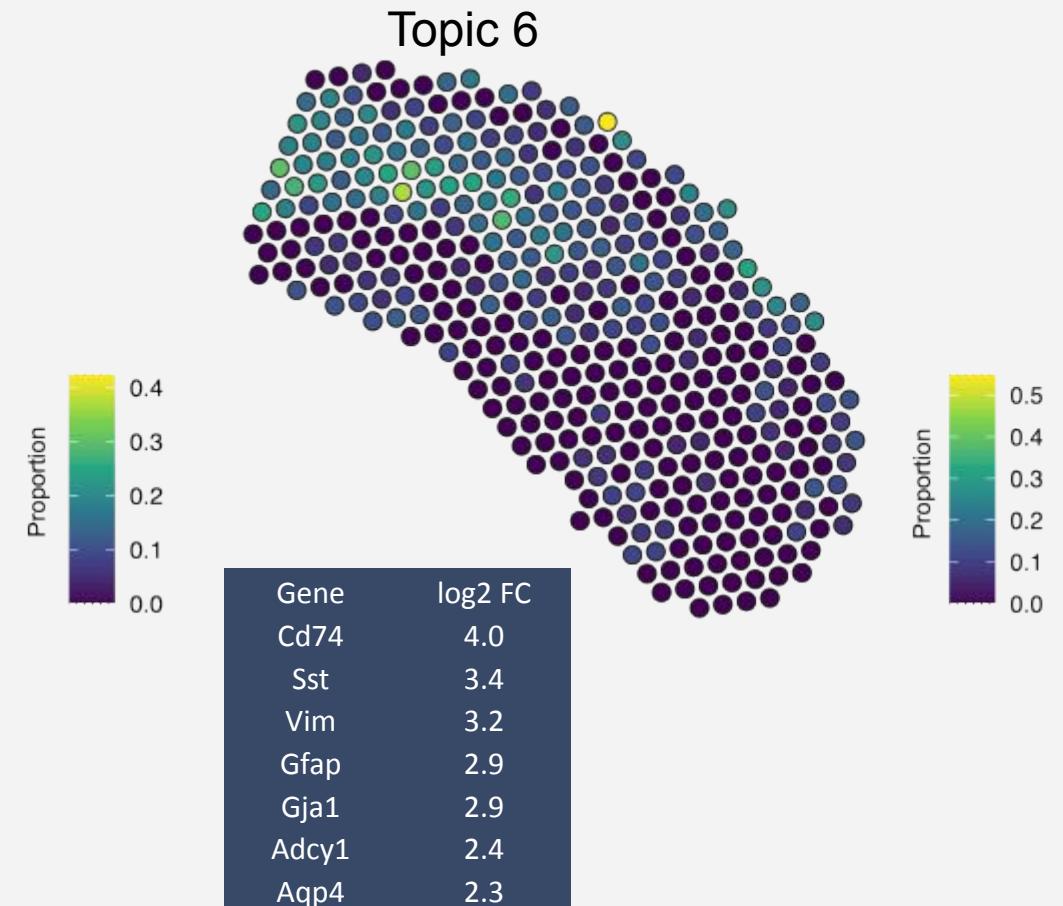
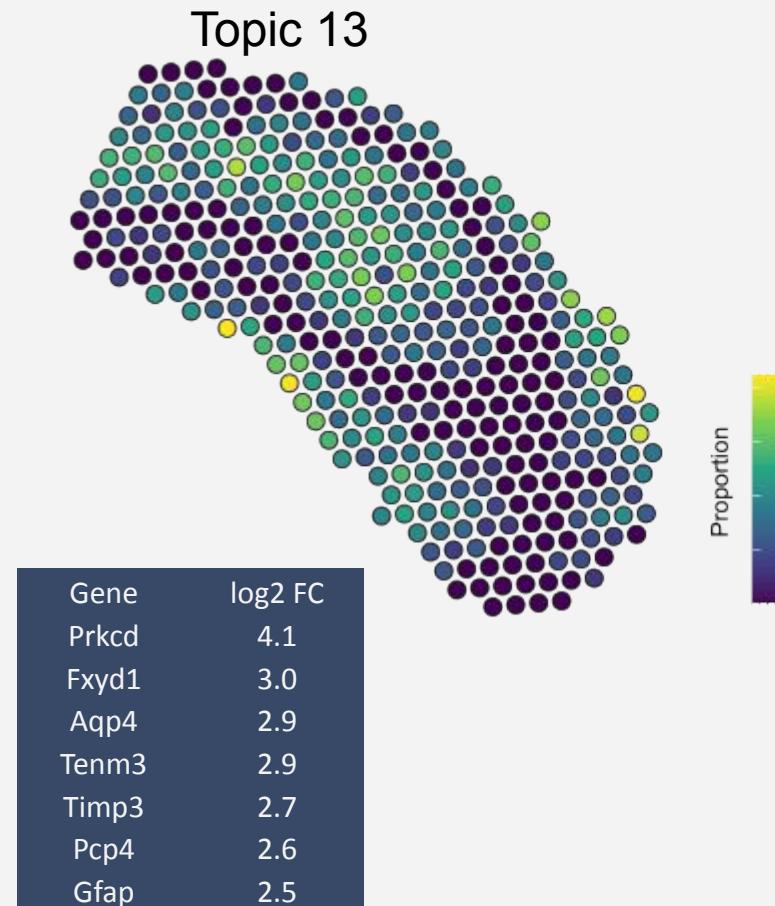
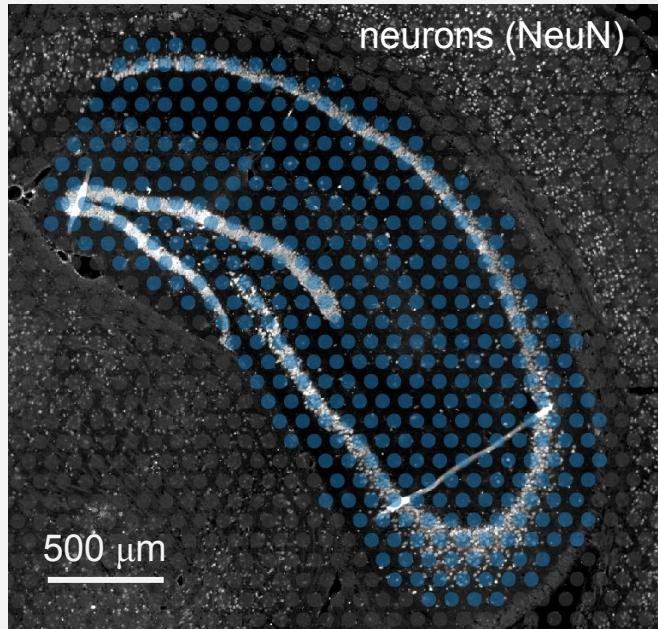


Gene	log2 FC
C1ql2	4.0
Prox1	3.5
Sema5a	3.1
Glis3	3.1
Lrrtm4	3.0
Rasl10a	2.8
Smoc2	2.7



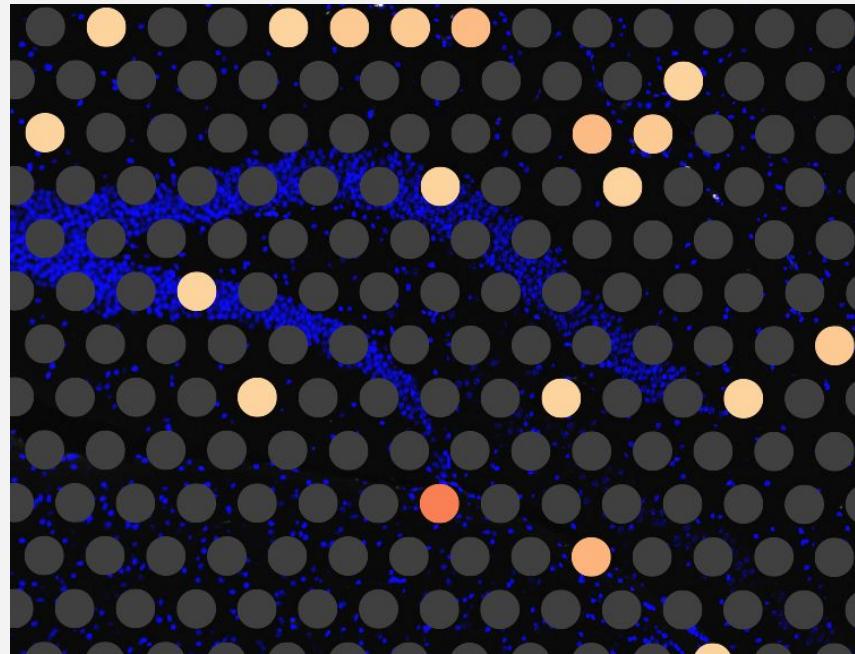
Gene	log2 FC
Camkk1	2.0
Sst	2.0
Diras2	1.9
Cacna2d1	1.9
Syn1	1.8
Atp6v1c1	1.8
Cck	1.8

# Exploring the Results Part 2

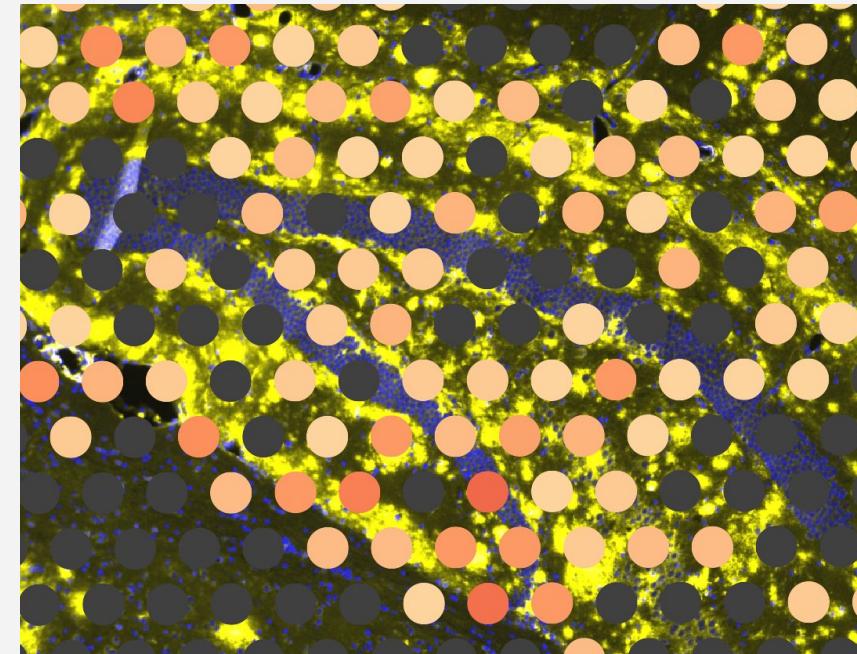


**Results:** Astrocytes (Topic 13) and Reactive Astrocytes (Topic 6)

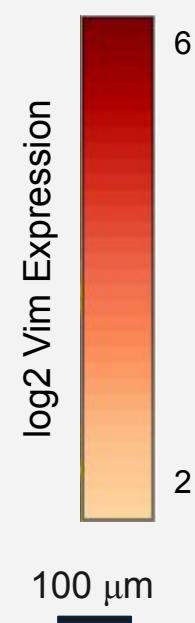
# Comparison: Transgenic to Wild Type Mice



13.2-month-old  
Mouse Brain



17.9-month-old  
TgCRND8 Mouse Brain



Blue is dapi nuclei and Yellow is beta-amyloid aggregates

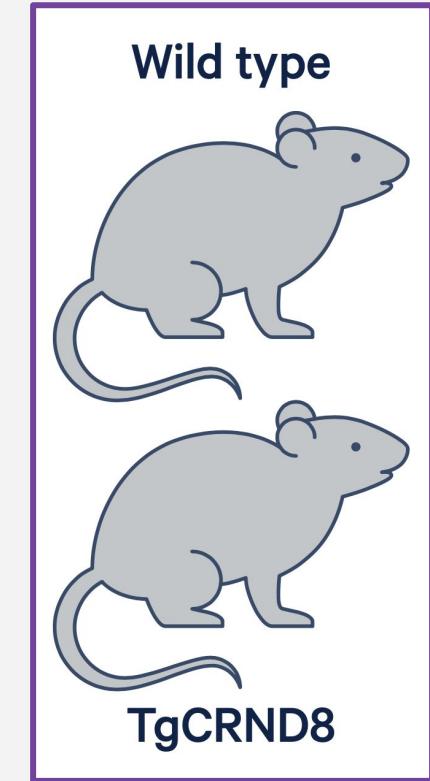
# Paired scRNAseq data

## 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.

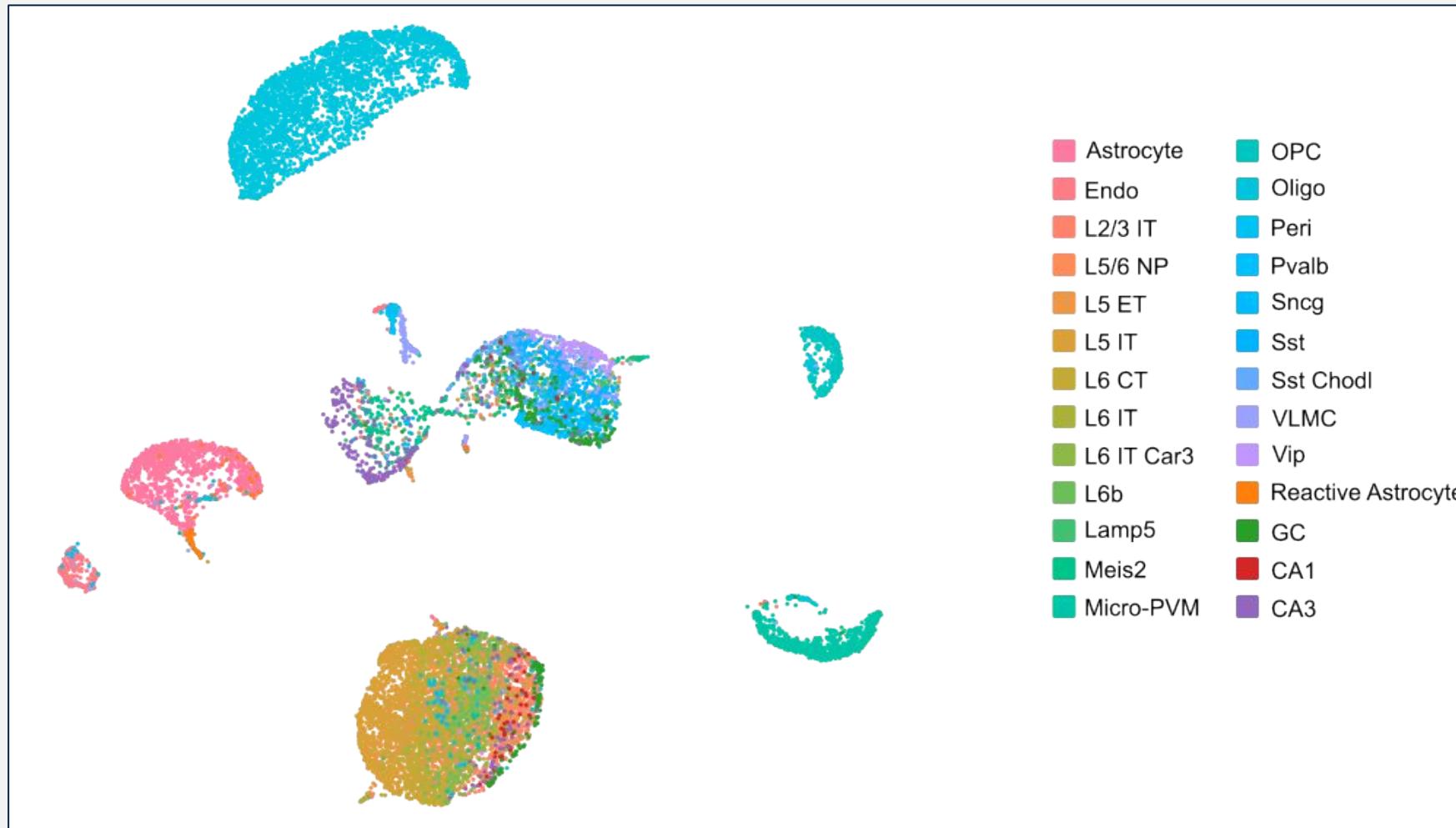
## Single cell RNAseq data added benefits

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



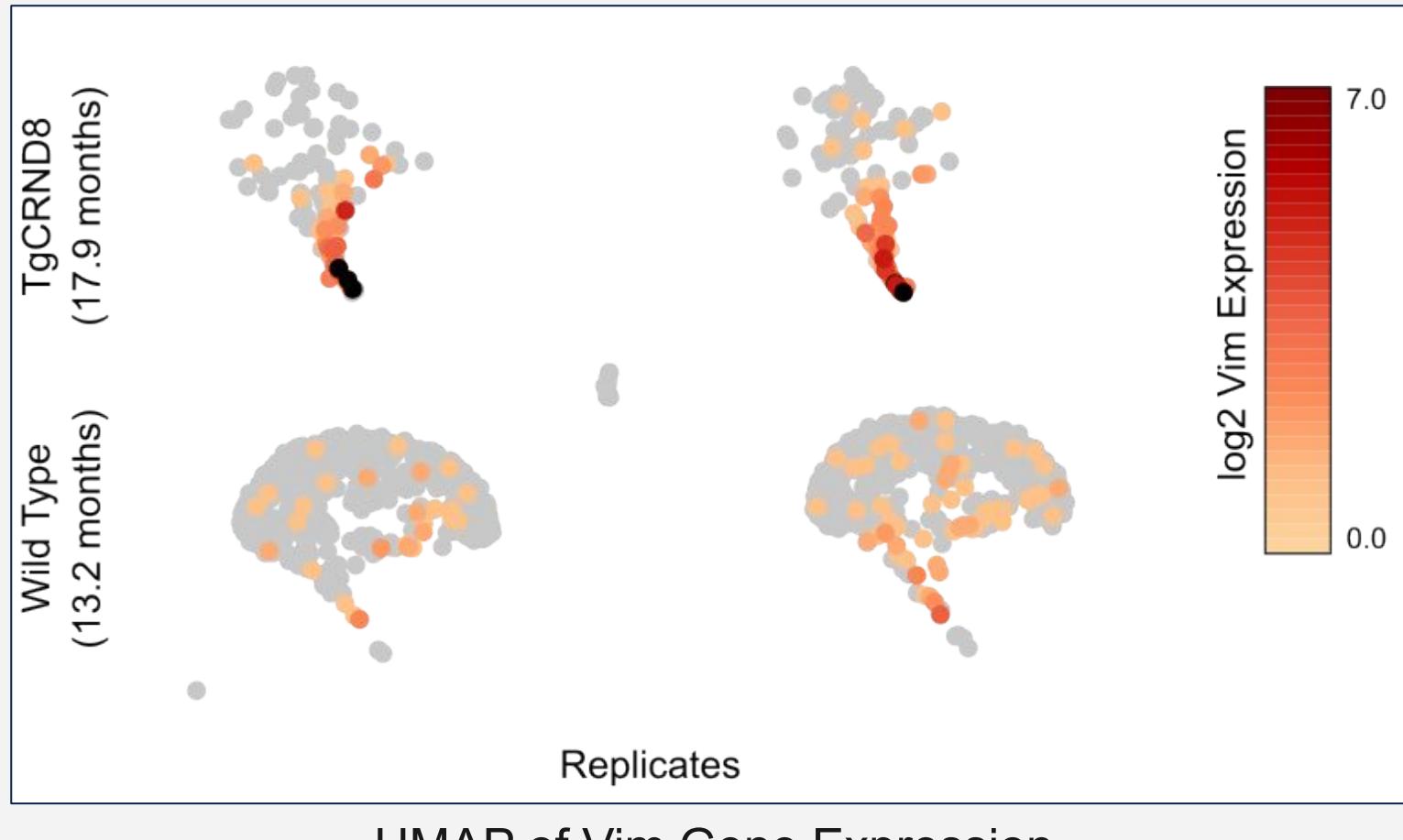
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# Paired scRNAseq data



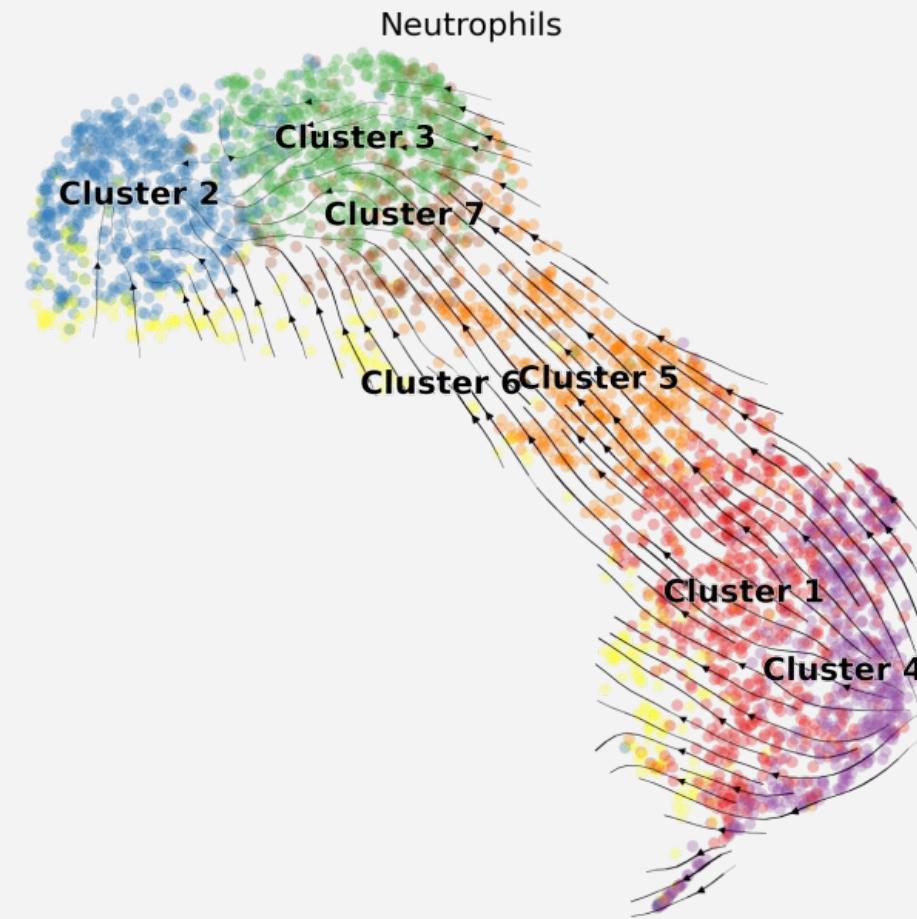
UMAP of Wildtype (13.2 months) and TgCRND8 (17.9 months) (N=2 per condition) clustering

# Paired scRNAseq data



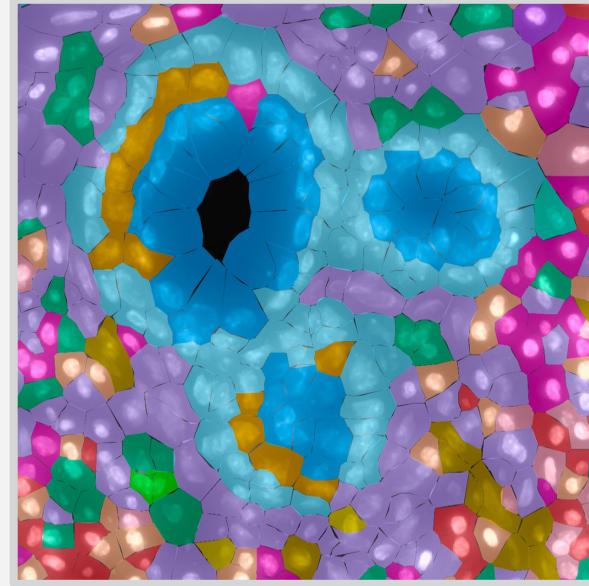
# Paired scRNASeq data: Mechanistic Studies

- **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/2023\\_Exploring\\_Your\\_Visium\\_Data.ipynb](https://colab.research.google.com/github/10XGenomics/analysis_guides/blob/main/2023_Exploring_Your_Visium_Data.ipynb)