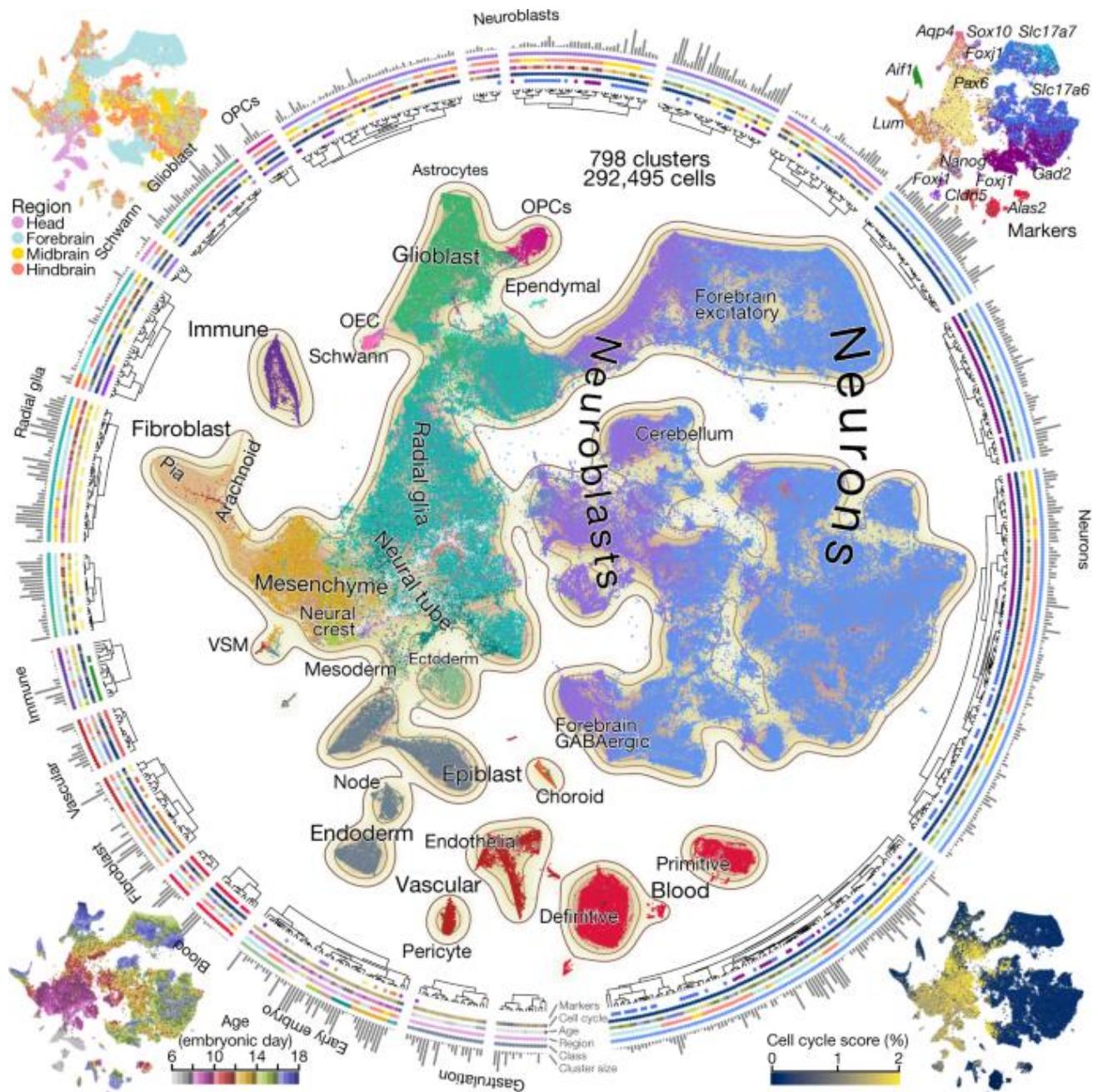


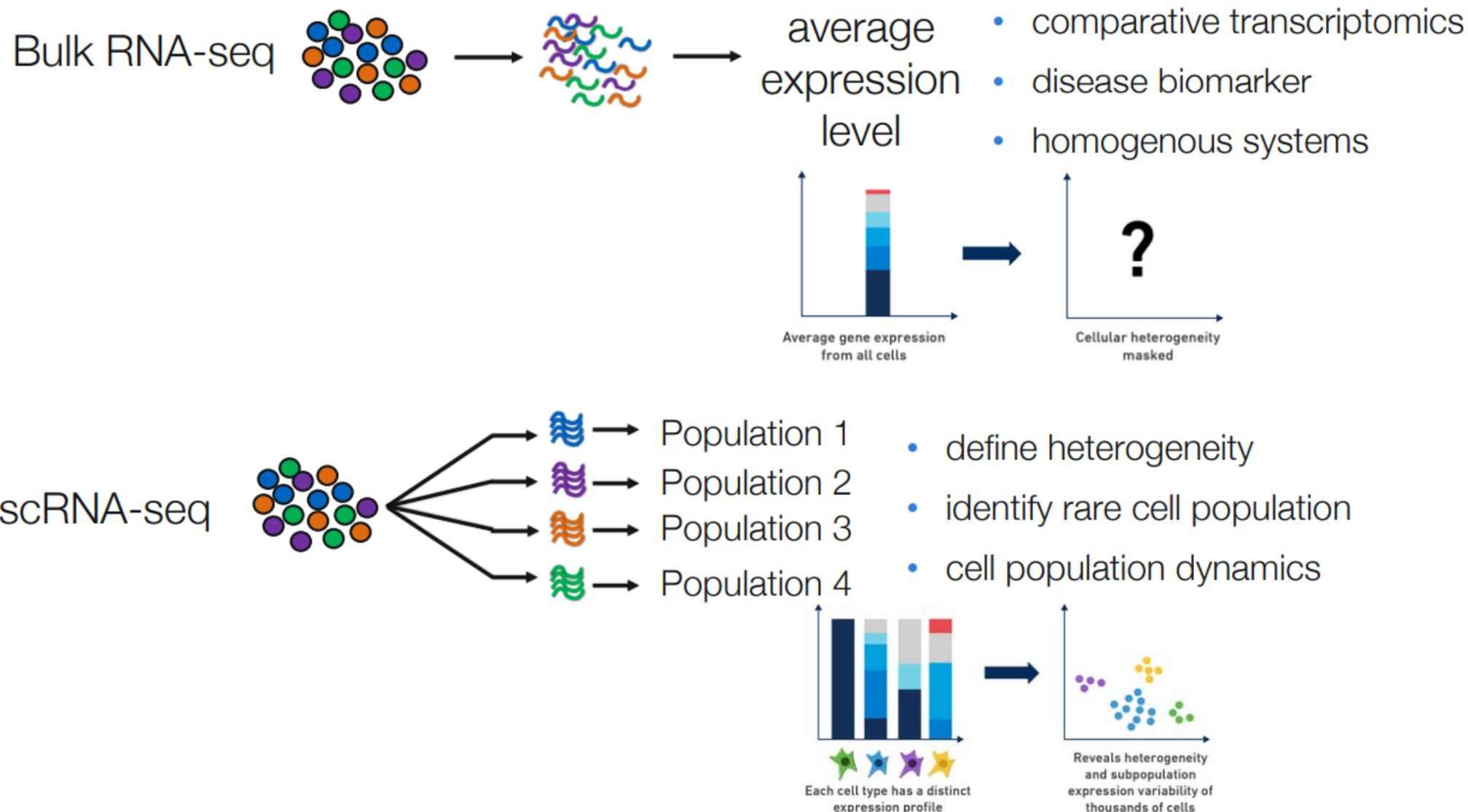
IBRO school single-cell RNAseq

Oct, 2024
Aline Rangel

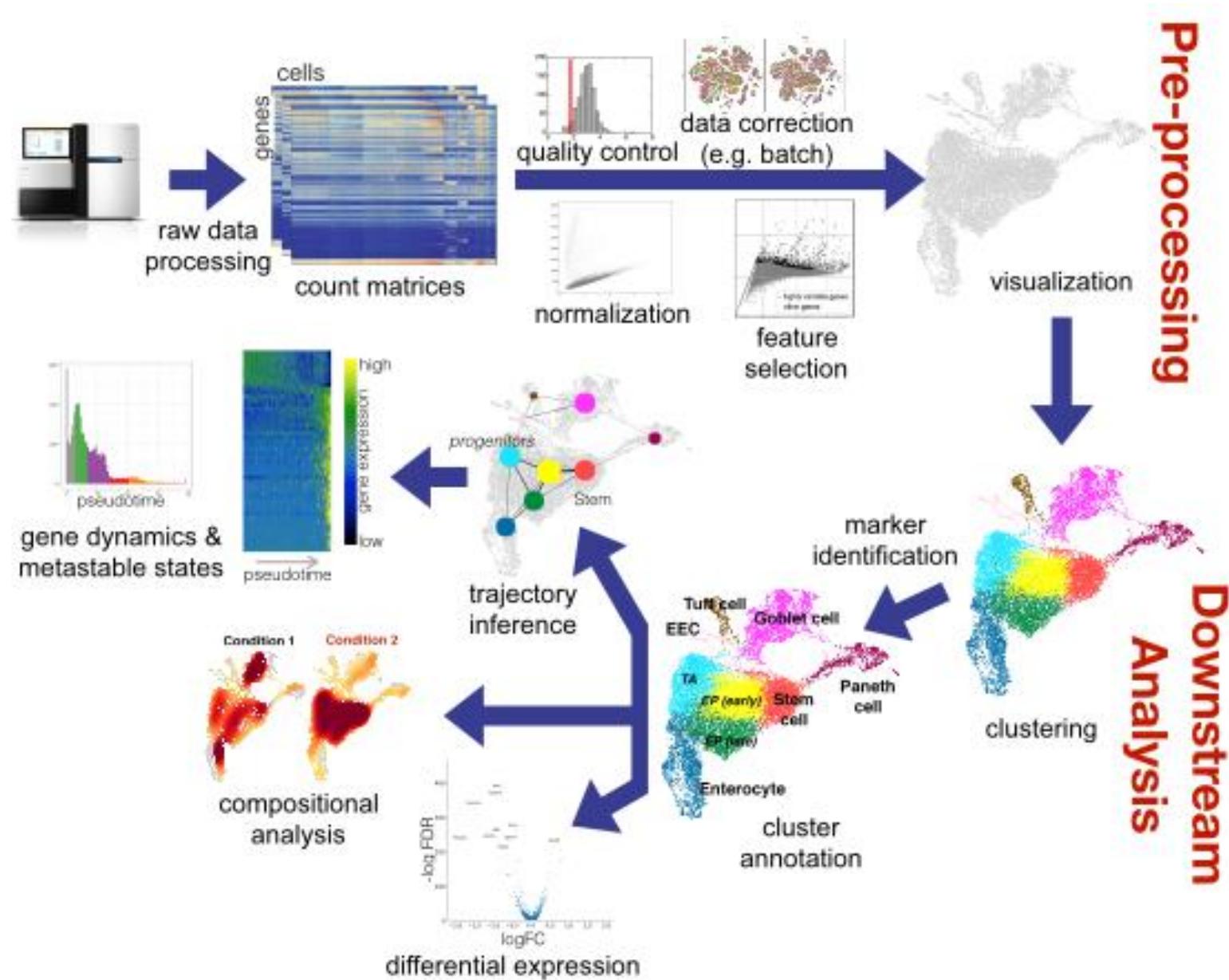


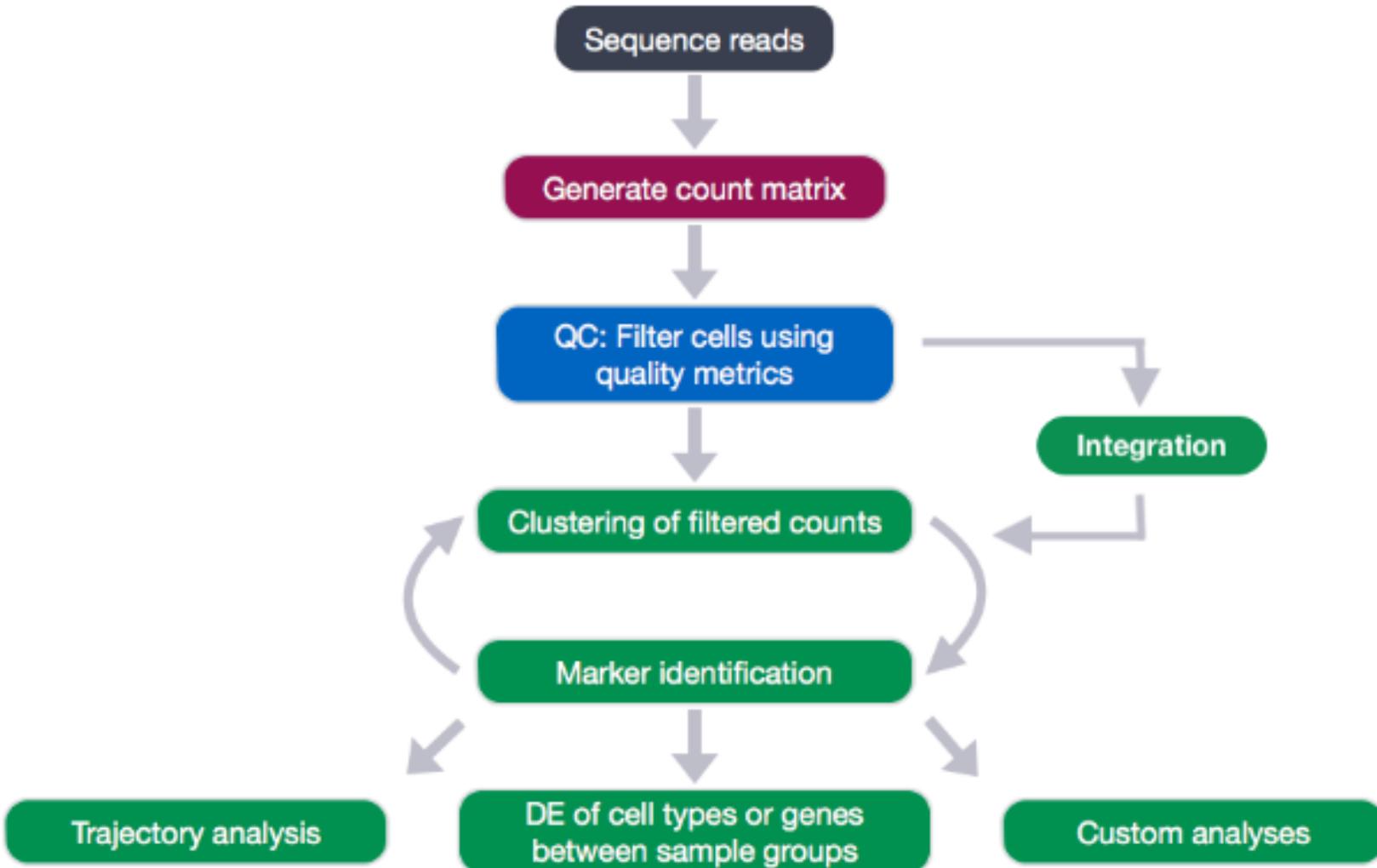
Manno et al., 2021

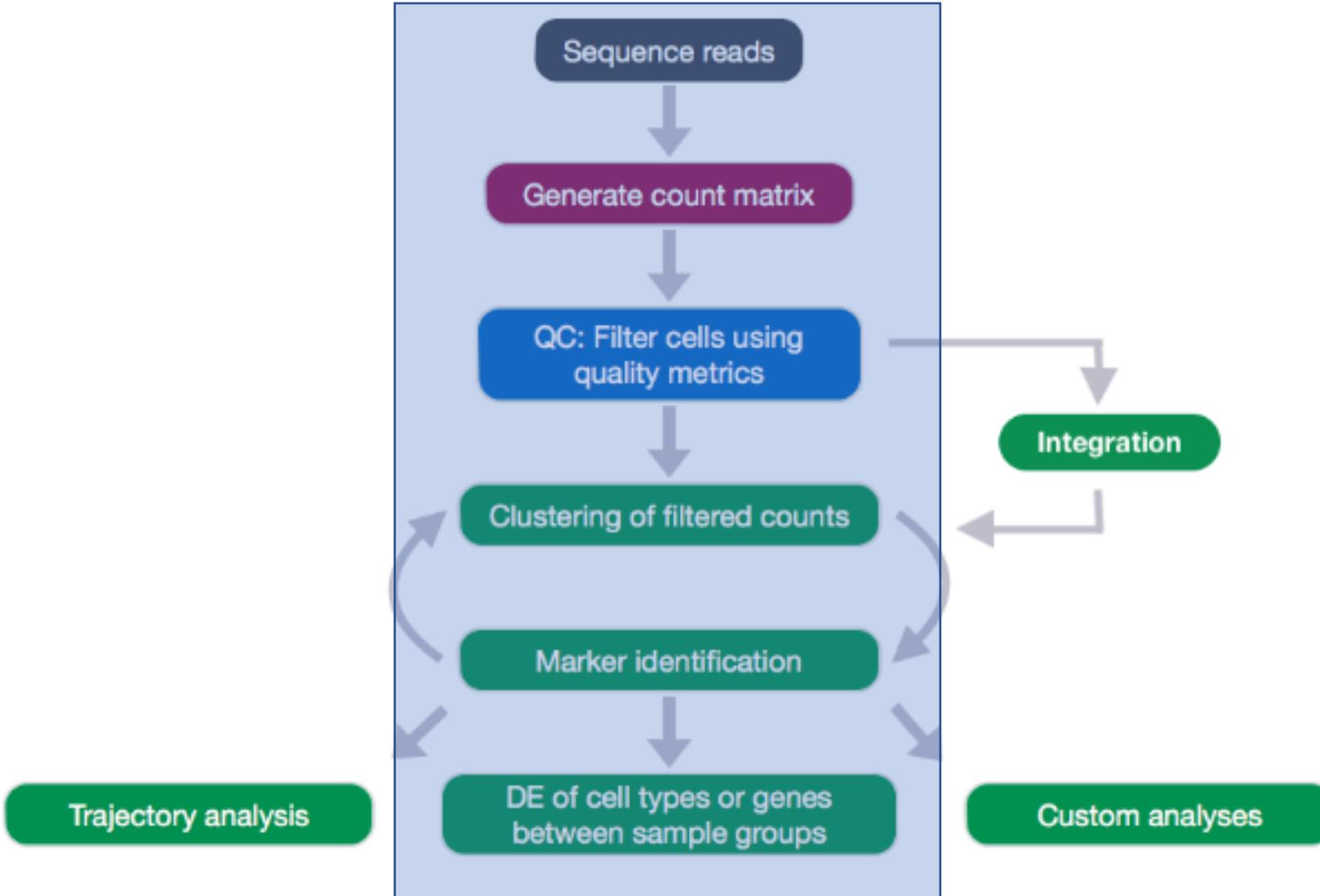
Bulk vs single-cell RNA sequencing



Standard analysis



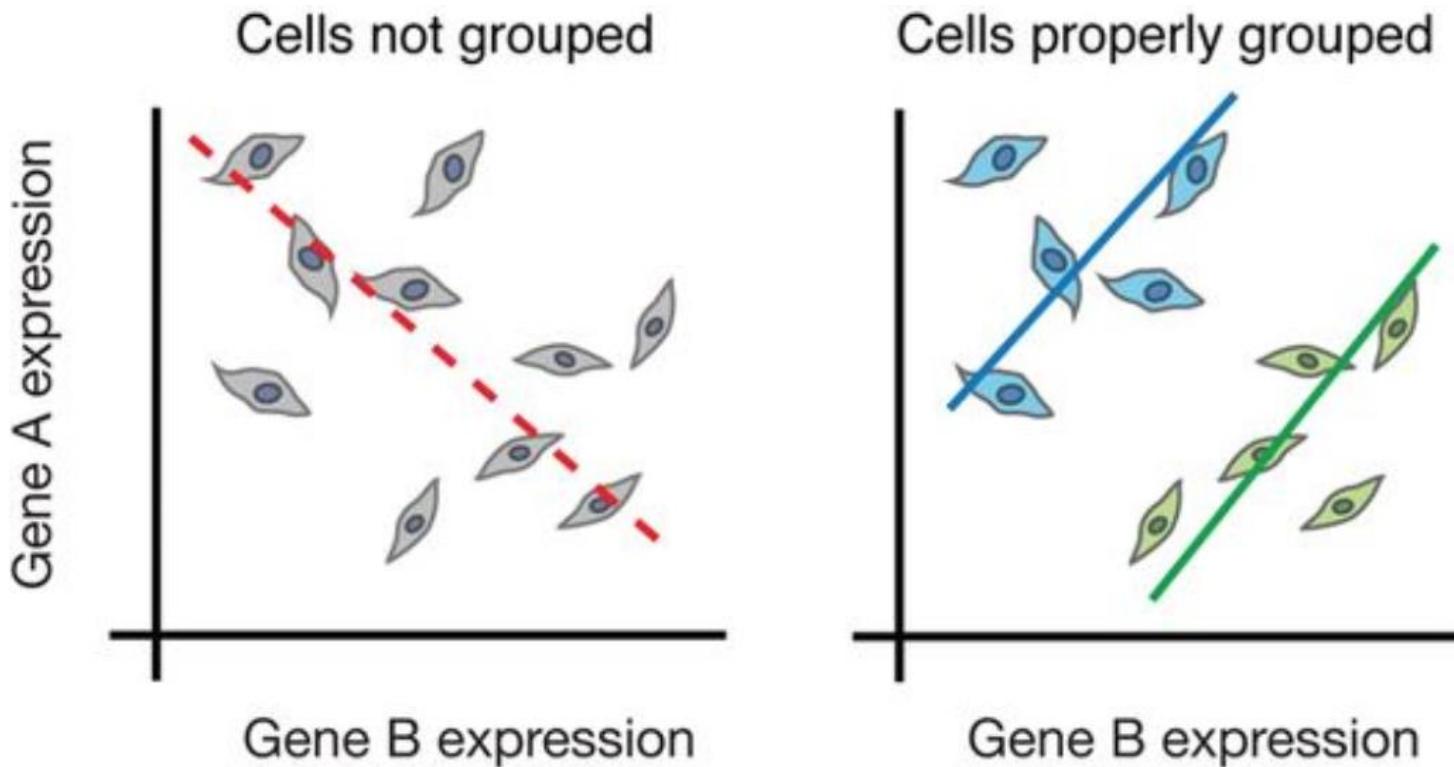




Filter cells using quality metrics

- Low-quality libraries in scRNA-seq data:
 - cell damage during dissociation
 - failure in library preparation (e.g., inefficient reverse transcription or PCR amplification).
 - “cells” with low total counts
 - few expressed genes
 - high mitochondrial or spike-in proportions
 - For example, we might consider cells to be low quality if they have library sizes below 100,000 reads; express fewer than 5,000 genes; have spike-in proportions above 10%; or have mitochondrial proportions above 10%.

Clustering and marker identification



Data accessibility

- [Allen Institute](#)



- [Broad Institute](#)



- [GEO](#) (Gene Expression Omnibus)



To find general information of a gene:

- MGI for mice

The screenshot shows the MGI website's search interface. At the top, there's a navigation bar with links like Home, Genes, Phenotypes, Human Disease, Expression, Recombinases, Function, Strains / SNPs, Homology, Tumors, and several dropdown menus for Search, Download, More Resources, and Analysis Tools. A search bar at the top right contains the placeholder "Type your search here". Below the search bar is a "Quick Search" button. To the right is the "ALLIANCE OF GENOME RESOURCES FOUNDING MEMBER" logo. The main area is titled "Genes and Markers Query Form". It features a search input field with a placeholder "Search for genes and markers by name, feature type, location, GO terms, protein domains, etc." and "Gene/Marker Symbol/Name" fields. A "Feature type" section includes a tree view of categories like "all feature types", "gene", "protein coding gene", "non-coding RNA gene", etc. A "Genome location" section has fields for "Chromosome(s)" and "cM Position".

- Genecards for human

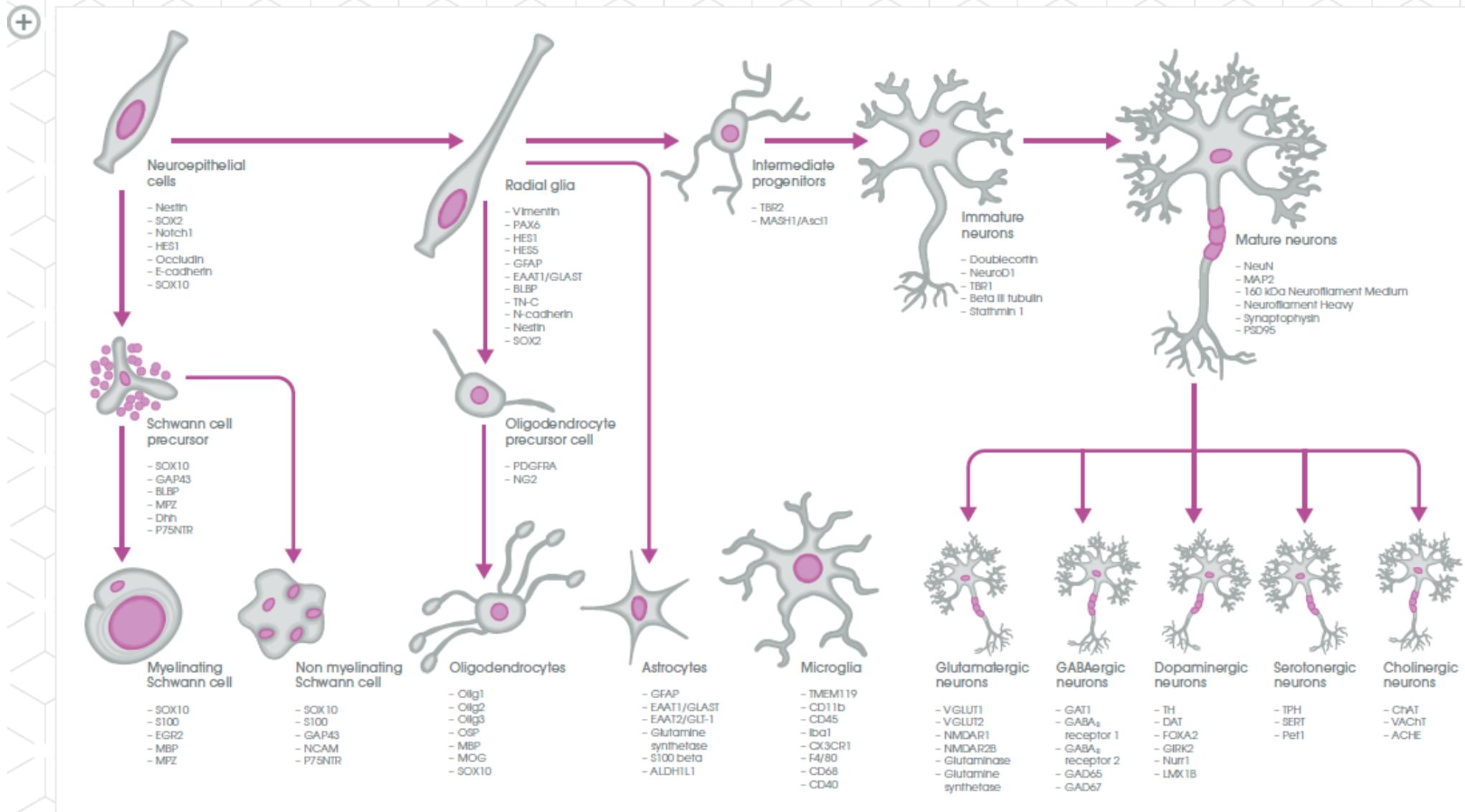
The screenshot shows the GeneCards website. At the top, there's a header with the GeneCards logo ("THE HUMAN GENE DATABASE"), a search bar, and logos for WEIZMANN INSTITUTE OF SCIENCE and LifeMap SCIENCES. The header also includes links for Home, User Guide, Analysis Tools, Release Notes, About, Data Access, GeneCards Team, My Genes, and Log In / Sign Up. Below the header, a section titled "GeneCards® : The Human Gene Database" describes the database as a searchable, integrative database for human genes. A search bar at the bottom contains the query "cdh4", with a magnifying glass icon and an "Advanced" link below it. The page also includes a link to "Examples: TYK2, 'prostate cancer', collagen, metabolism".

scRNA-seq analysis software

- [Seurat](#) in R
- [Bioconductor](#) in R
- [Scanpy](#) in Python

Neural lineage markers at a glance

abcam



To find out more, please visit abcam.com/neuralmarkers

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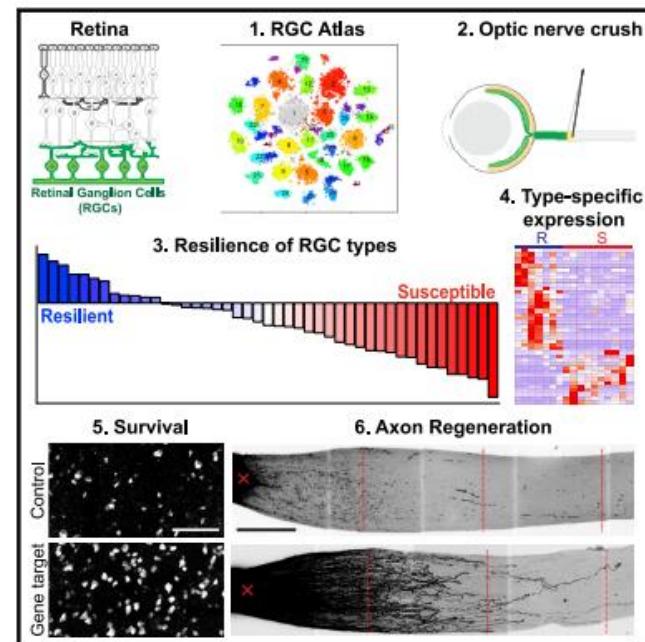
RGC single-cell data

NeuroResource

Neuron

Single-Cell Profiles of Retinal Ganglion Cells Differing in Resilience to Injury Reveal Neuroprotective Genes

Graphical Abstract



Authors

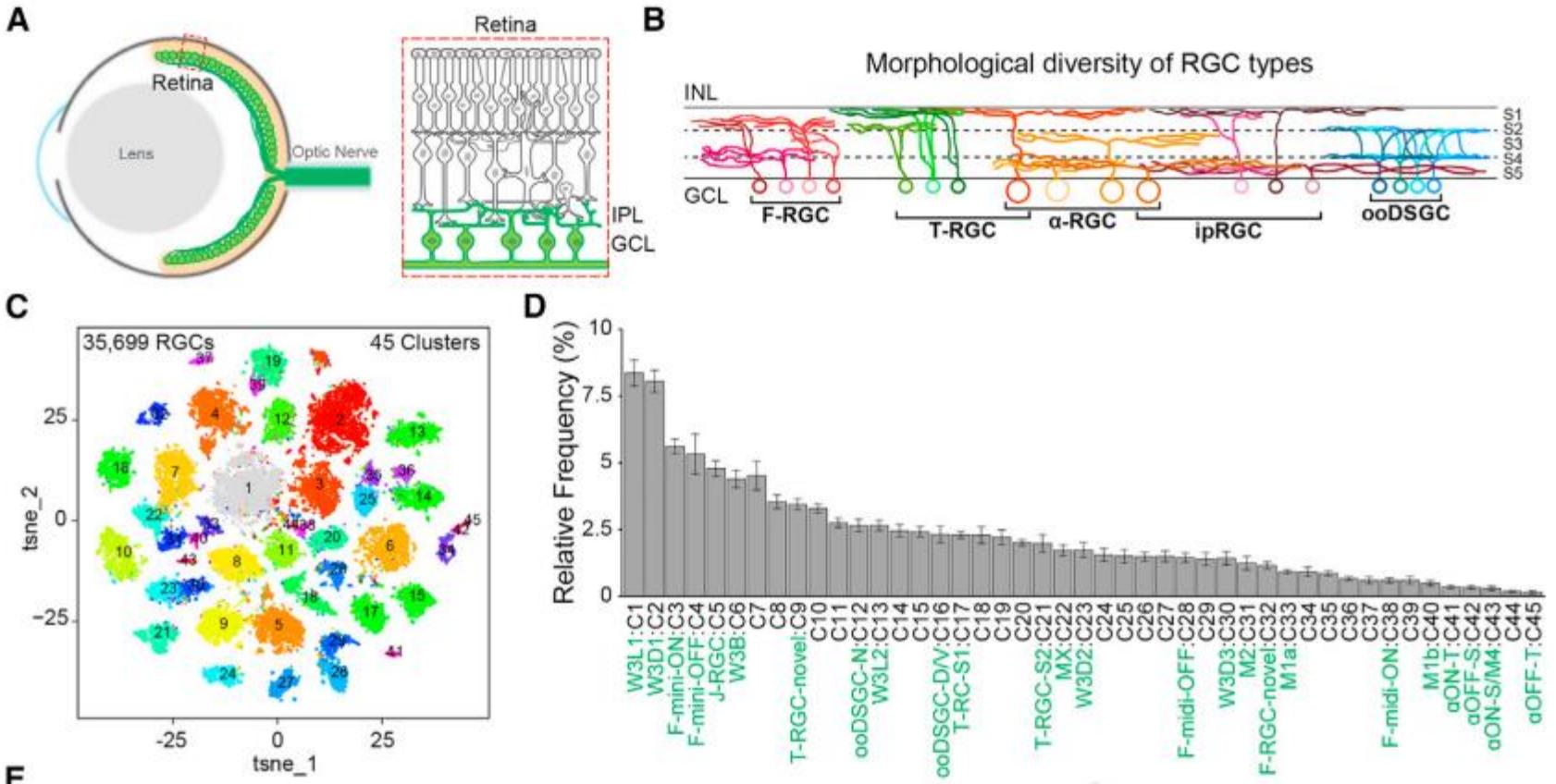
Nicholas M. Tran, Karthik Shekhar,
Irene E. Whitney, ..., Aviv Regev,
Zhigang He, Joshua R. Sanes

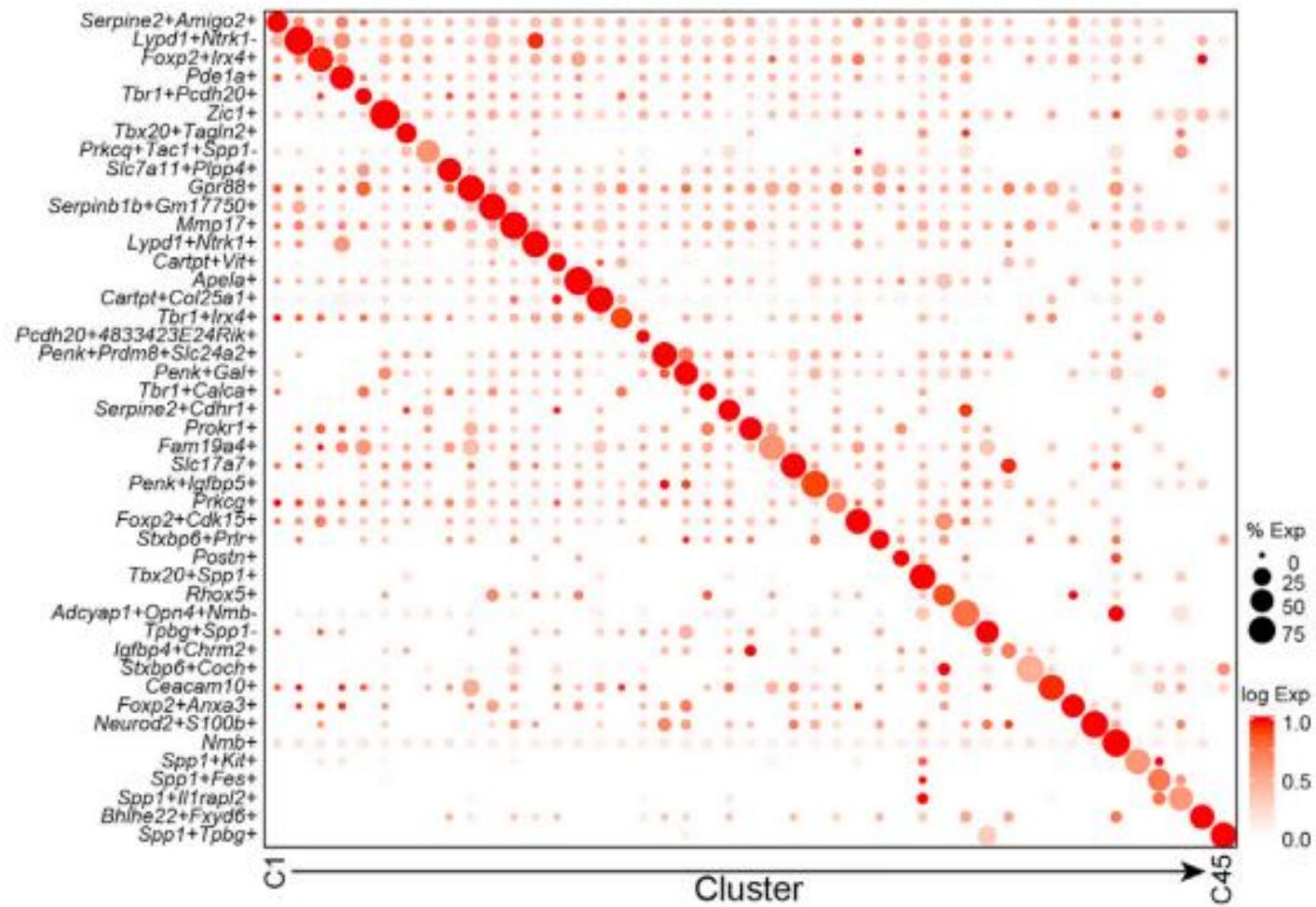
Correspondence

sanesj@mcb.harvard.edu

In Brief

High-throughput single-cell RNA-seq characterizes 46 types of adult mouse retinal ganglion cells and documents dramatic differences among them in their ability to survive axotomy. Manipulation of genes differentially expressed between resilient and vulnerable types enhances survival and axon regeneration.





RGC single-cell data

Data and Code Availability

Submission of all the raw and processed datasets reported in this study has been initiated to the Gene Expression Omnibus (GEO). The accession number for the sequencing data reported in this paper is GEO: GSE137400. The single cell data can be visualized in the Broad Institute's Single Cell Portal at

https://singlecell.broadinstitute.org/single_cell/study/SCP509/mouse-retinal-ganglion-cell-adult-atlas-and-optic-nerve-crush-time-series, and the code for mapping injured RGCs is available on <https://github.com/klarman-cell-observatory/RetinalGanglionCell-ONC>.

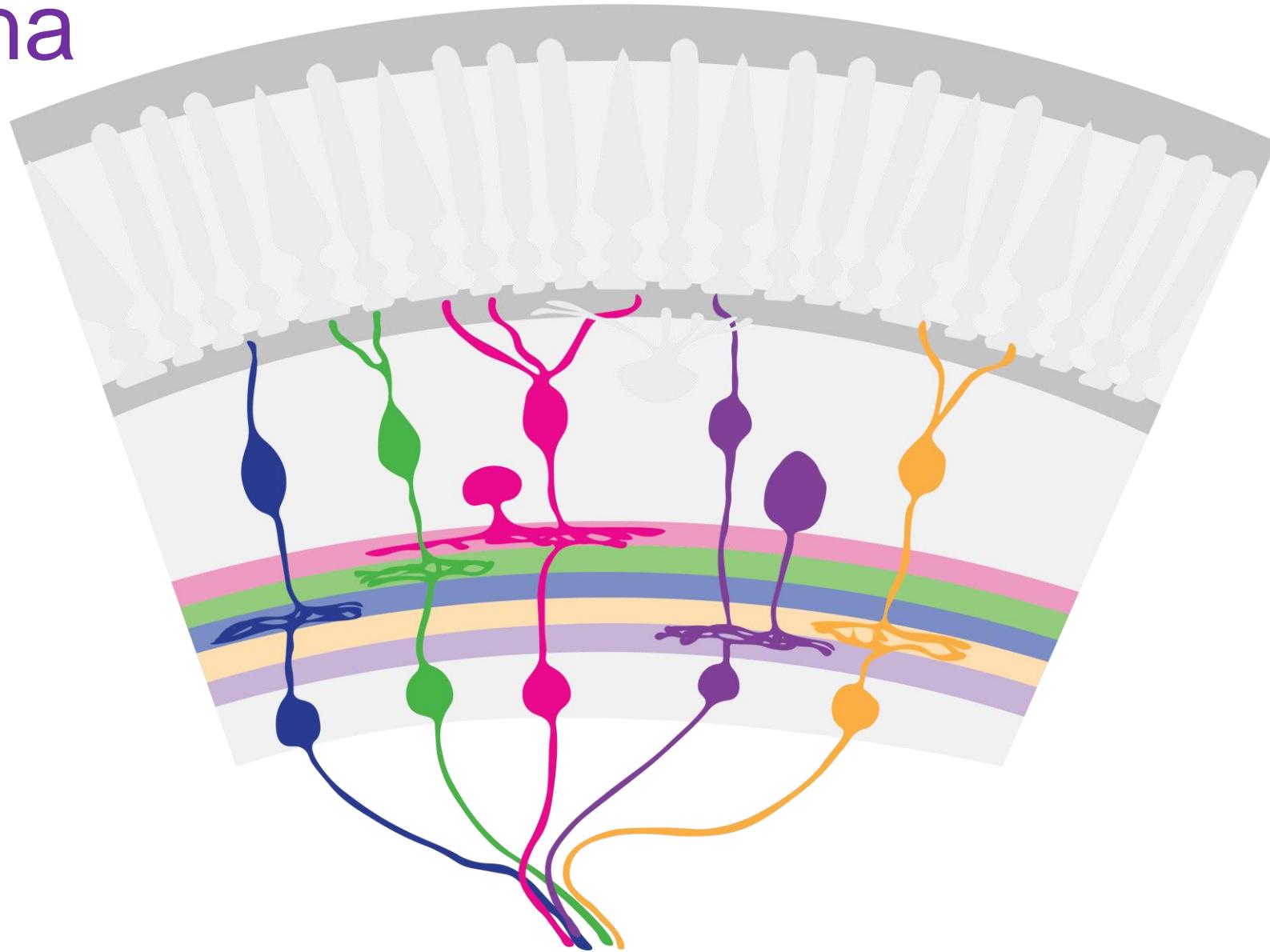
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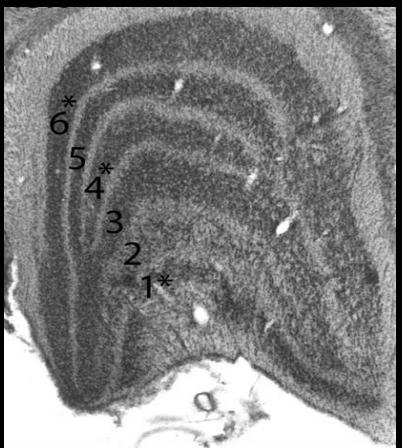
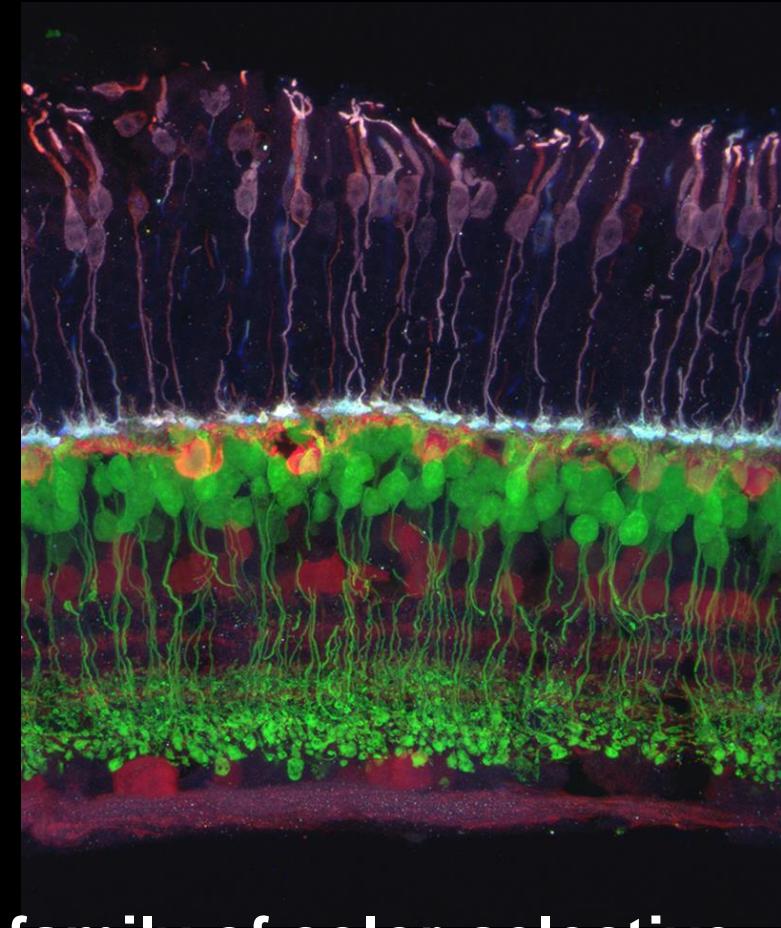
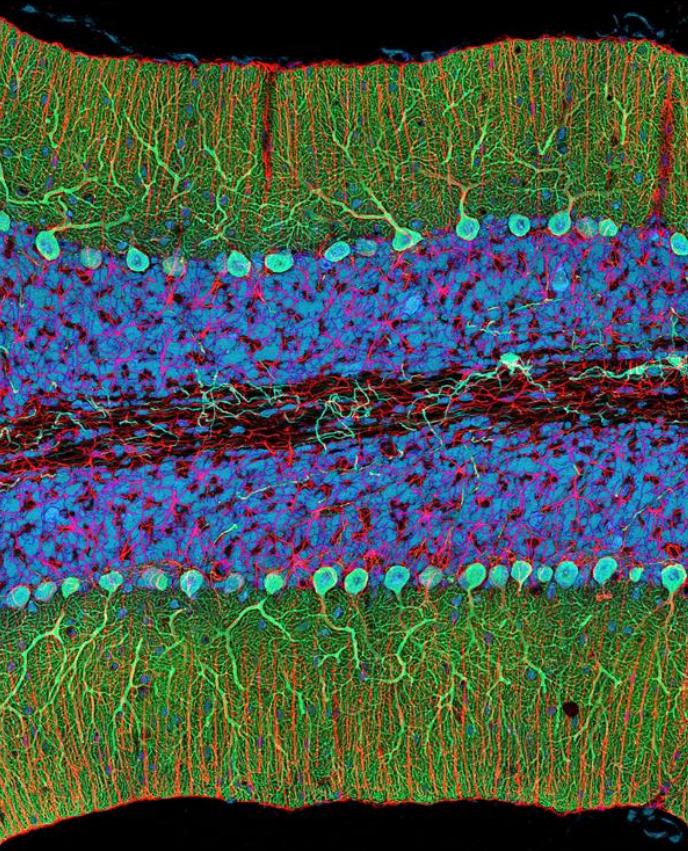
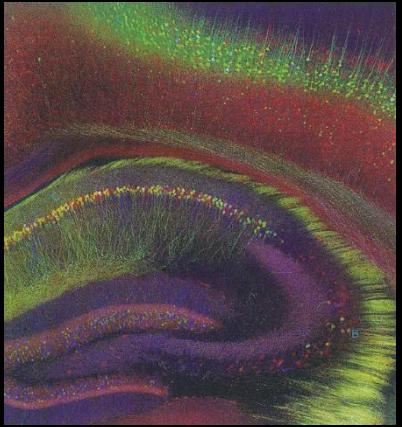
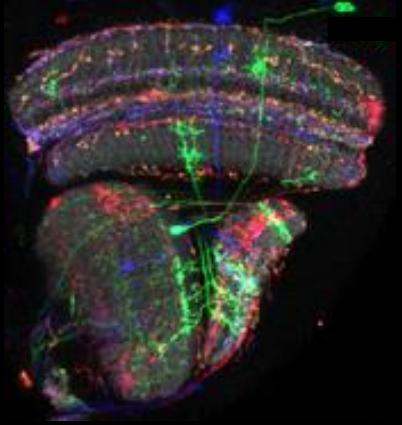
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- **Counts:**

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137400>

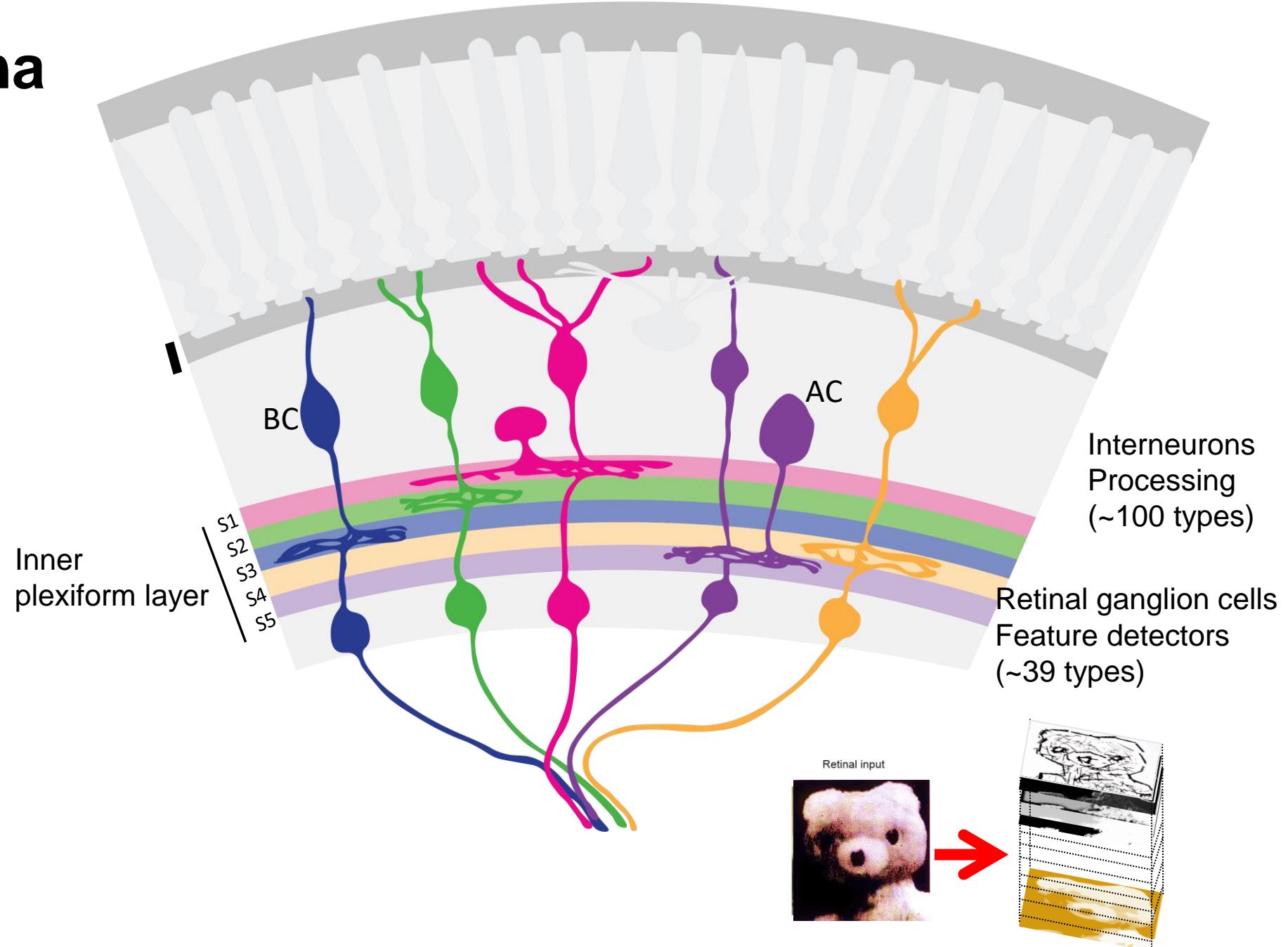
The retina

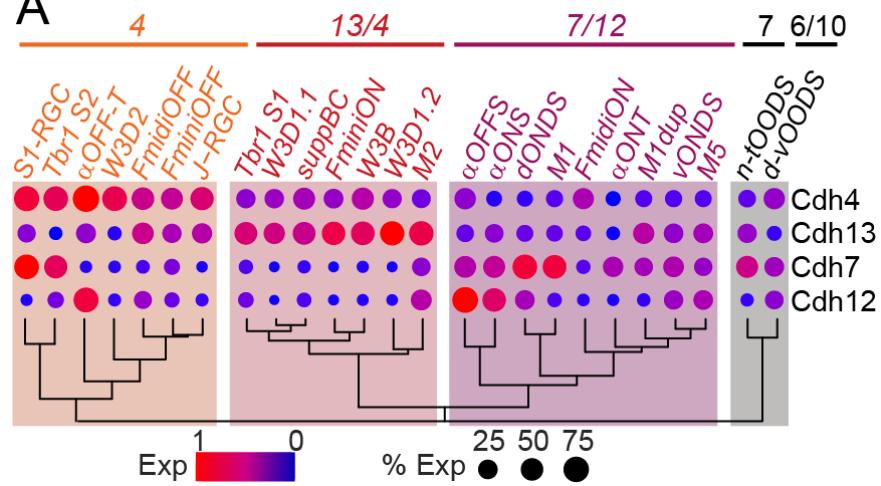


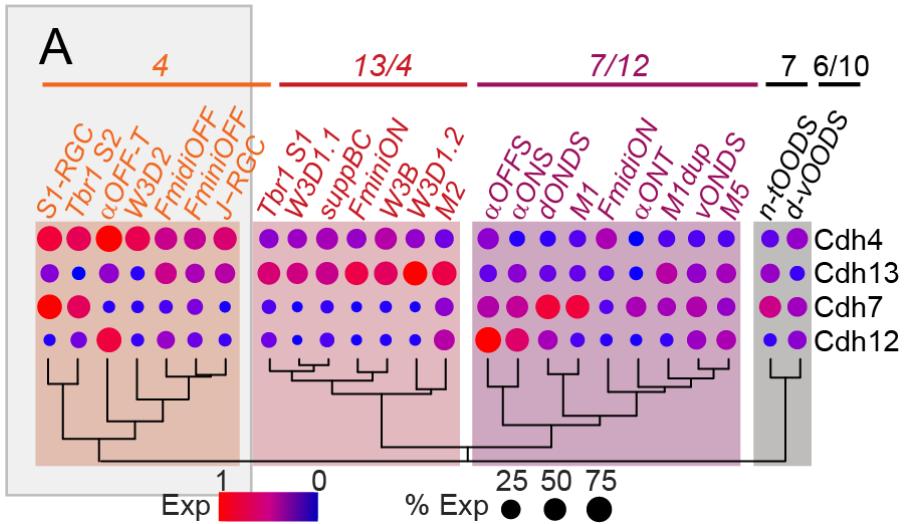


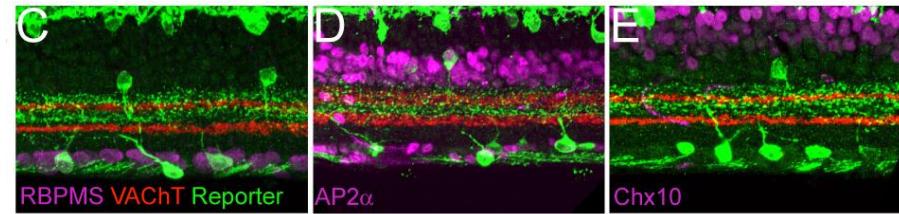
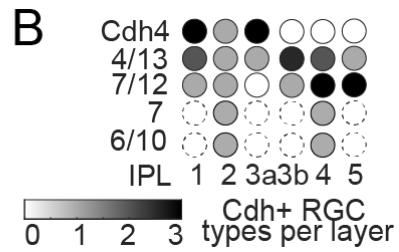
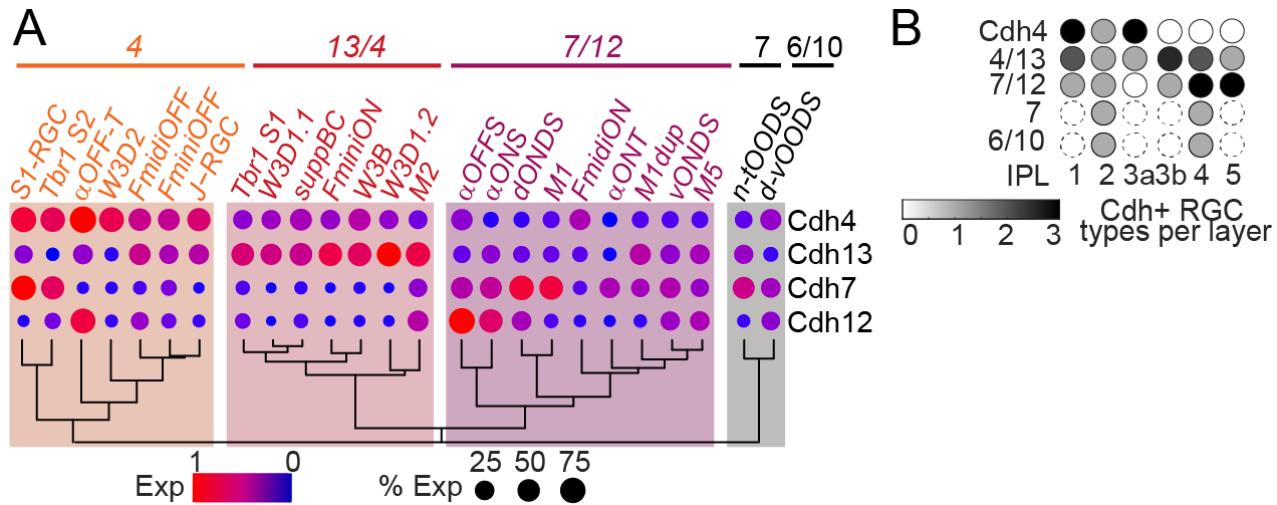
**Cadherin 4 assembles a family of color-selective
retinal circuits that respond to light offset**

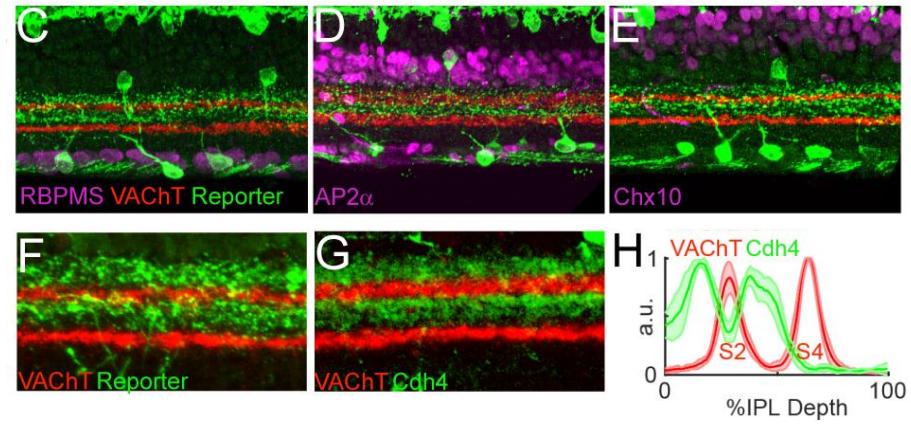
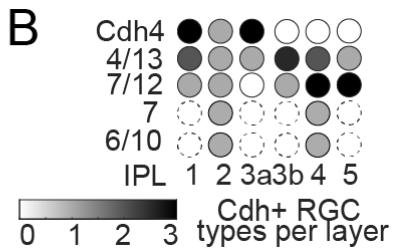
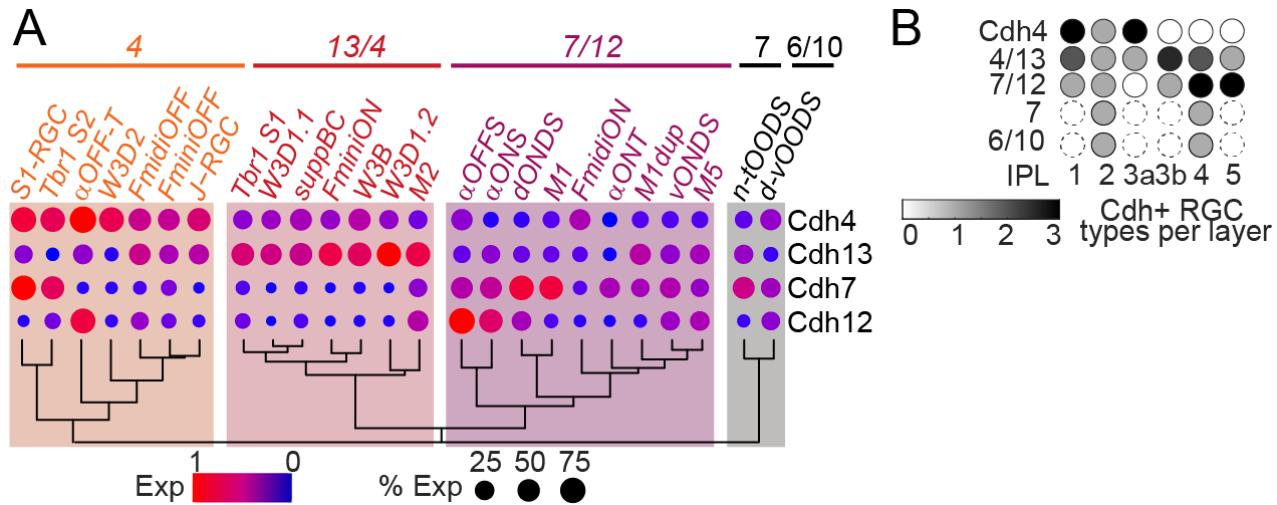
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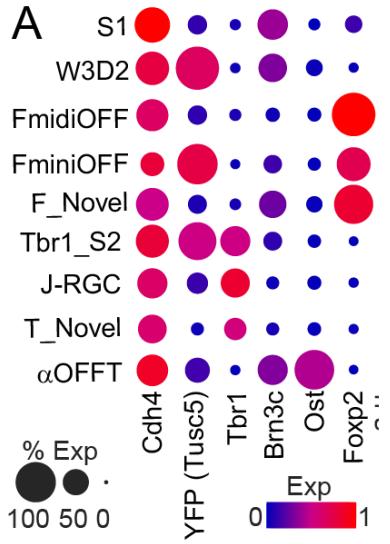


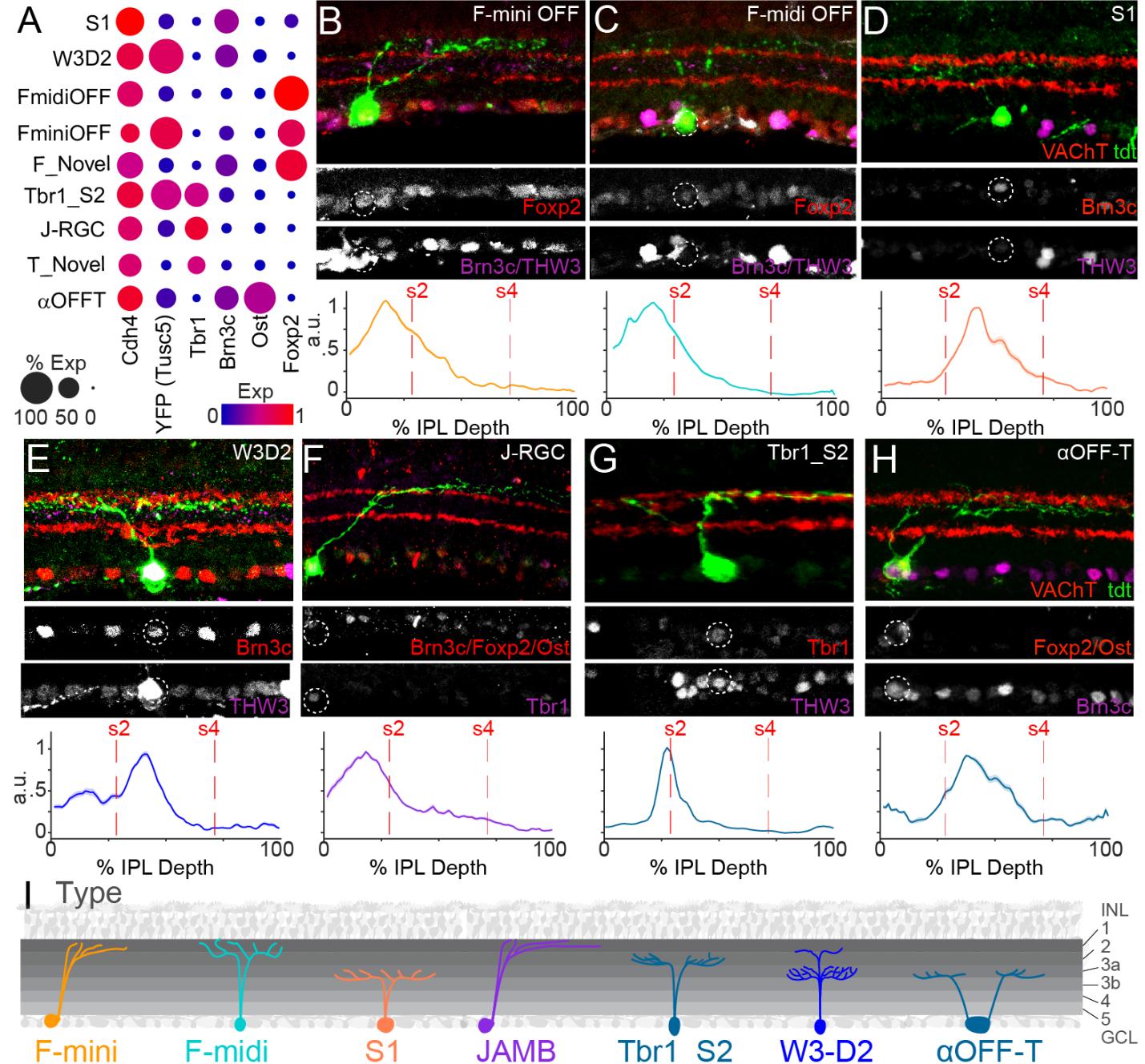
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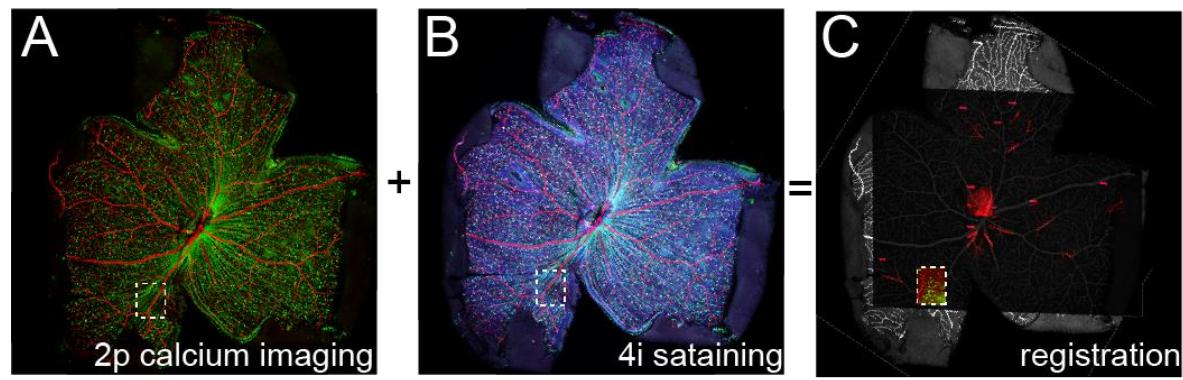


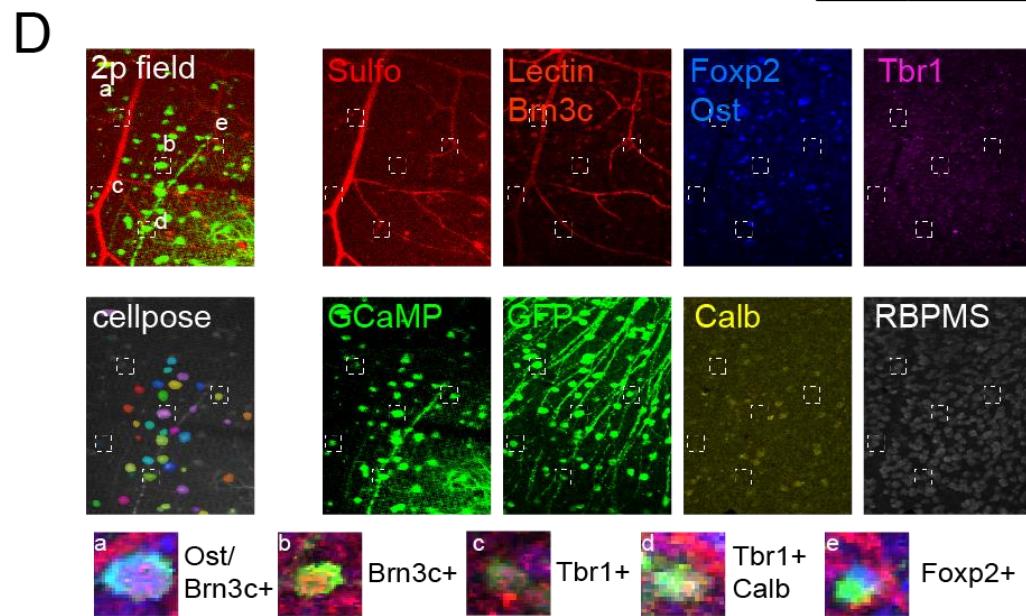
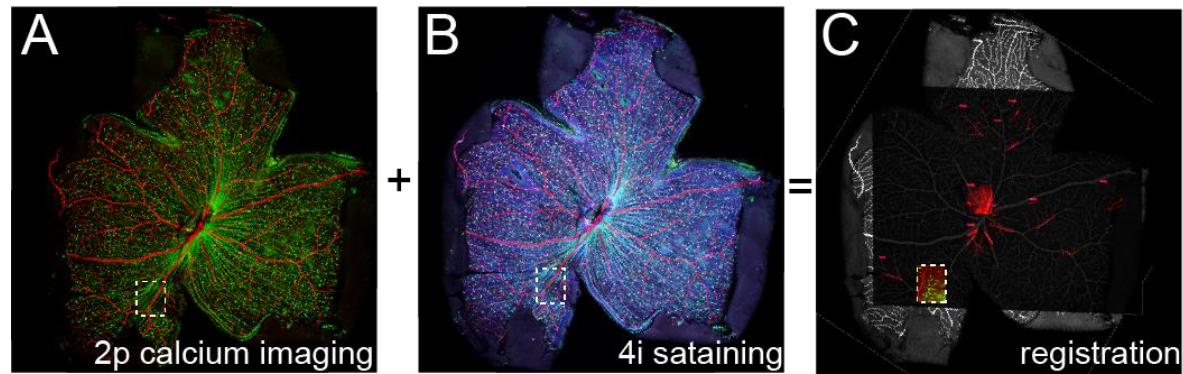


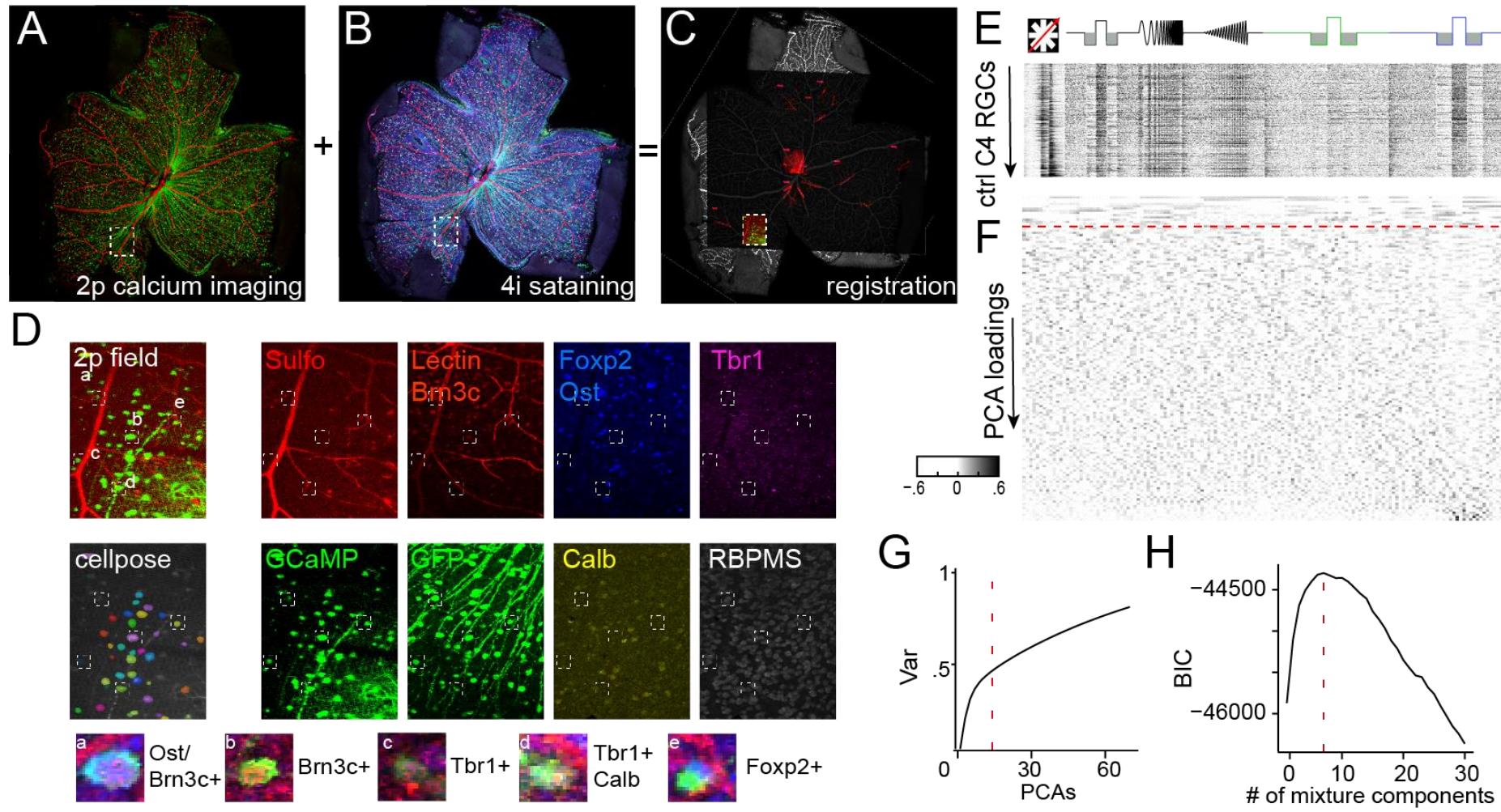


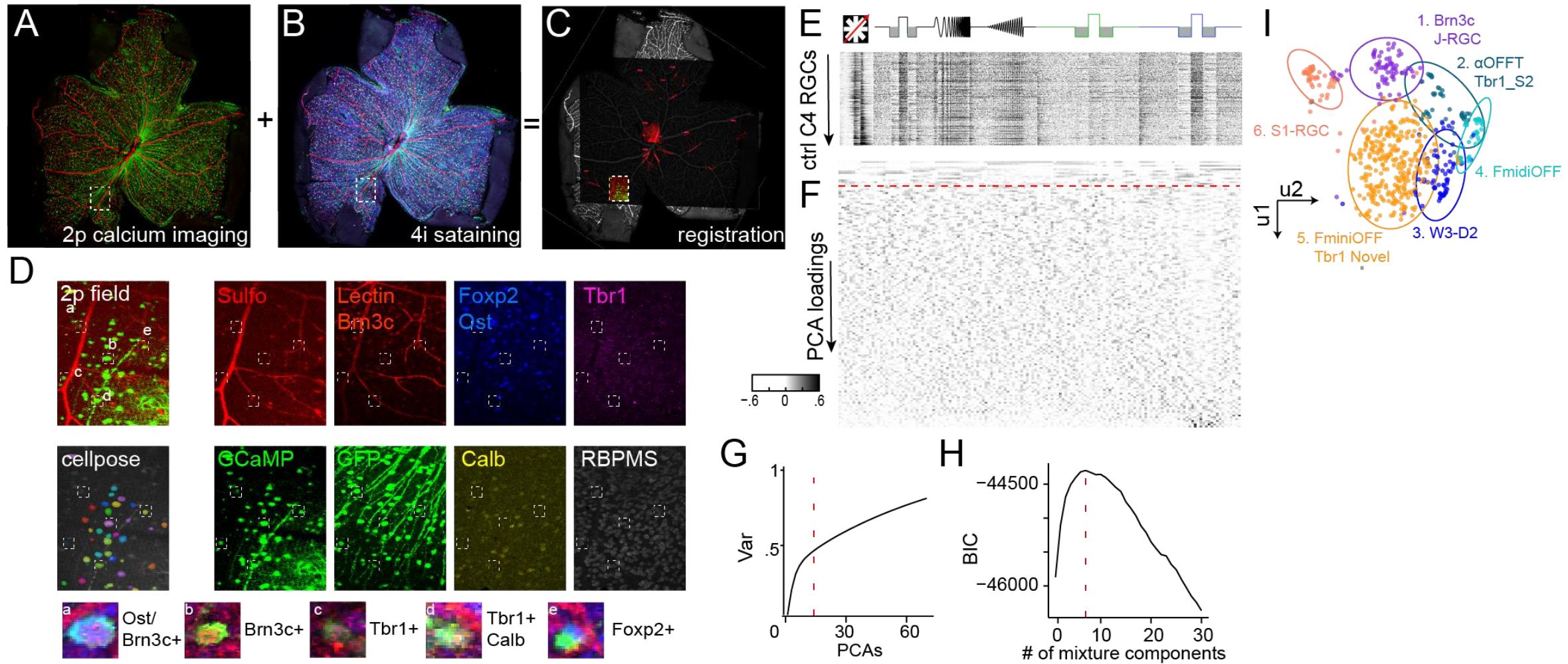


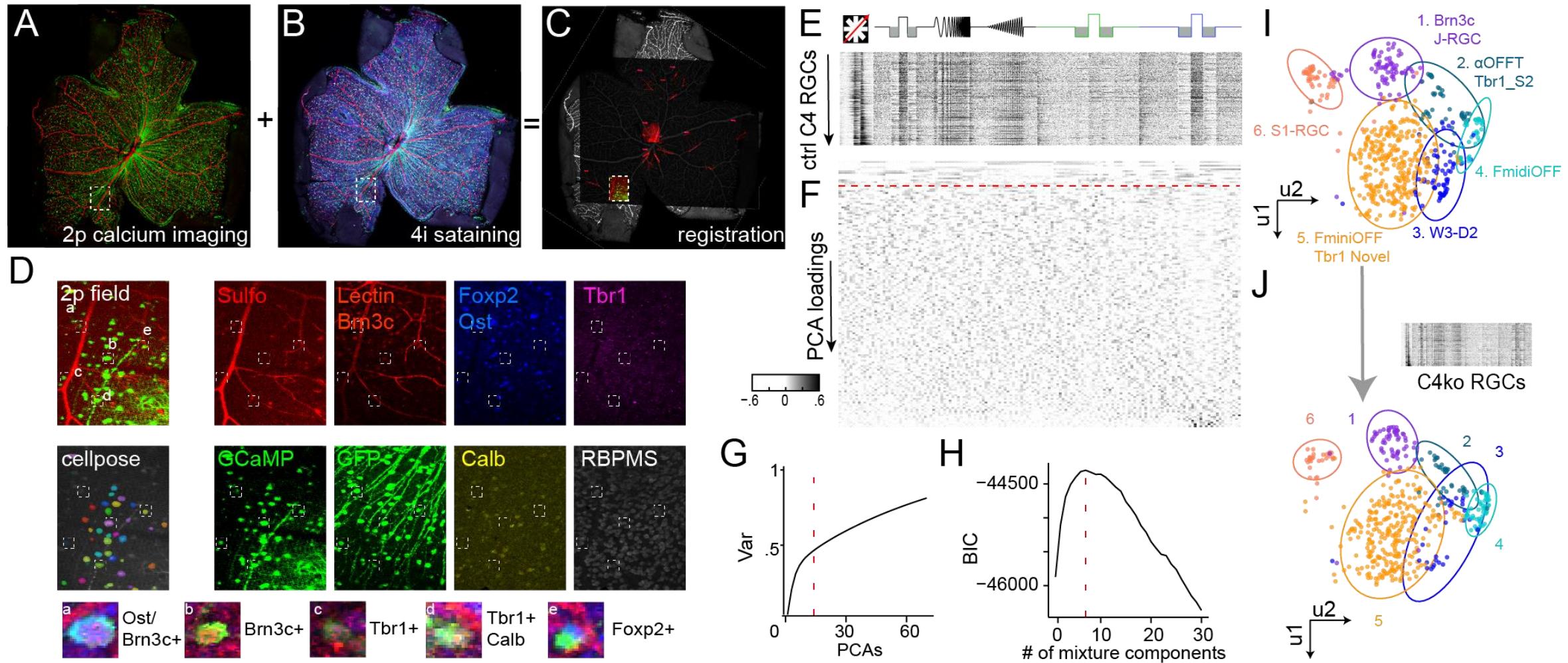


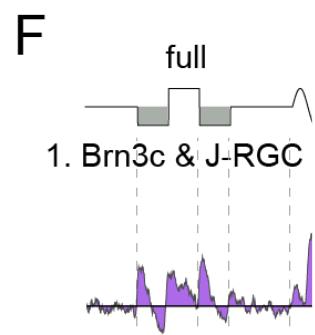


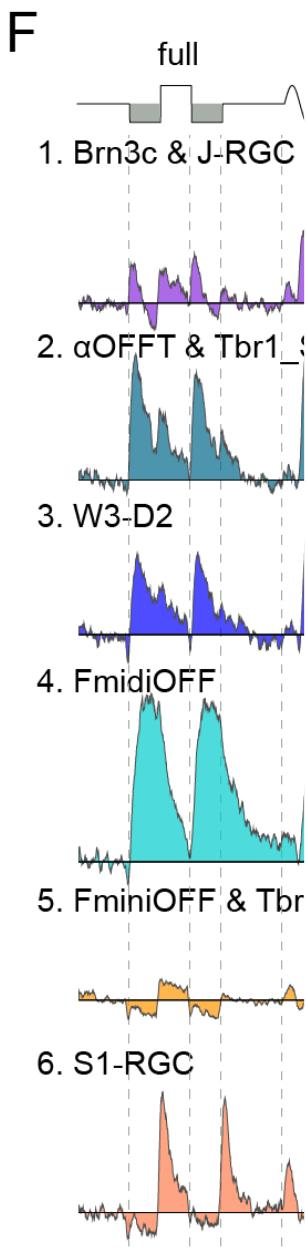




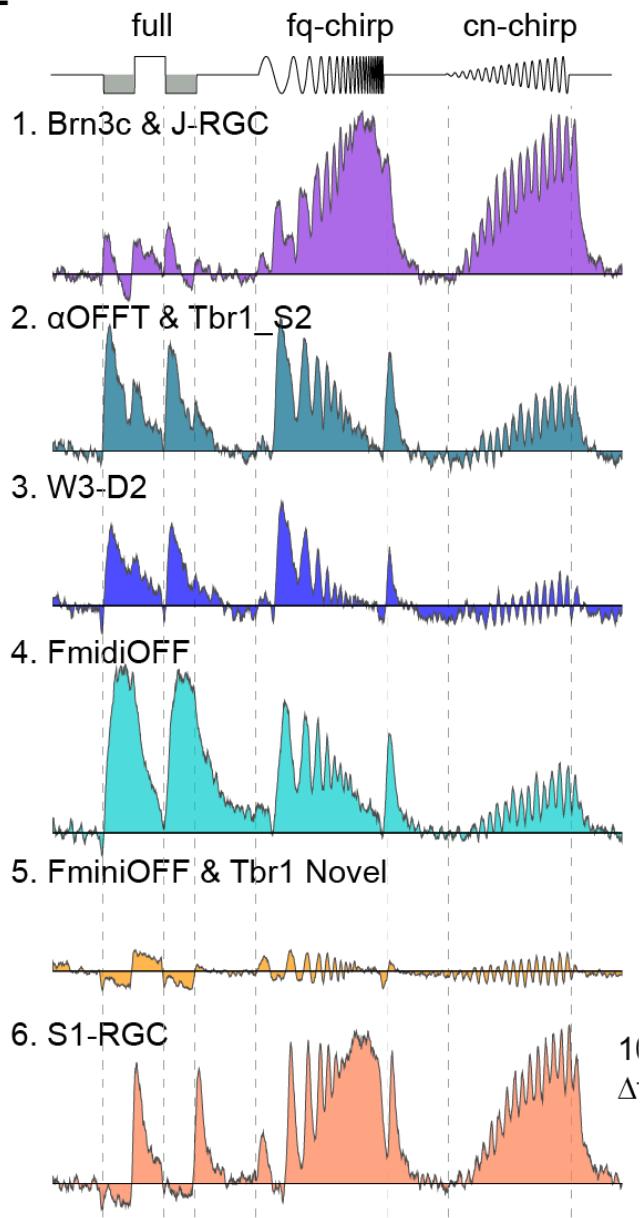




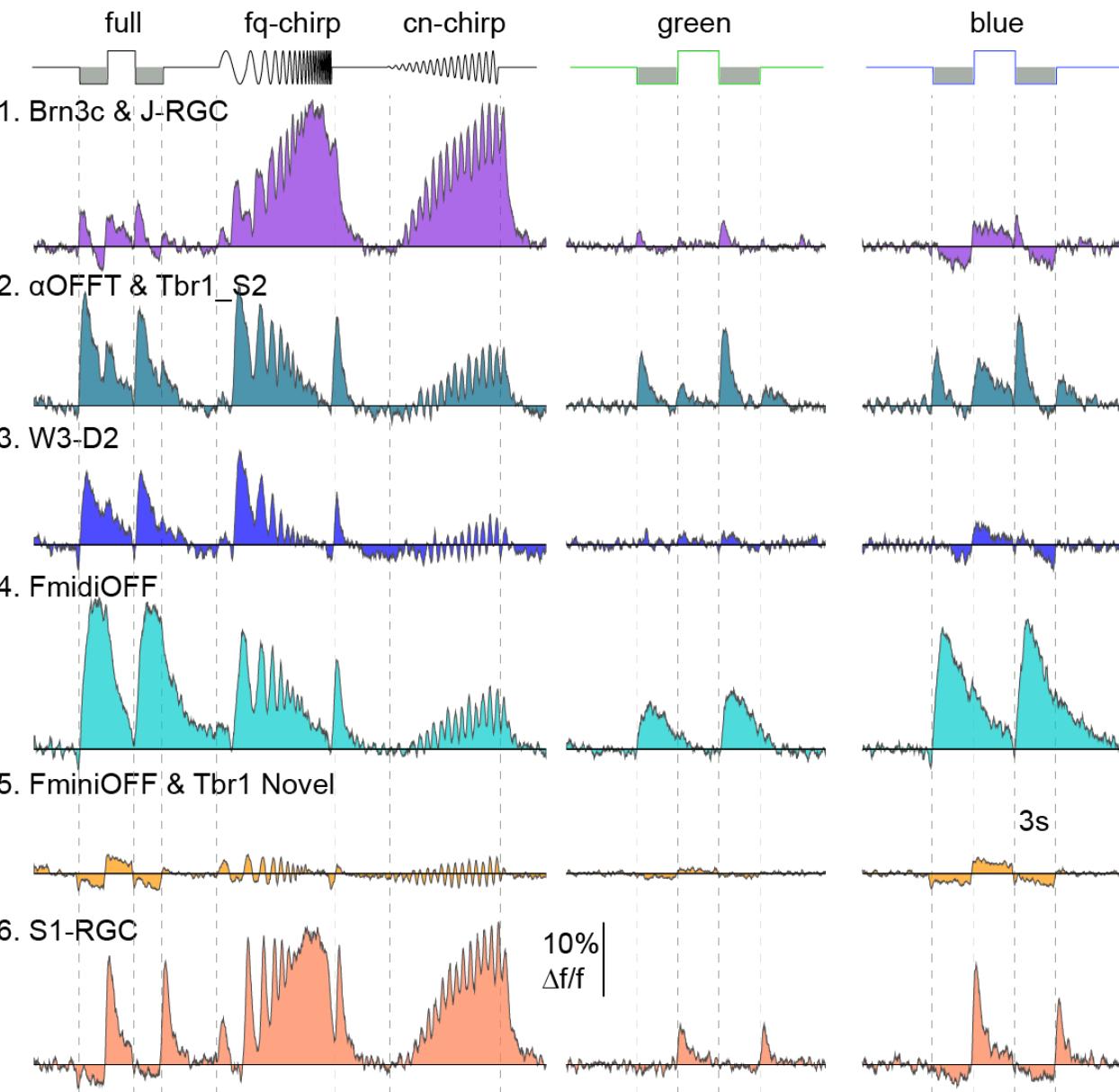


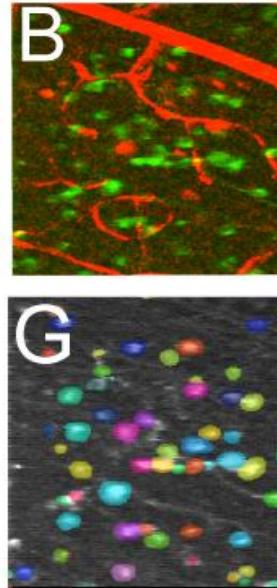
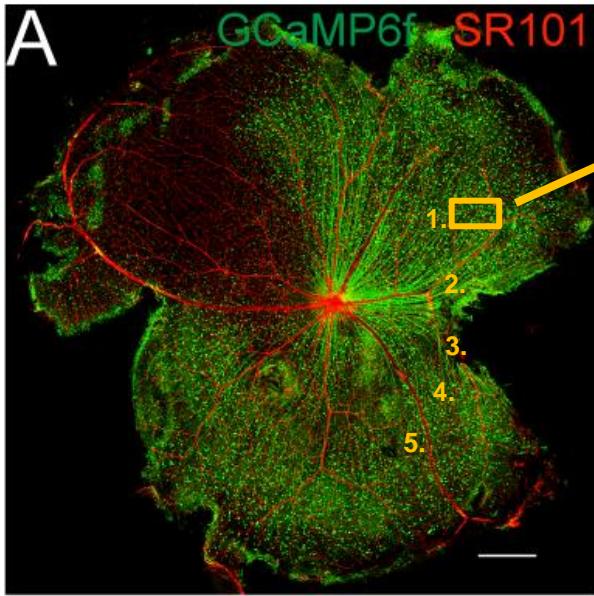
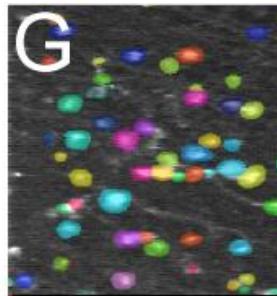


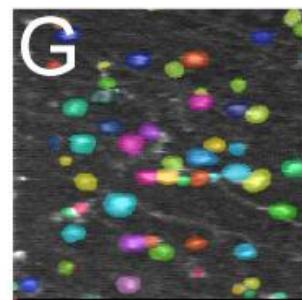
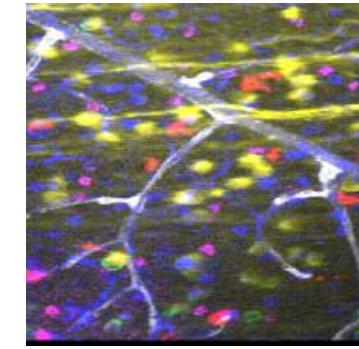
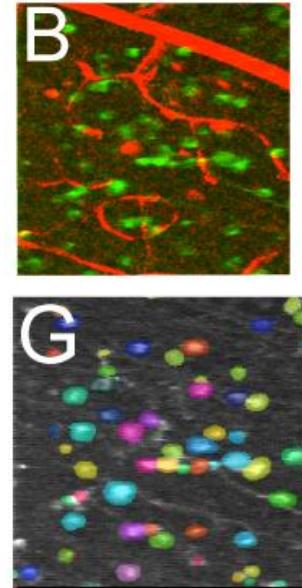
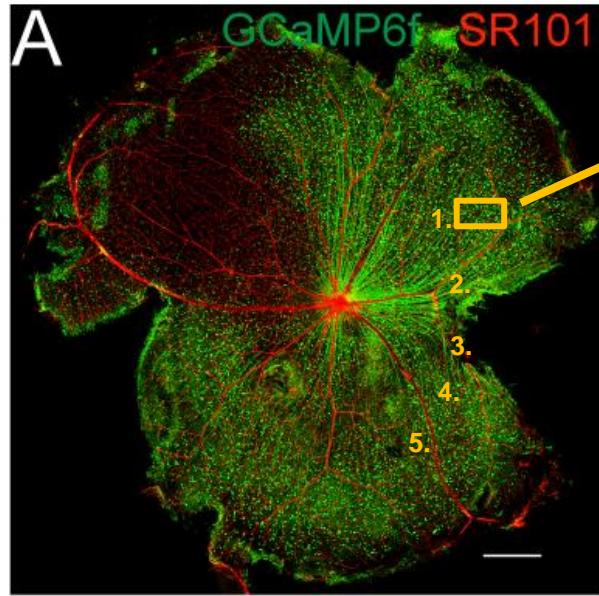
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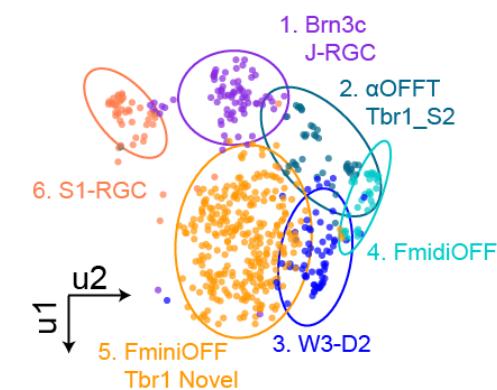
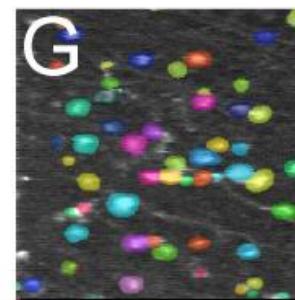
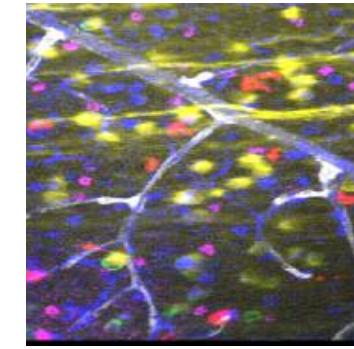
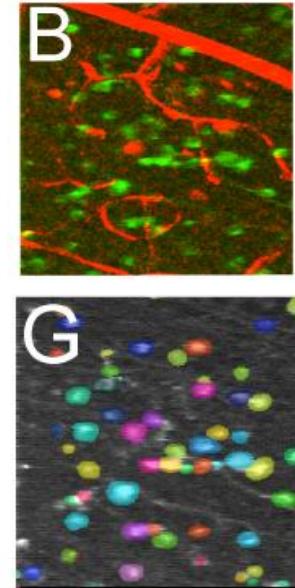
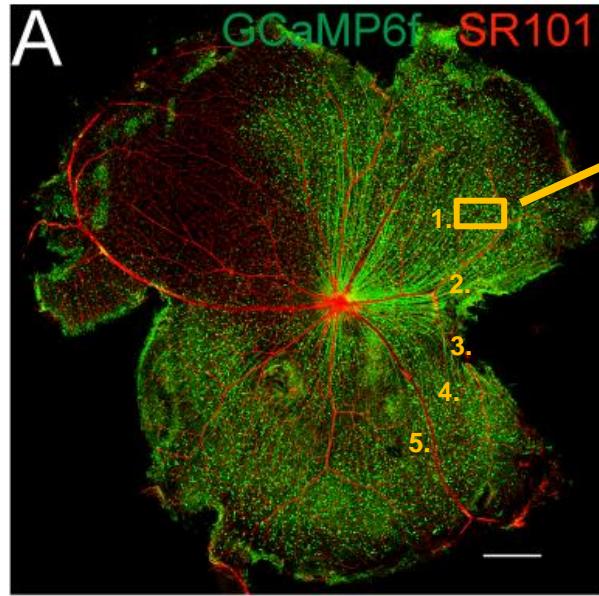
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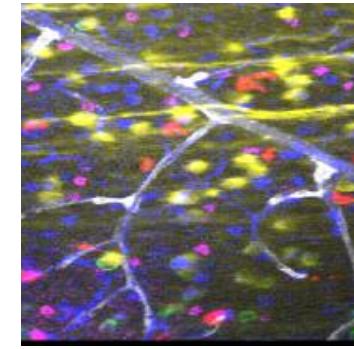
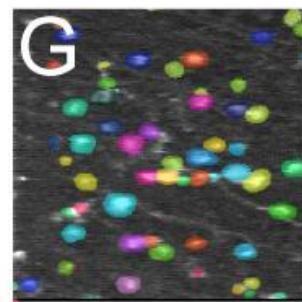
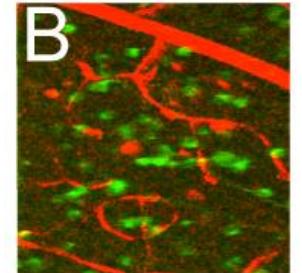
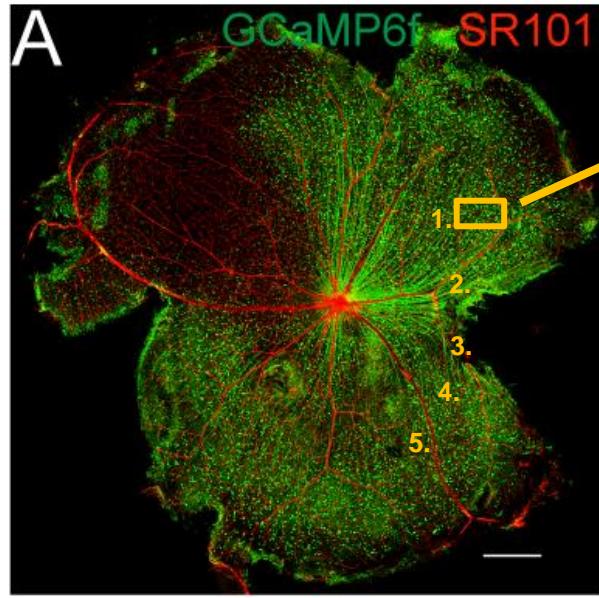


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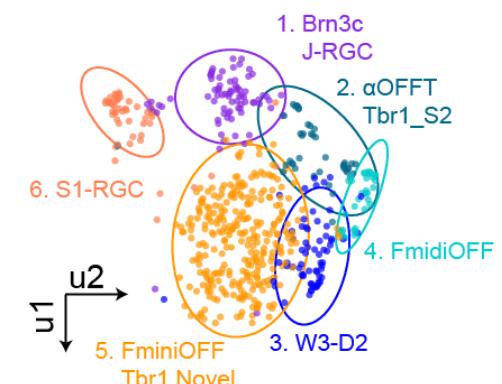
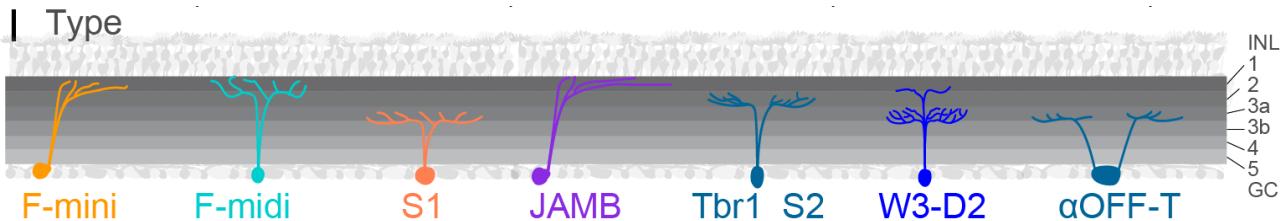


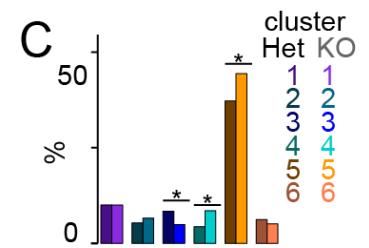
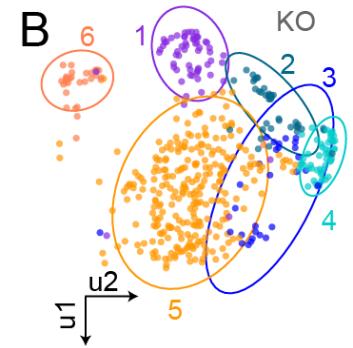
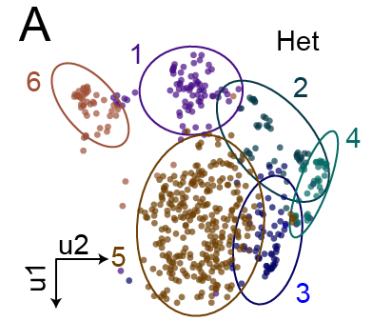
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Foxp2	Lectin
GFP	SR101

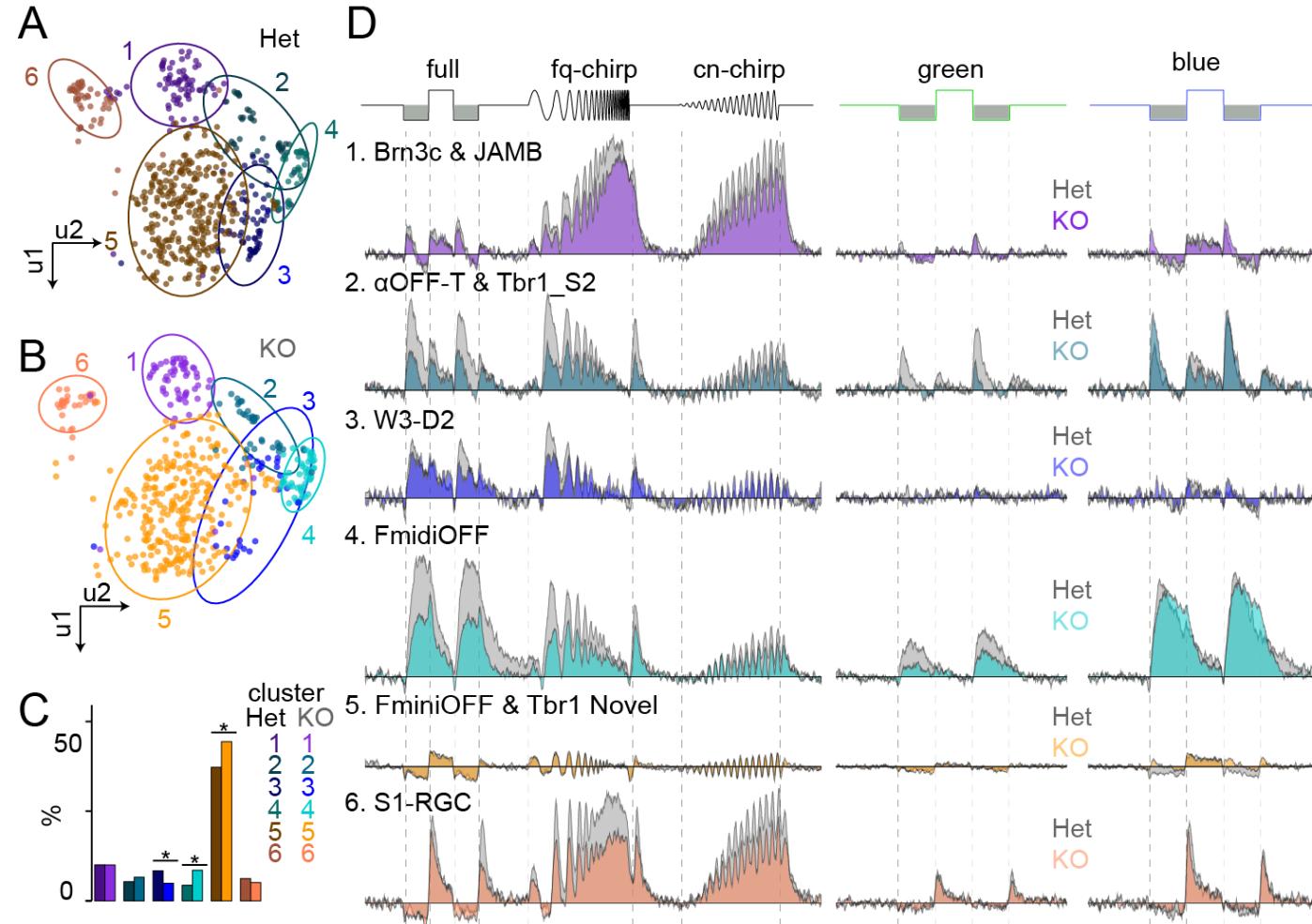


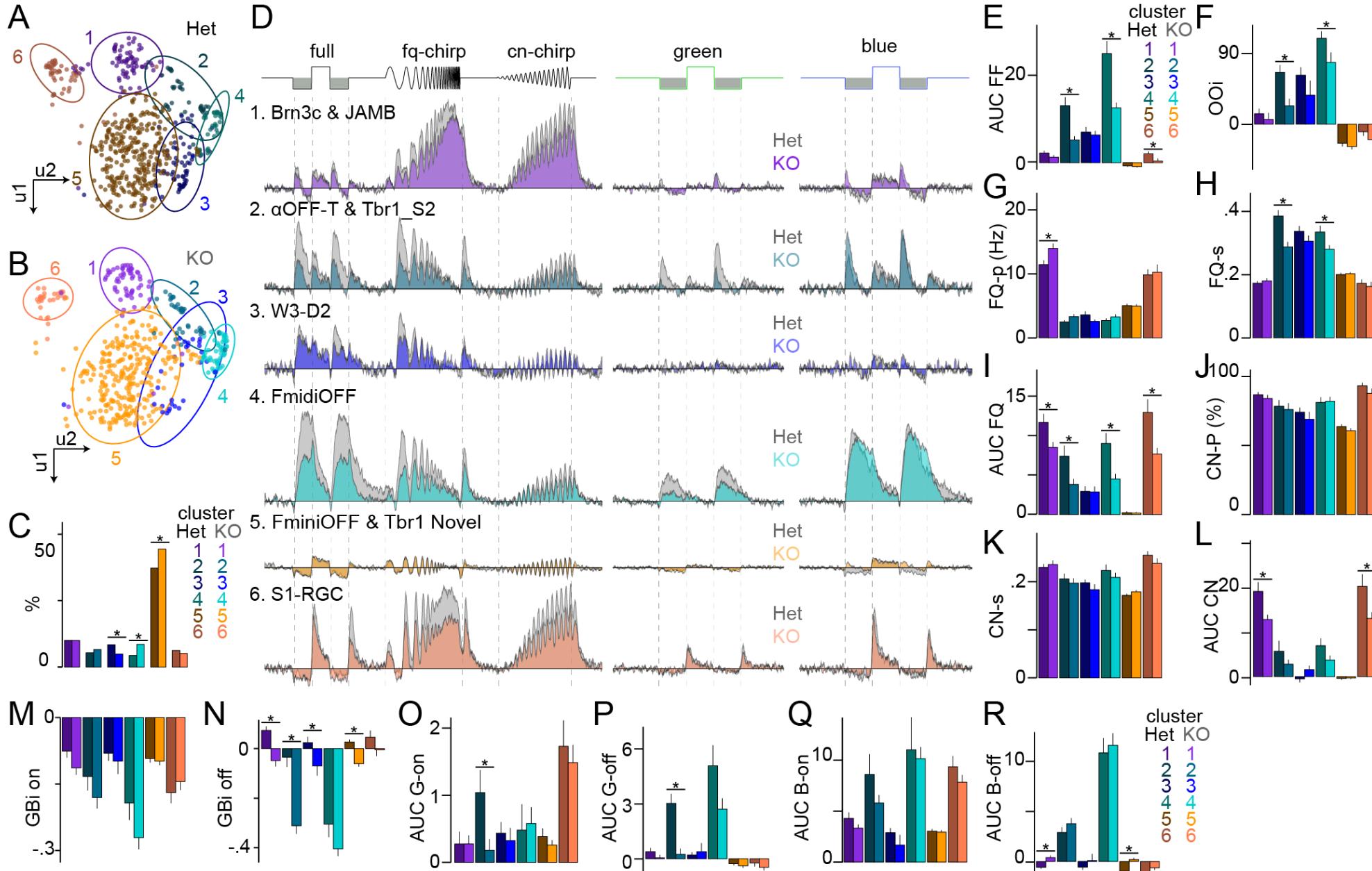


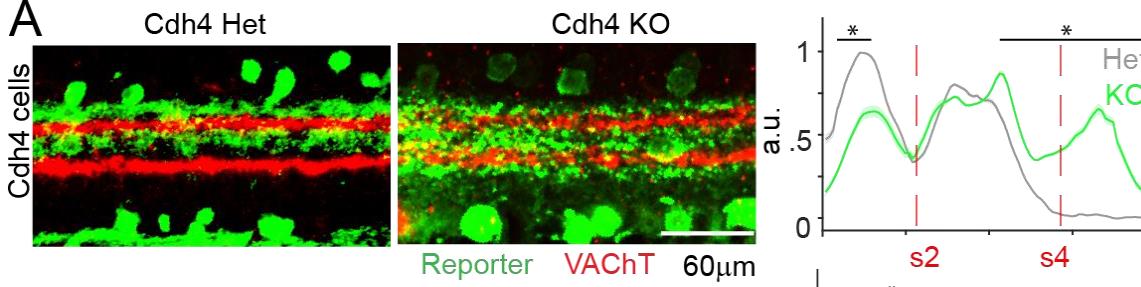
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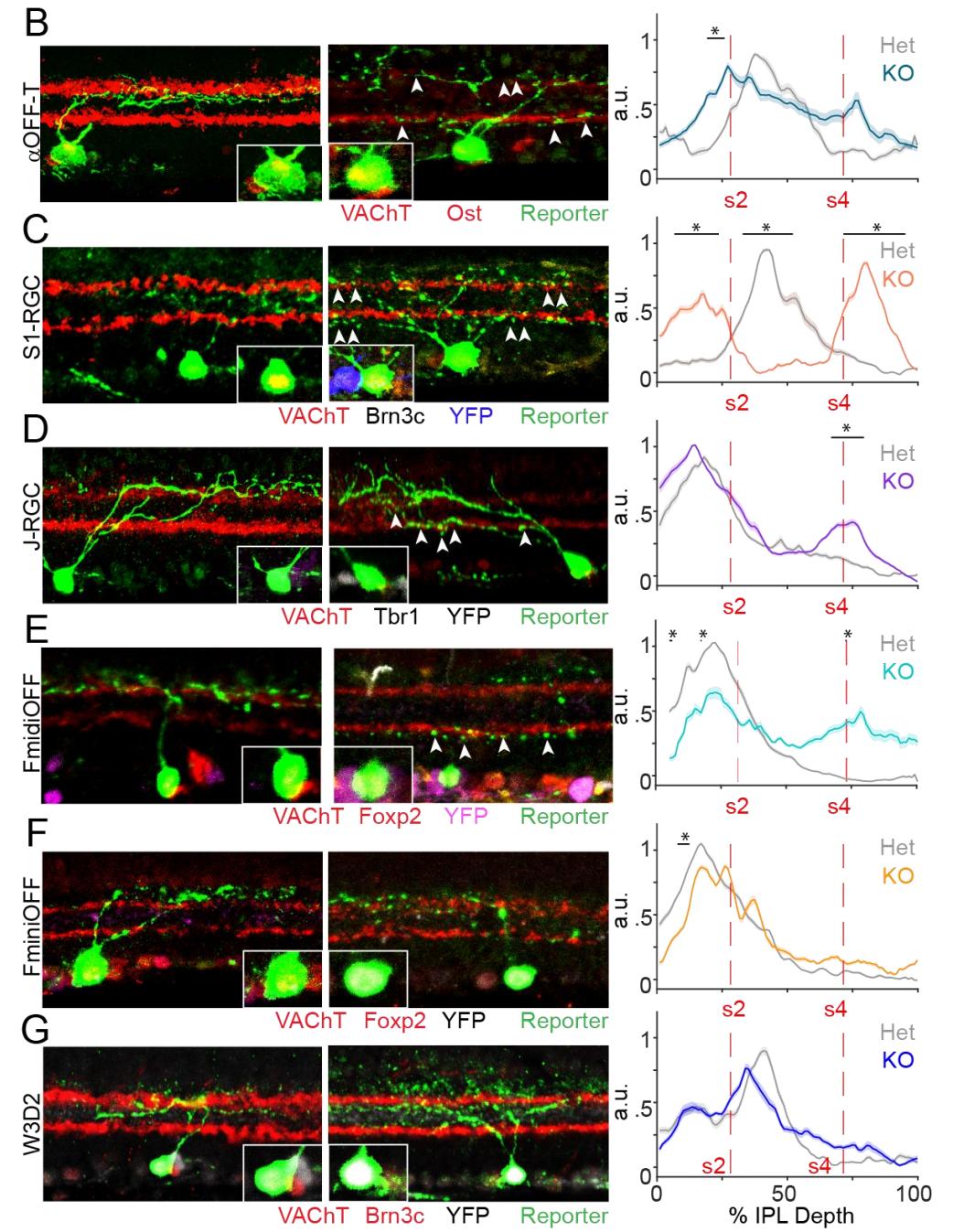
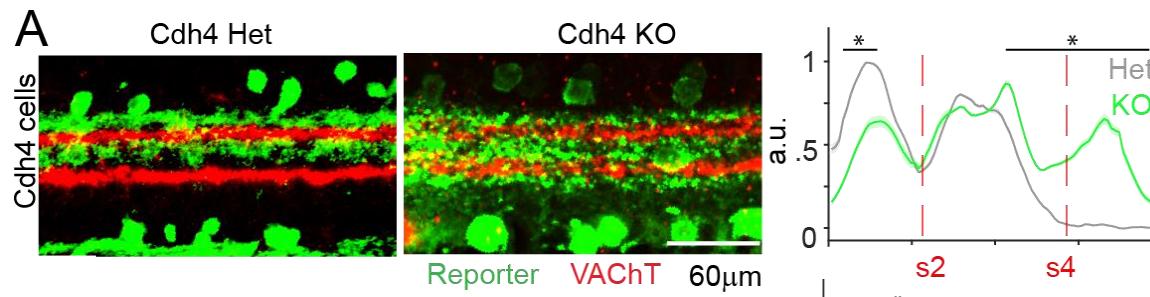


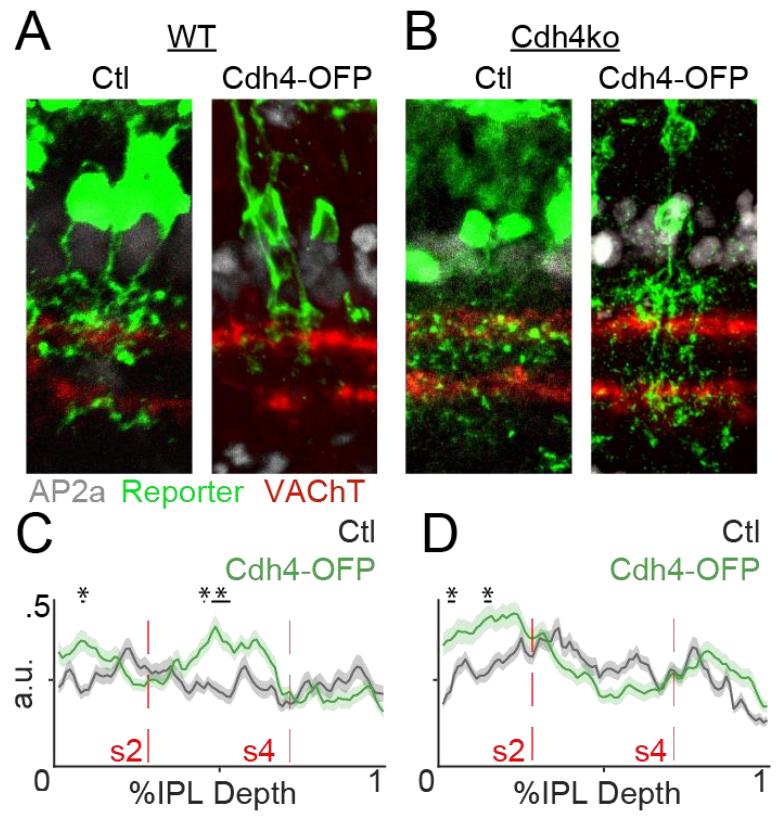


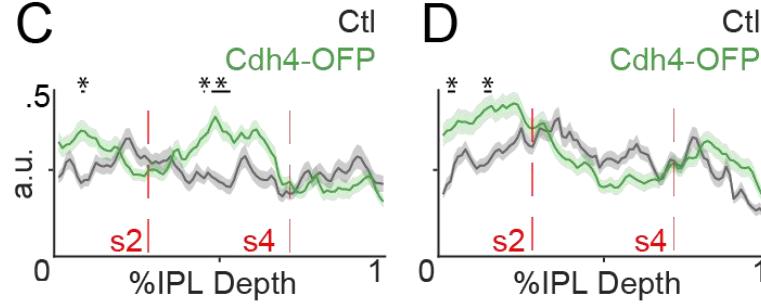
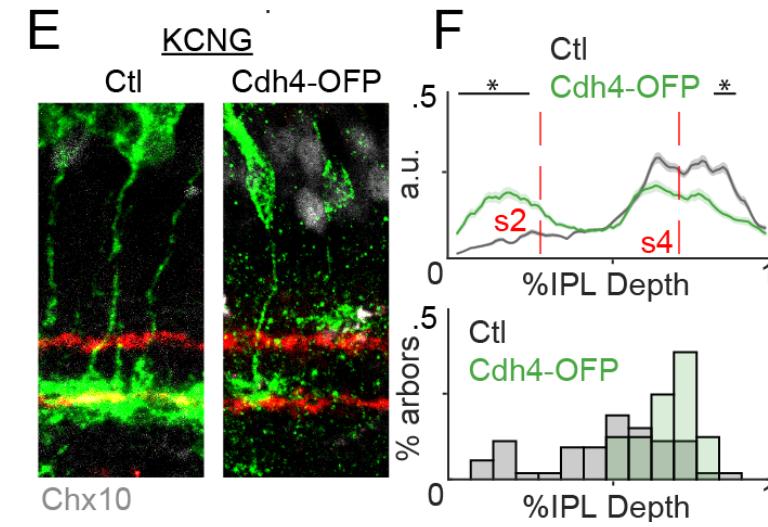
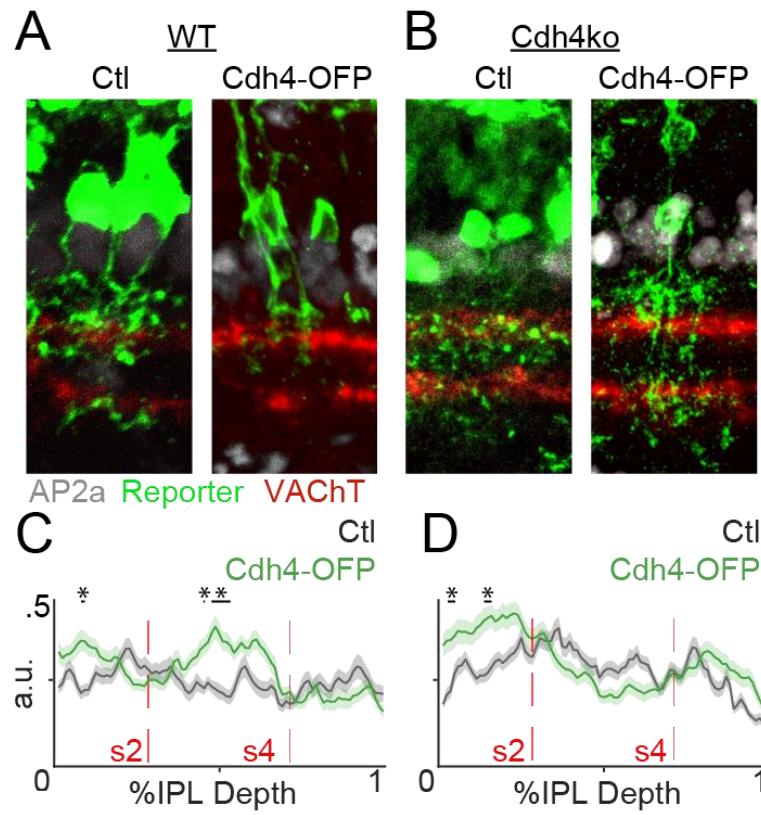


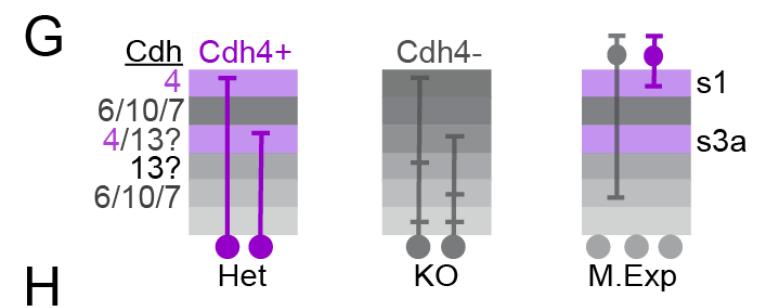
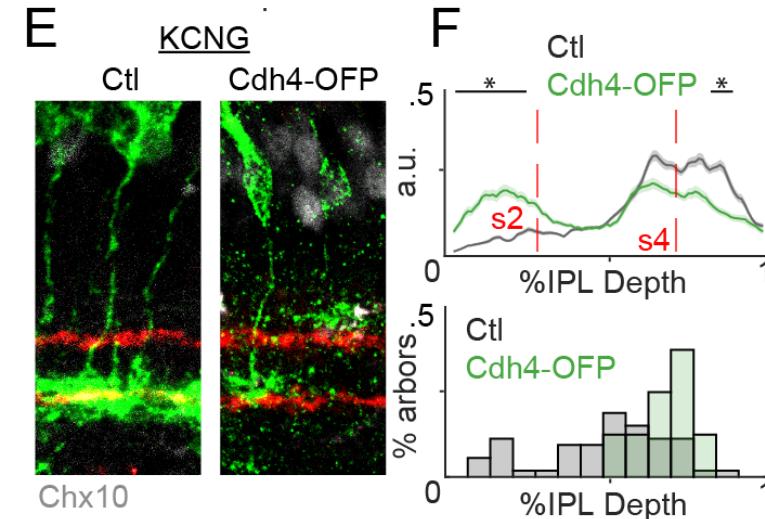
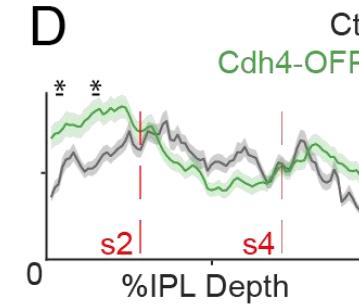
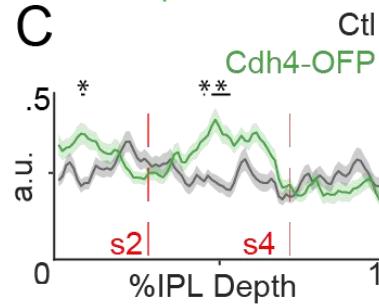
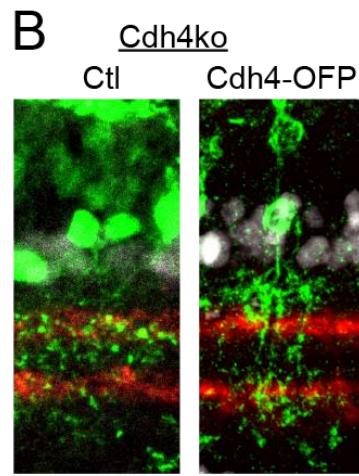
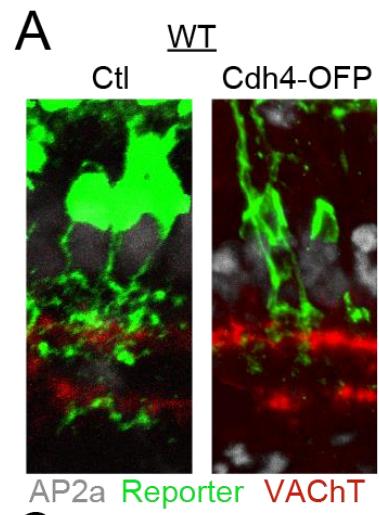


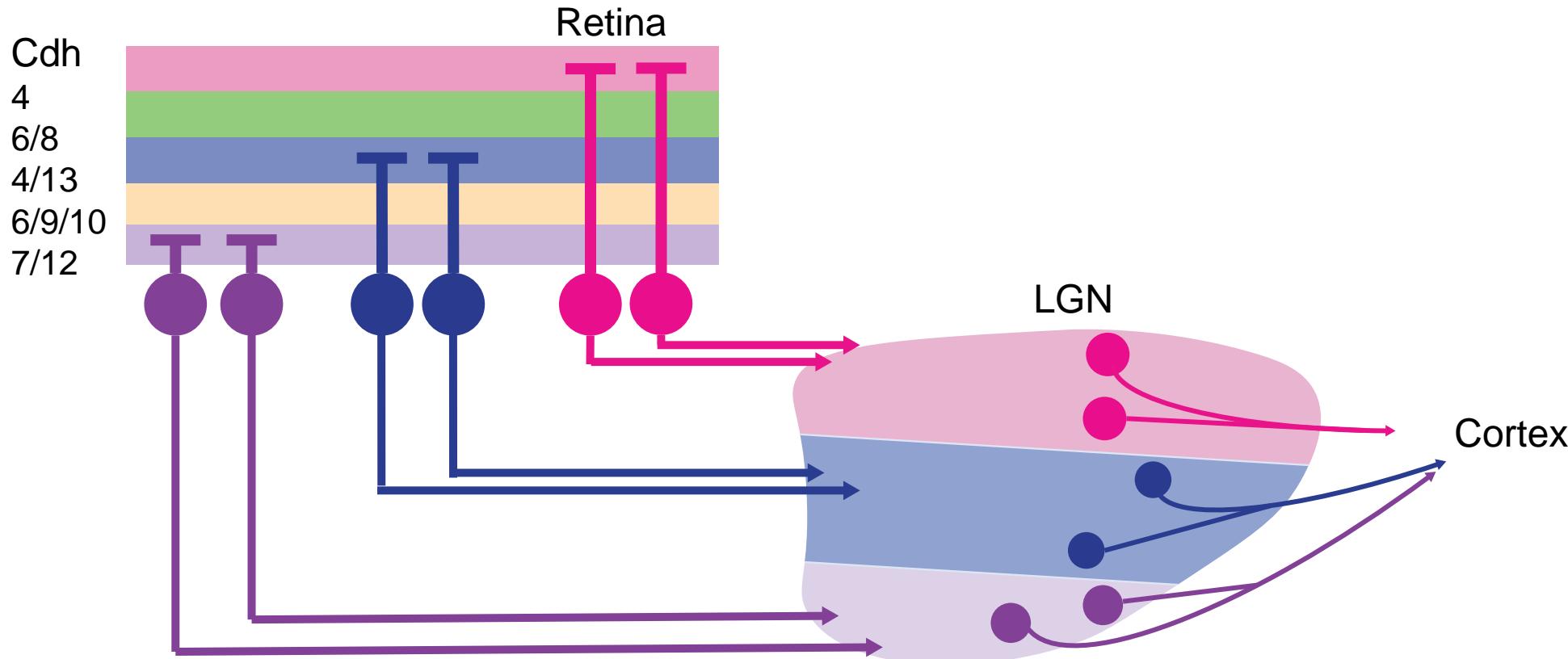
A











Acknowledgements

Swamy lab:

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Jonas Lehnert

Dr. Kuwook Cha

Dr. Eric Cook



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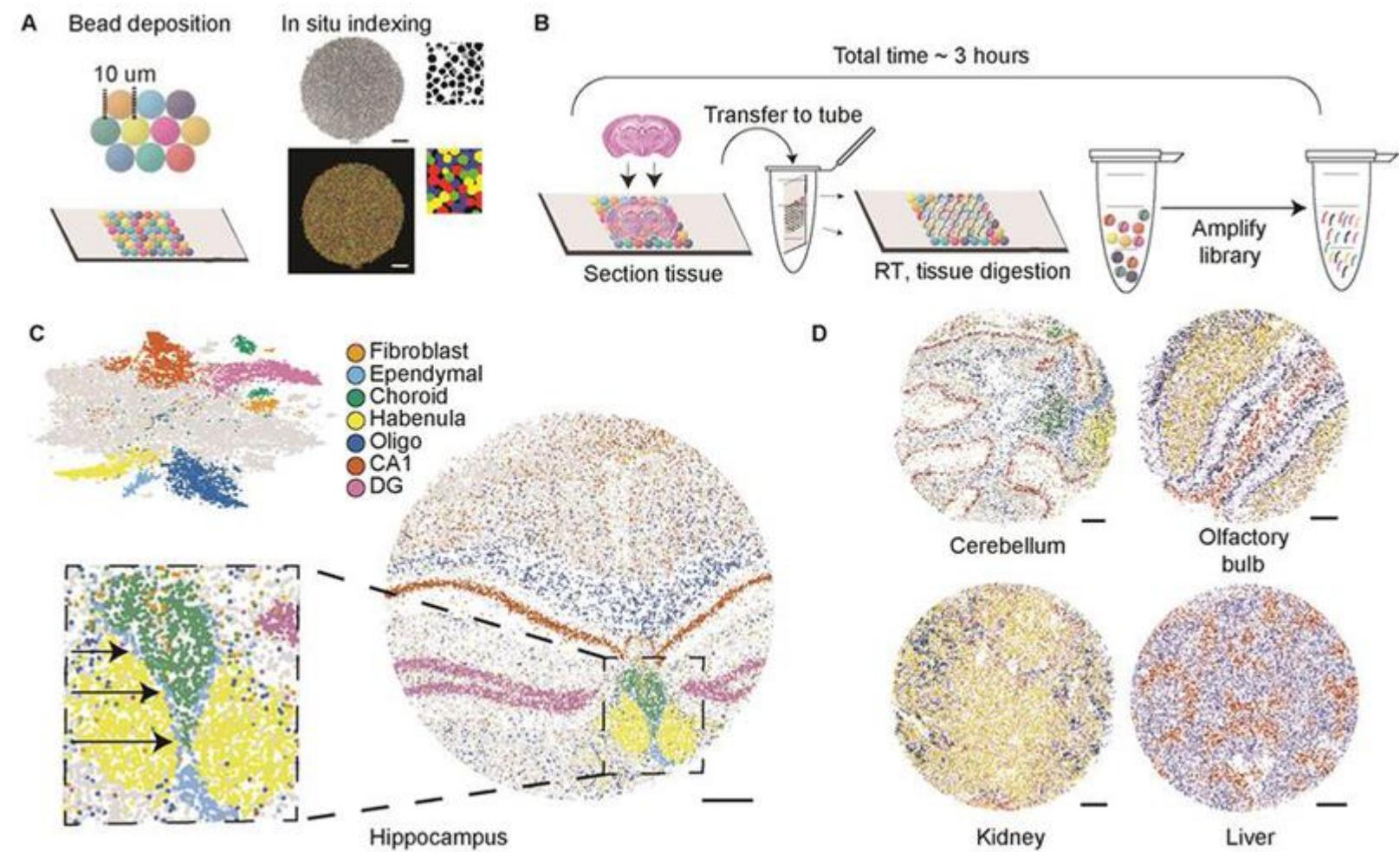
RÉSEAU DE RECHERCHE
EN SANTÉ DE LA VISION

VISION HEALTH
RESEARCH NETWORK


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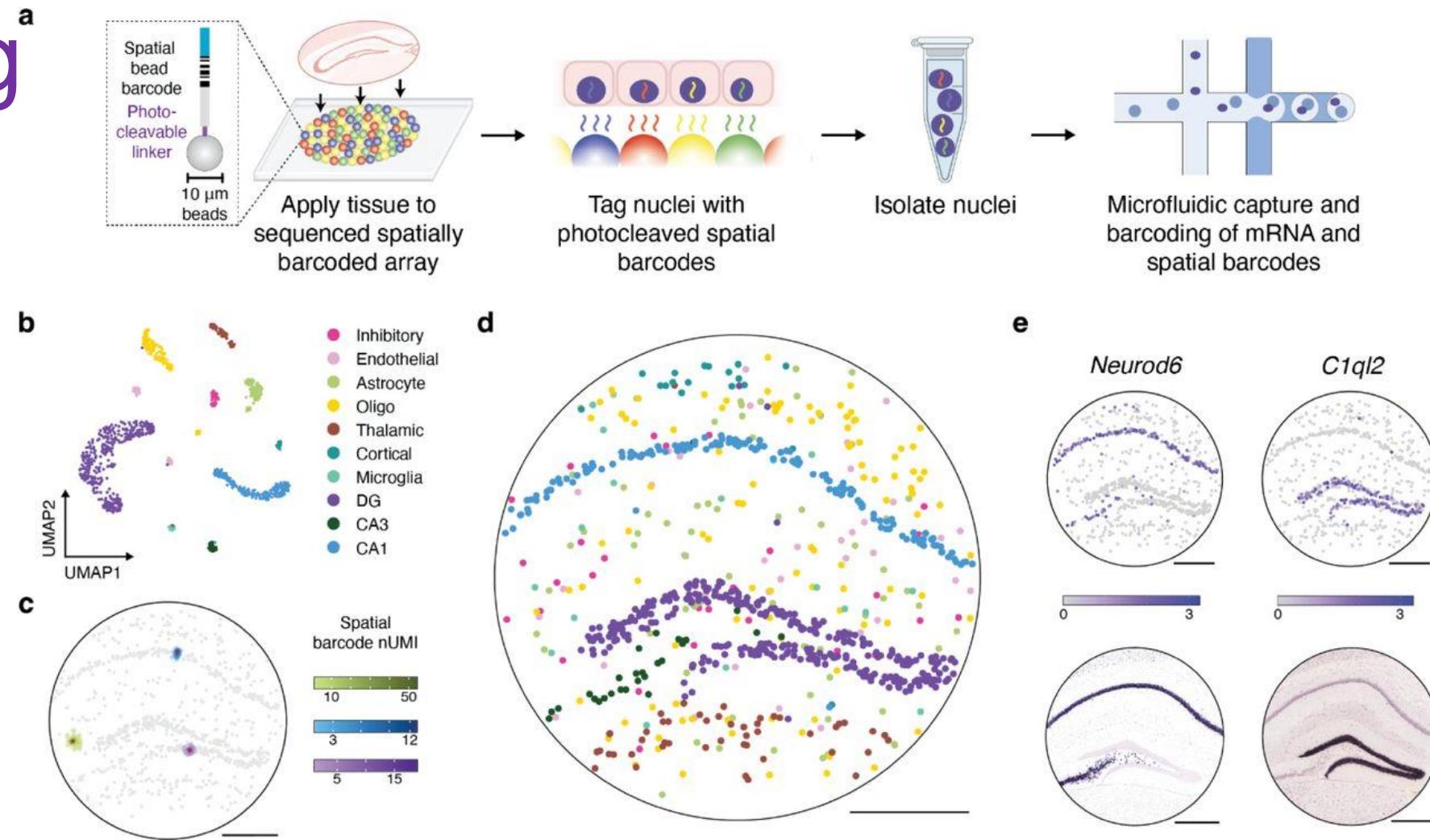


Slide-seq



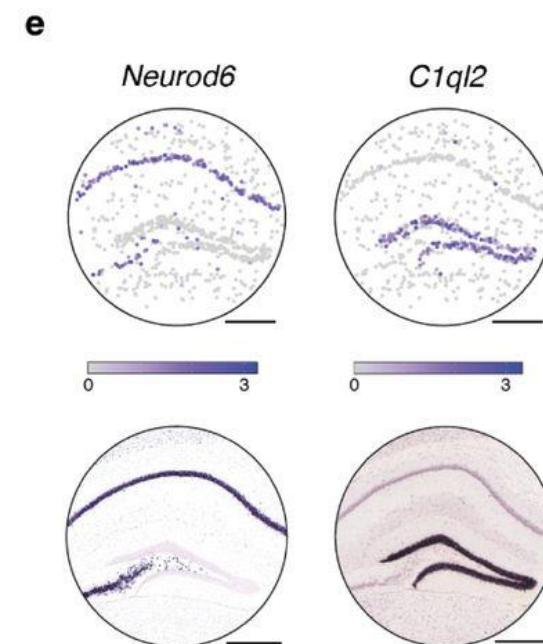
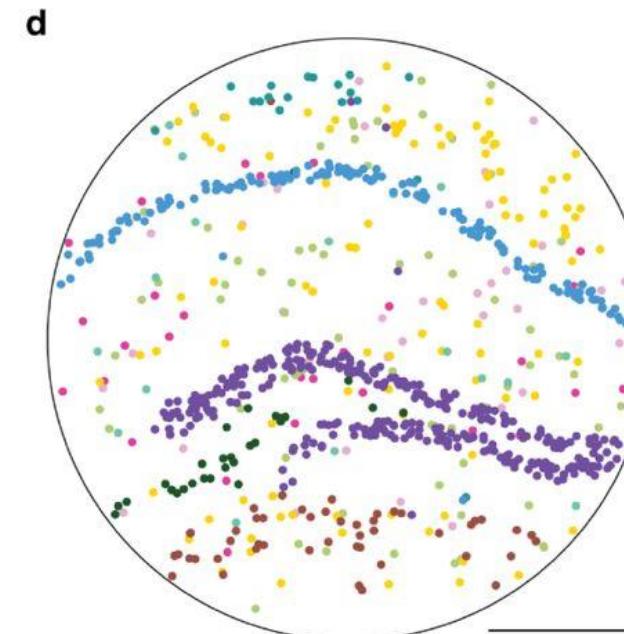
Rodrigues et al., 2019

Slide-tag



Spatial barcode nUMI

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3	12
5	15

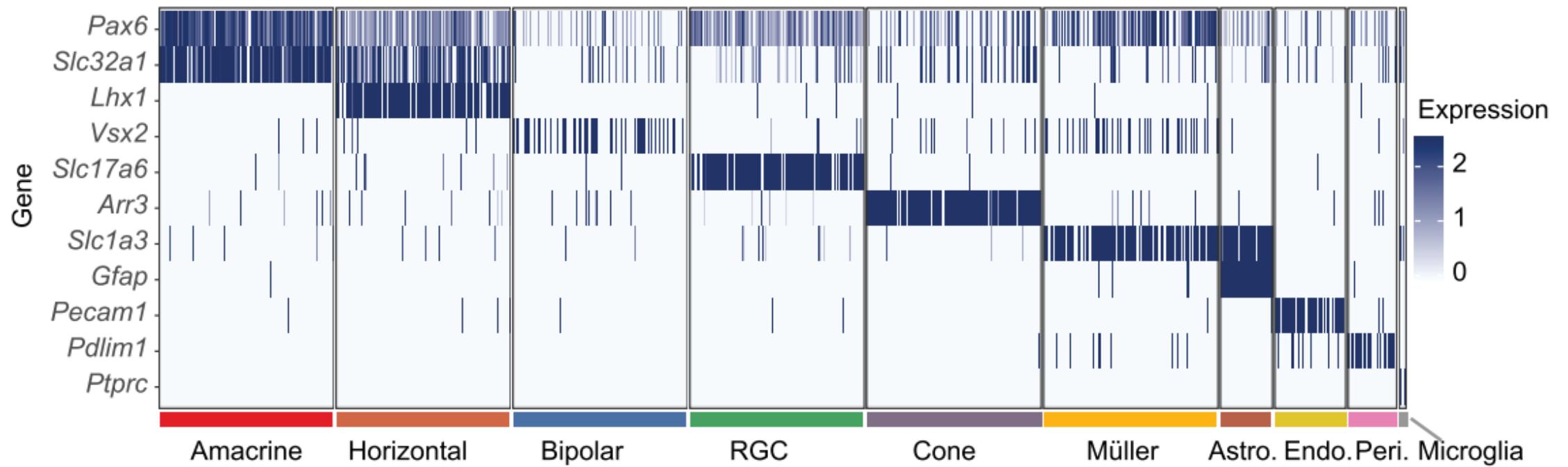


Mini introduction to R

- Open file “IntroToR.html”
“IntroToR.RMD” to run code along the explanation

Intro to Seurat

- Open file “RGC_singleCell_Analysis.html”
“RGC_singleCell_Analysis.RMD” to run code along the explanation



Hands on!!!

- On your computer you will find 5 different directories that correspond to brain regions. Your job is to choose the region that interest you the most and analyze it in Seurat.
- Each directory contain a brief description of the data and the paper that used that dataset.

Brain regions of interest for exercises

- Hypothalamus: [counts \(Zhang et al., 2021\)](#)
- spine cord: [counts \(Delile et al., 2019\)](#)
- Hippocampus: [TPMs \(Habib et al., 2016\)](#)
- Cortex: [TPMs and counts](#), also in the [Allen \(Tasic et al., 2016\)](#)
- LGN: [counts \(Bakken et al., 2021\)](#)

Questions?

- Contact: aline.rangelolguin@mail.mcgill.ca