



# The Cellular and Molecular Landscape of the Octopus chierchiai Central Brain

Jessica Stock<sup>1</sup>, Alyson Hally<sup>2</sup>, Chaichontat Sriworarat<sup>3</sup>, Kyla Woyshner<sup>2</sup>, Dominick Dickerson<sup>4</sup>,  
Rachel Latanich<sup>3</sup>, Güldölen<sup>4</sup>, Caroline Albertin<sup>1,\*</sup>, Loyal A. Goff<sup>2,3,5,6,\*</sup>

## Abstract

The pygmy zebra octopus (*Octopus chierchiai*) is an attractive emerging model organism to interrogate brain function and development, owing in part to its small size, early adult behaviors, and its capacity for multi-generational breeding in culture.

Building on the foundation of our recently assembled high-quality reference genome and transcriptome, we have begun to characterize the cellular and molecular organization of the central brain of *O. chierchiai*. Using a flexible, ultra-high-throughput method which we have optimized for cephalopod cellular physiology, we have established a preliminary atlas of single-nucleus transcriptional profiles, revealing the cellular diversity and transcriptional states within the adult optic lobe of *O. chierchiai*. In parallel, we have conducted unbiased spatial transcriptomic analysis at cellular resolution within the entire central brain, to provide a spatial map of cellular composition and resolve patterns of gene co-regulation across anatomical features. The integration of these data will provide a detailed view of the cellular and molecular organization of the brain, a critical step for unraveling the neurological underpinnings of cephalopod intelligence and behavior.

The availability of this atlas enhances the utility of *O. chierchiai* for targeted functional studies and provides a valuable asset for comparative neurobiology and the continued development and application of modern, high-throughput molecular biology assays in cephalopods.

## Experimental Questions

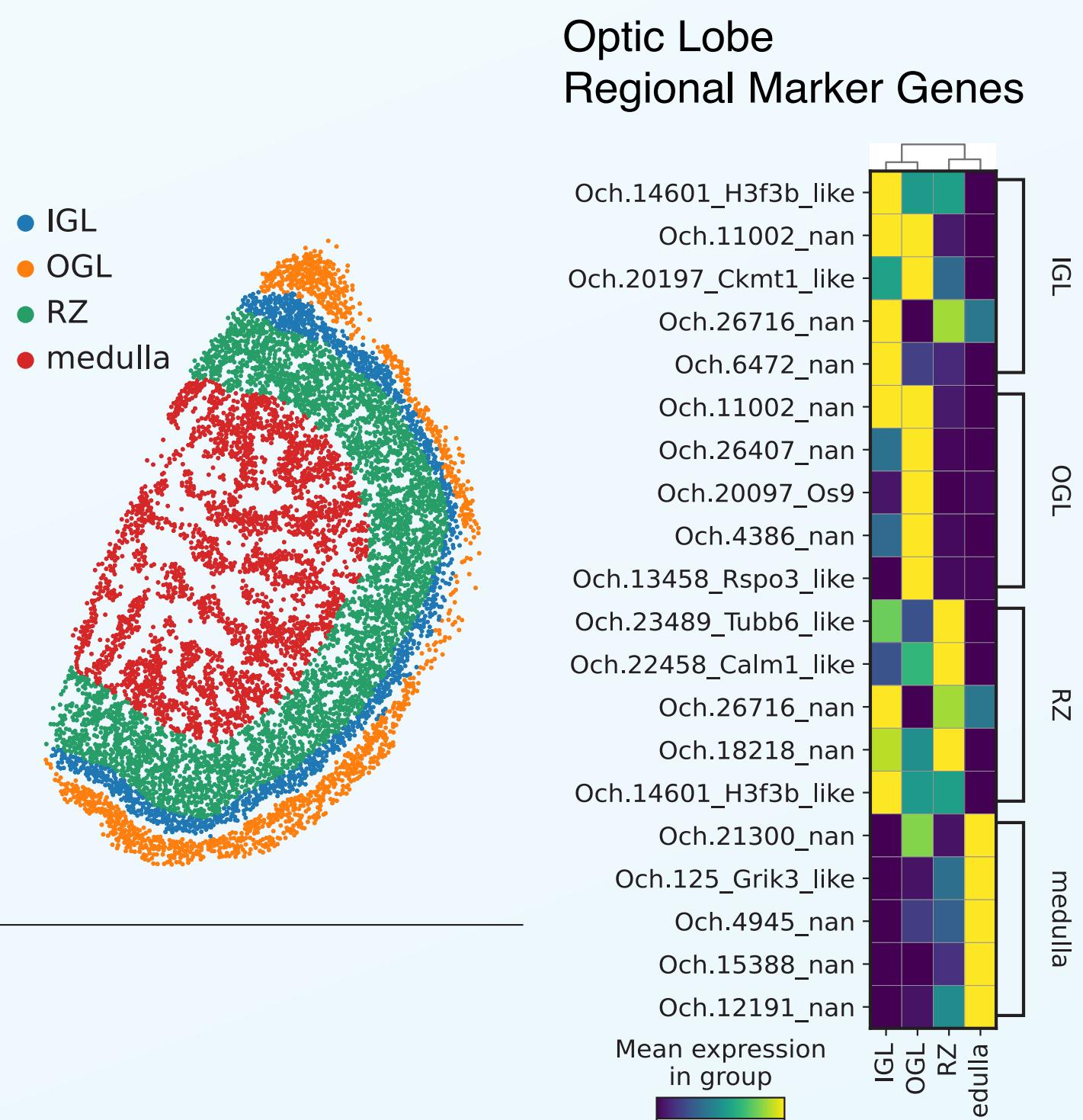
- Can we adapt the flexible, ultra-high-throughput sci-RNA-Seq3 method to cephalopod cellular physiology?
- Can we identify regional differences in gene expression across the *O. chierchiai* central brain?

## Unbiased Spatial Transcriptomics Analysis Using Curio Seeker

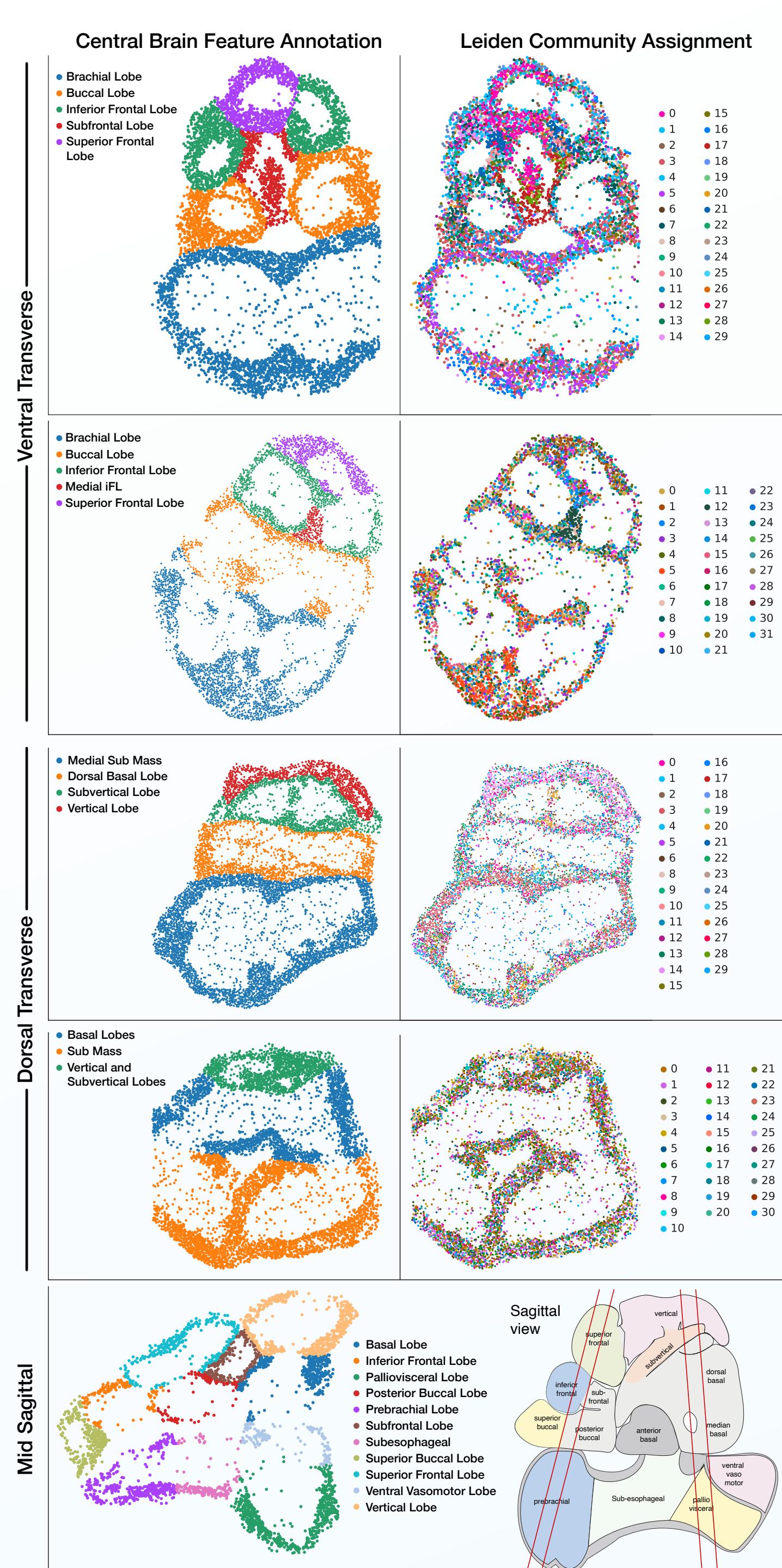


**Curio Seeker Workflow**  
Tissue section is placed onto specialty slide boards and then cut into 10 µm sections. These sections are then used for cDNA synthesis, bead recovery from substrate, NGS library preparation, and sequencing. The final product is a map of differential expression.

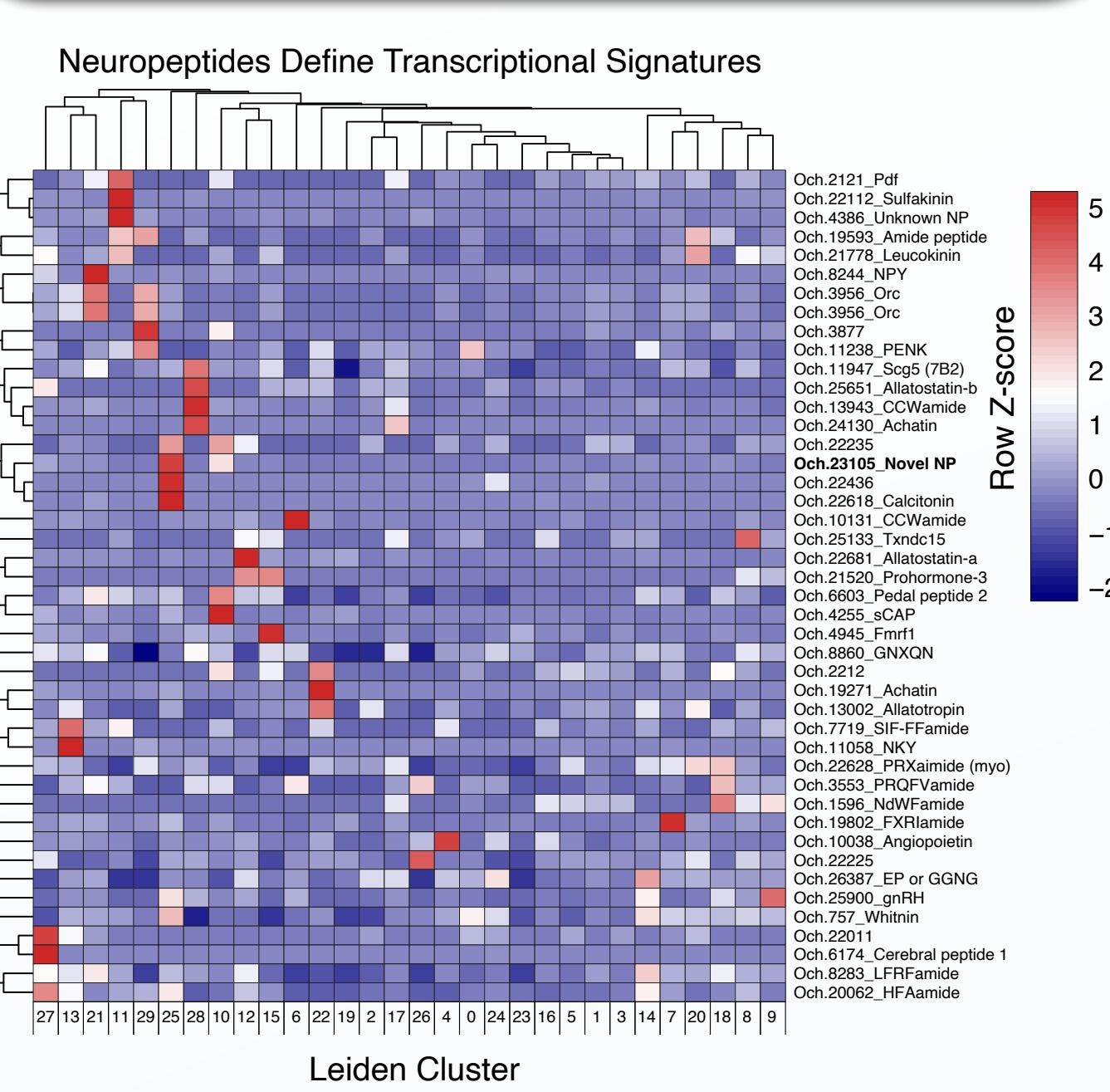
## Regional Variation in Optic Lobe



## Brain Feature Annotation

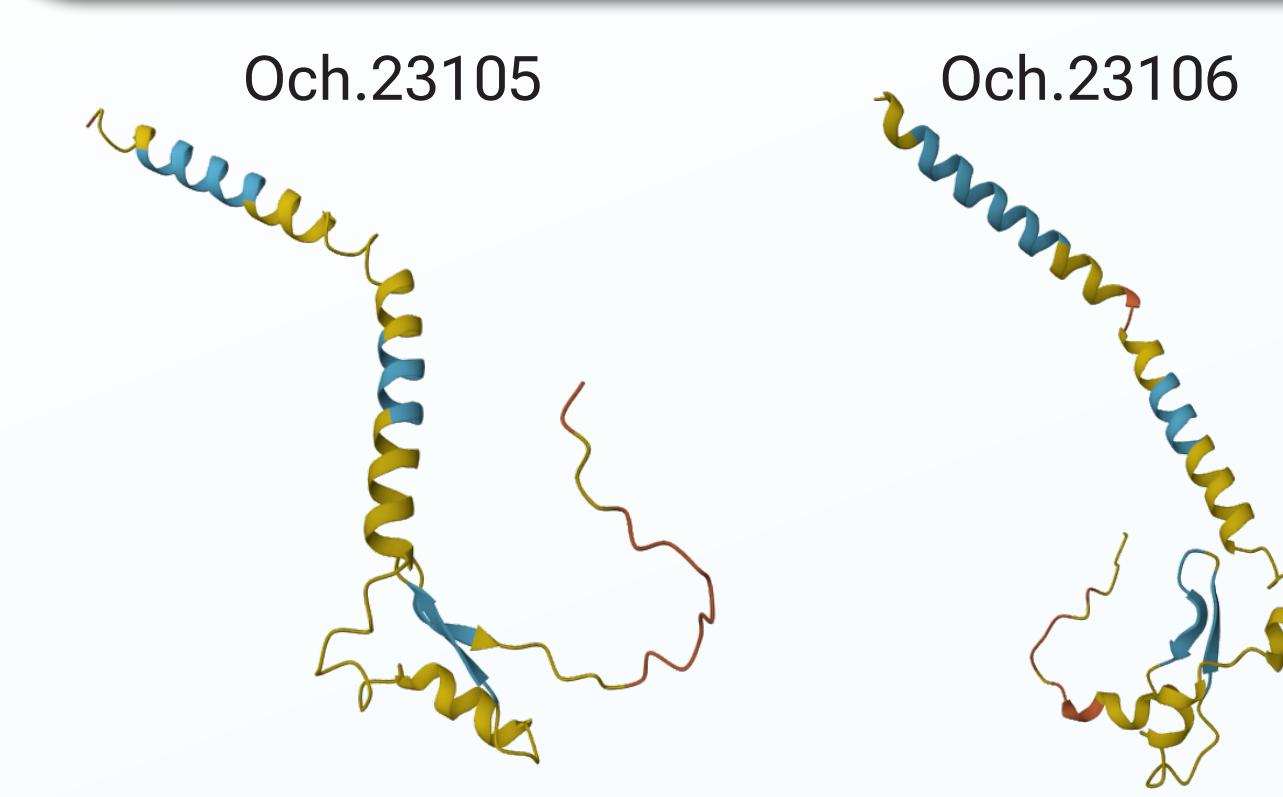


## Neuropeptides Drive Spatial Transcriptional Diversity



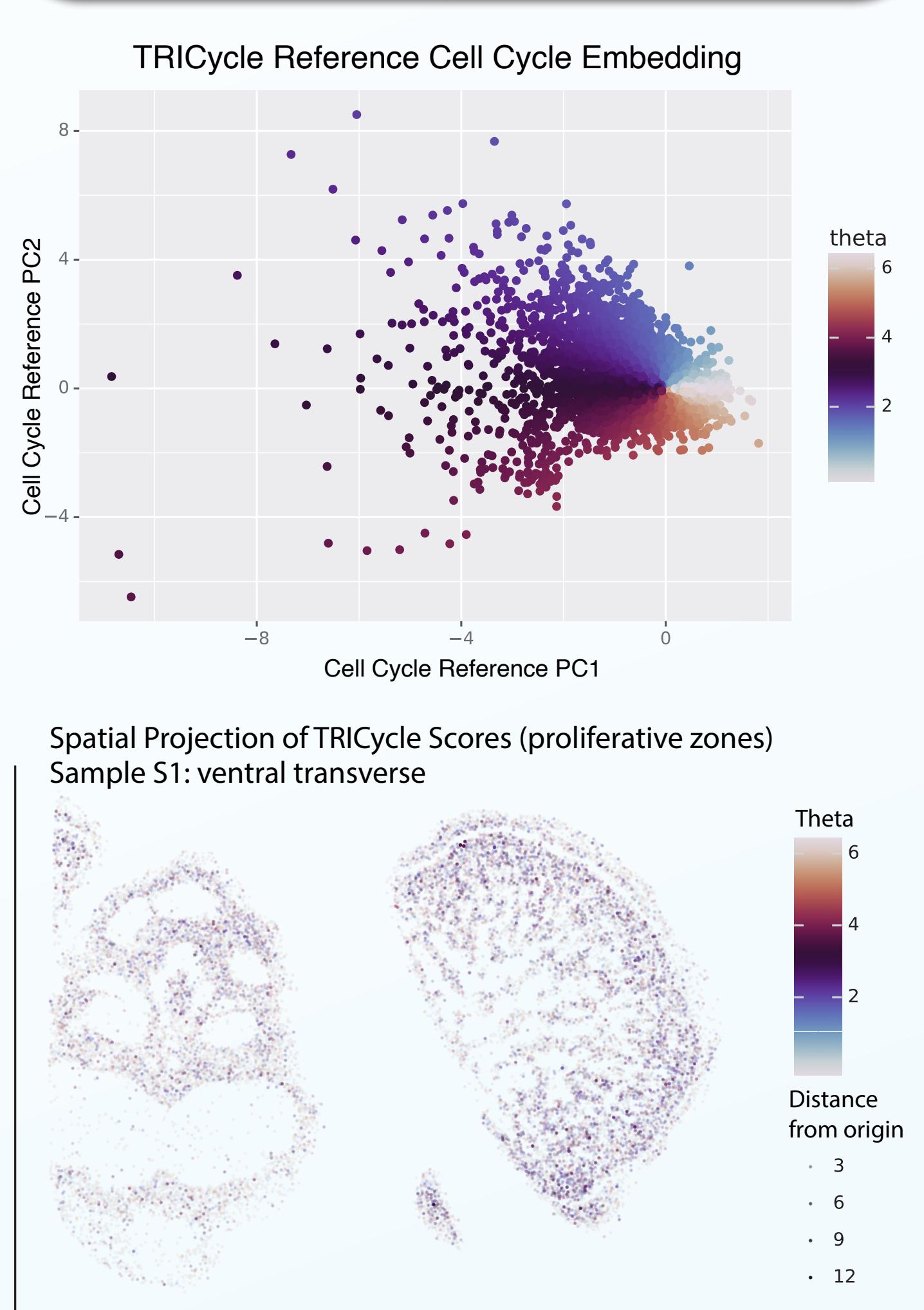
• Combinations of neuropeptides define many spot clusters

## Octopus-Specific Novel NKY-like Neuropeptide



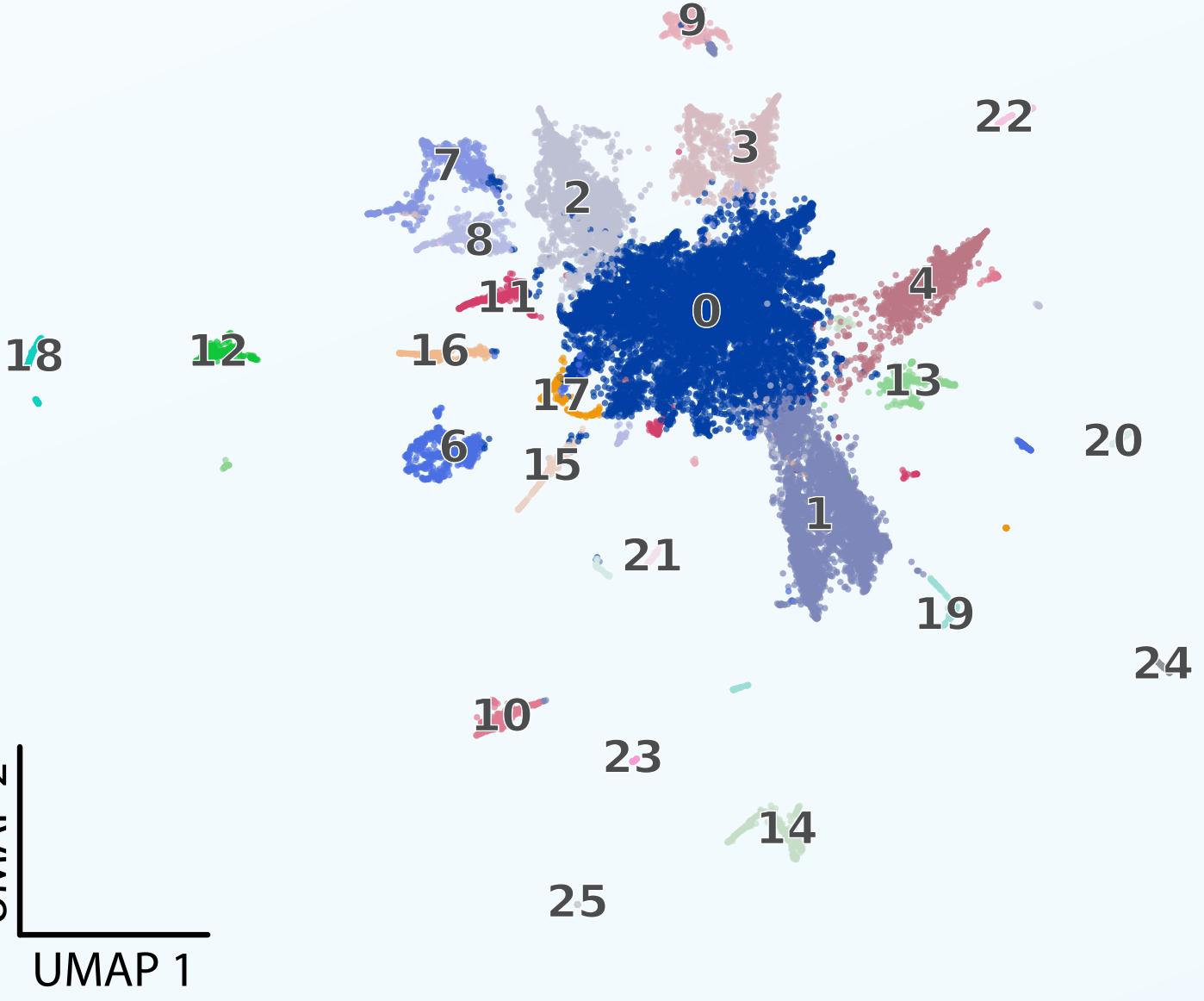
• Identification of octopus-specific neuropeptide with specific, regionalized expression in brain

## TRICycle analysis of cell proliferation

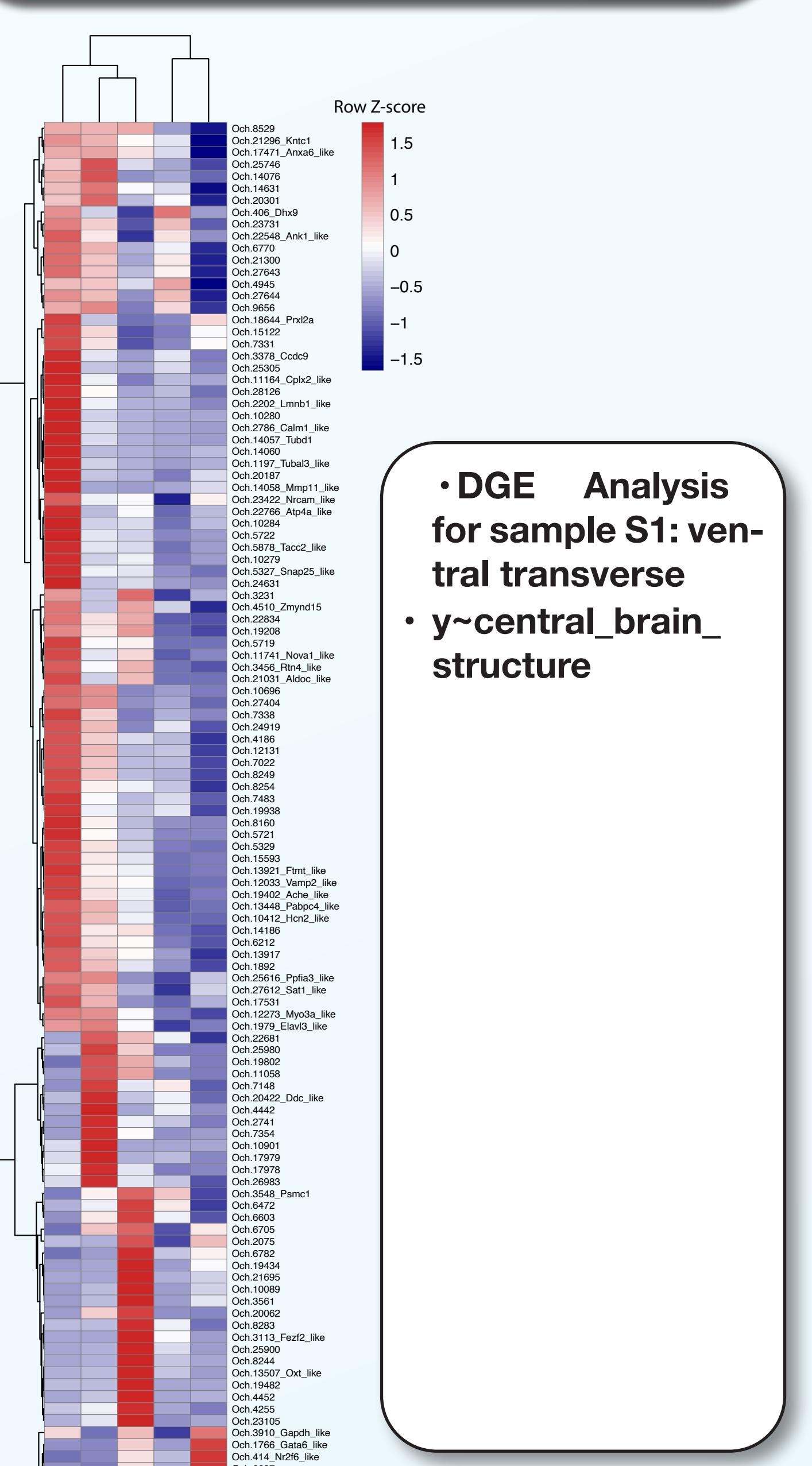


## Optimized Ultra-High-Throughput snRNA-Seq

### Leiden Cell Types



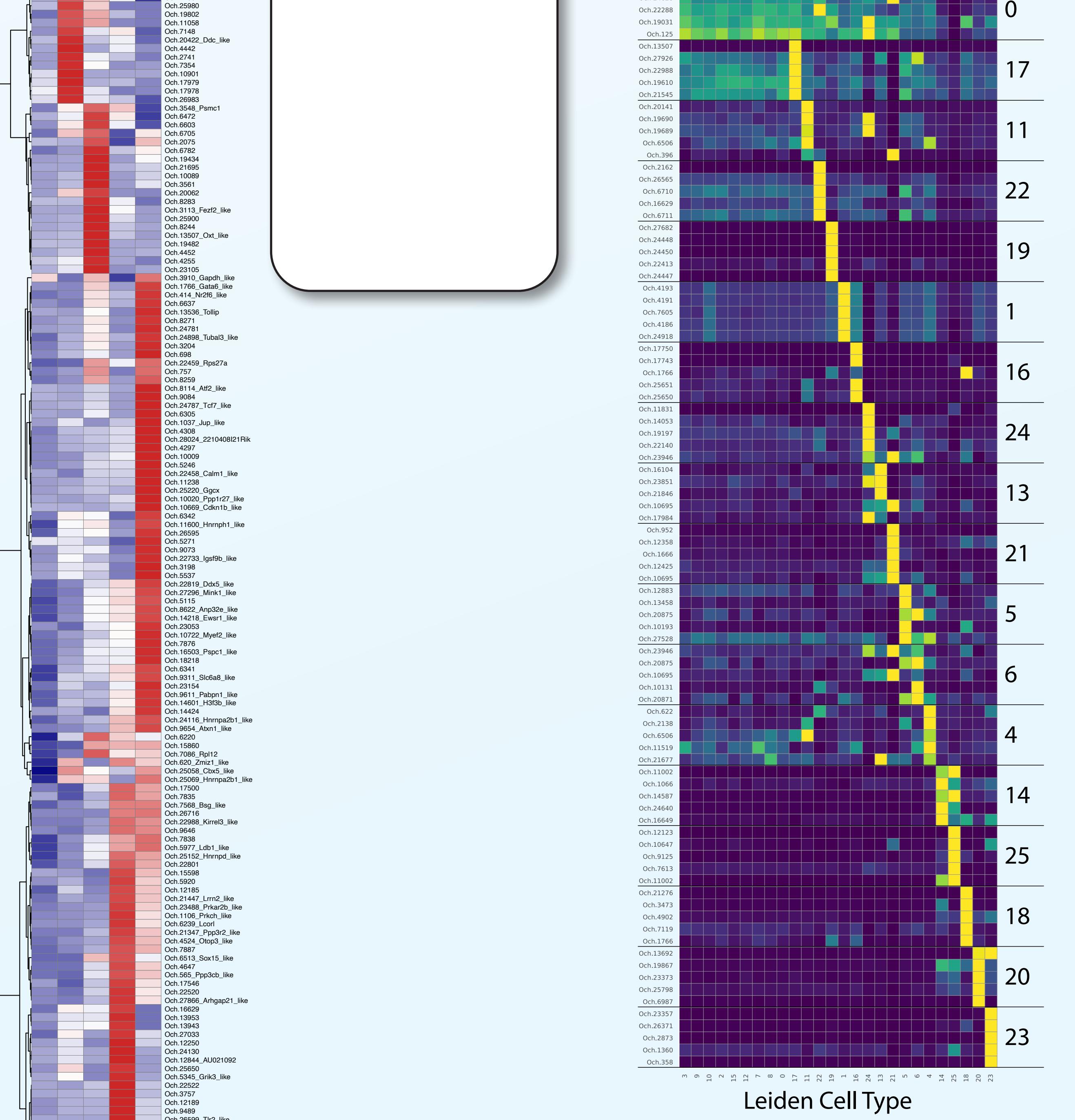
## Differential Gene Expression Analysis Across Brain Regions



## Future Directions

- Establish developmental trajectories for cell fate specification
- Integrative analysis of single cell and spatial data
- Cross-sample identification of patterns of gene co-regulation
- Comparative analysis across cephalopod clade

## Conclusions



## References

Tricycle manuscript

- We would like to acknowledge the help and support provided by all members of the Goff Lab (JHU), and the Albertin Lab (MBL).
- We thank the members of the Cephalopod Breeding Center at MBL for their continued support in developing and maintaining *O. chierchiai* in culture

We are looking for motivated postdocs for a variety of funded single cell and spatial transcriptomics-based projects in cephalopod neurobiology!



## Acknowledgments

- XXXX single nuclei from adult *O. chierchiai* optic lobe.
- Custom adaptation of sci-RNA-Seq3 for cephalopod snRNA-Seq
- Improved efficiency of barcode ligation in difficult samples increases fraction of usable reads
- Fixed and permeabilized nuclei allows for staging, multiplexing, and cell type enrichment via HCRFlow
- >80% cost reduction in library preparation