



Jessica Stock^{1,†}, Alyson Hally^{2,†}, Chaichontat Sriworarat³, Hope Orjuela², Kyla Woyschner², Dominick Dickerson⁴, Rachel Latanich³,
Gül Dölen^{3,4}, Caroline Albertin^{1,*}, Loyal A. Goff^{2,3,5,6,*}

¹ Woods Hole Marine Biological Laboratory, University of Chicago; ² McKusick-Nathans Department of Human Genetics, Johns Hopkins University; ³ Solomon H. Snyder Department of Neuroscience, Johns Hopkins University; ⁴ University of California, Berkeley; ⁵ Kavli Neurodiscovery Institute, Johns Hopkins University; ⁶ Presenting Author; [†] Authors contributed equally, * Co-corresponding Authors

Abstract

The pygmy zebra octopus (*Octopus chierchiae*) 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.

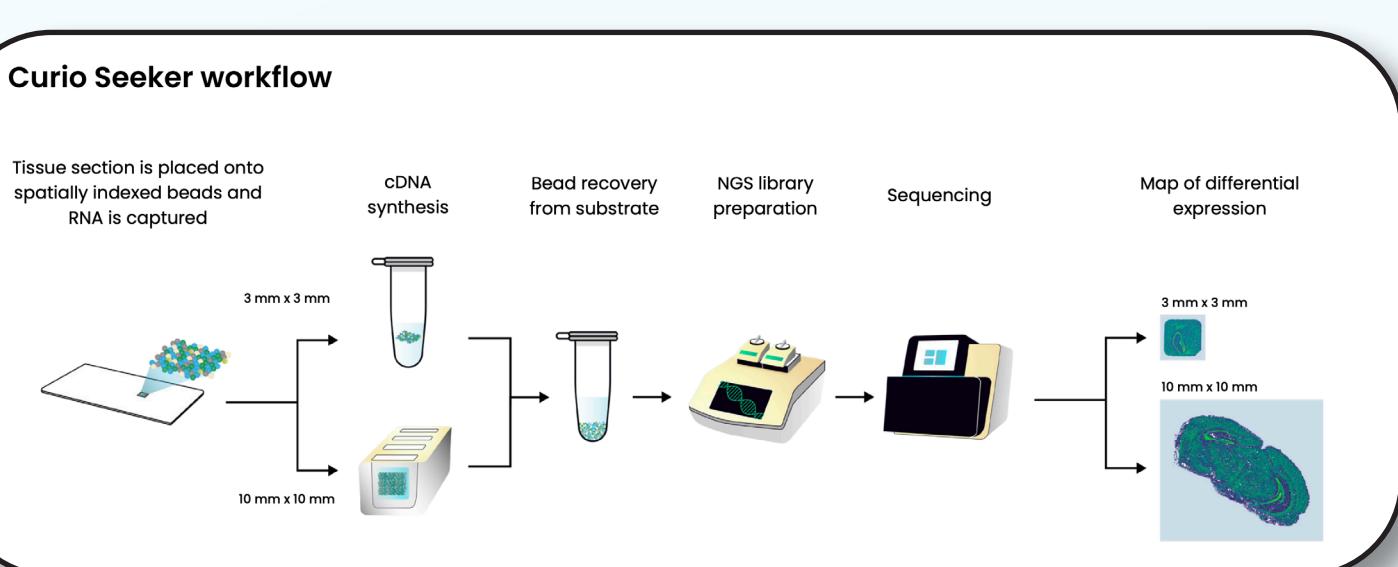
Building on the foundation of our recently assembled high-quality reference genome and transcriptome, we have begun to characterize the cellular and molecular organization of the central brain of *O. chierchiae*. Using a flexible, ultra-high-throughput method which we have optimized for cephalopod cellular 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. chierchiae*. 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. chierchiae* 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.

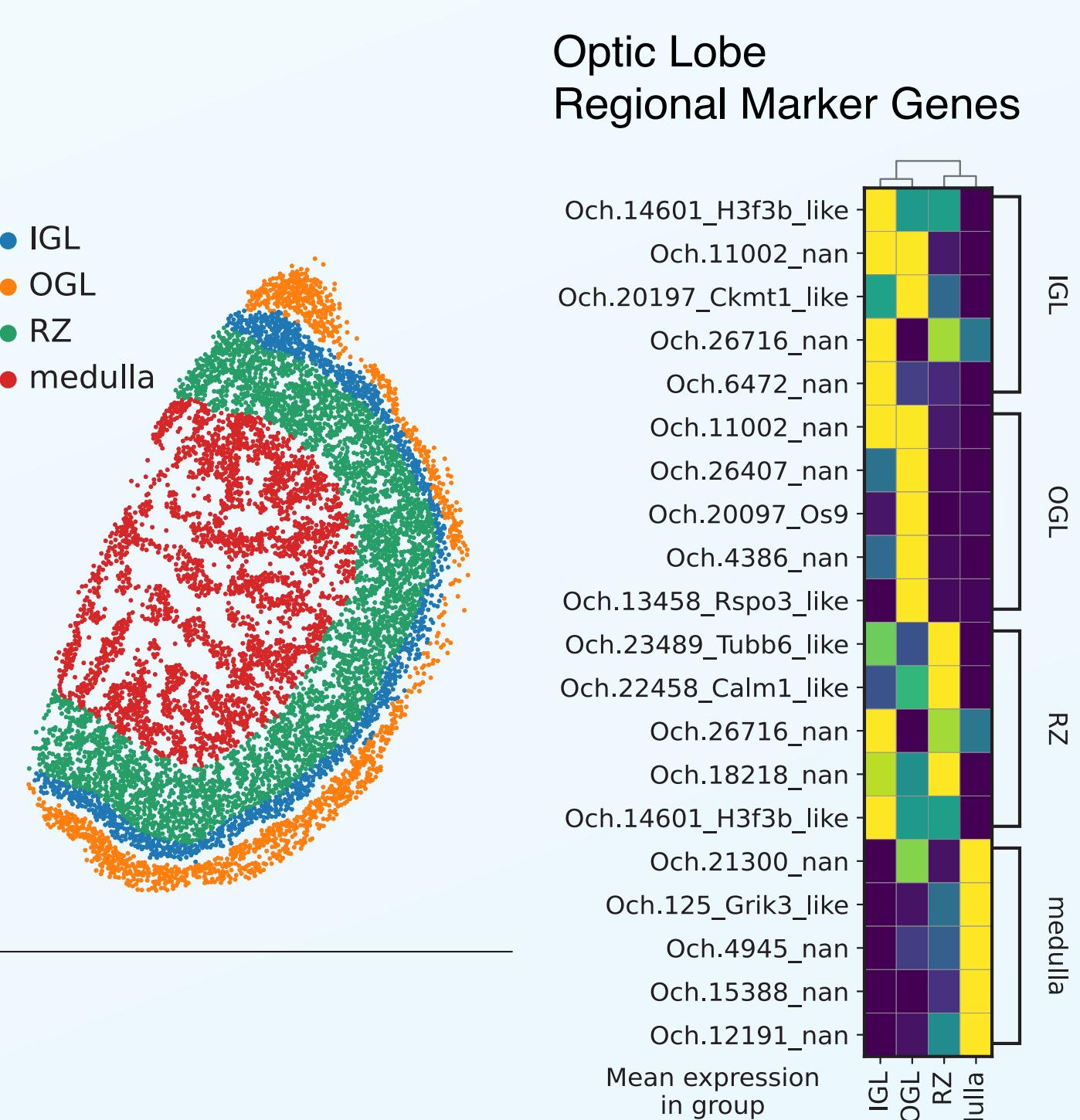
Experimental Questions

- What is the landscape of spatial gene expression variation across the cephalopod central brain?
- Can we identify regional variation in key developmental genes in *O. chierchiae*?
- Can we adapt the ultra-high-throughput scRNA-Seq3 method to cephalopod cellular physiology to identify cell types and states in neural tissues and improve sensitivity?

Unbiased Spatial Transcriptomics Analysis Using Curio Seeker



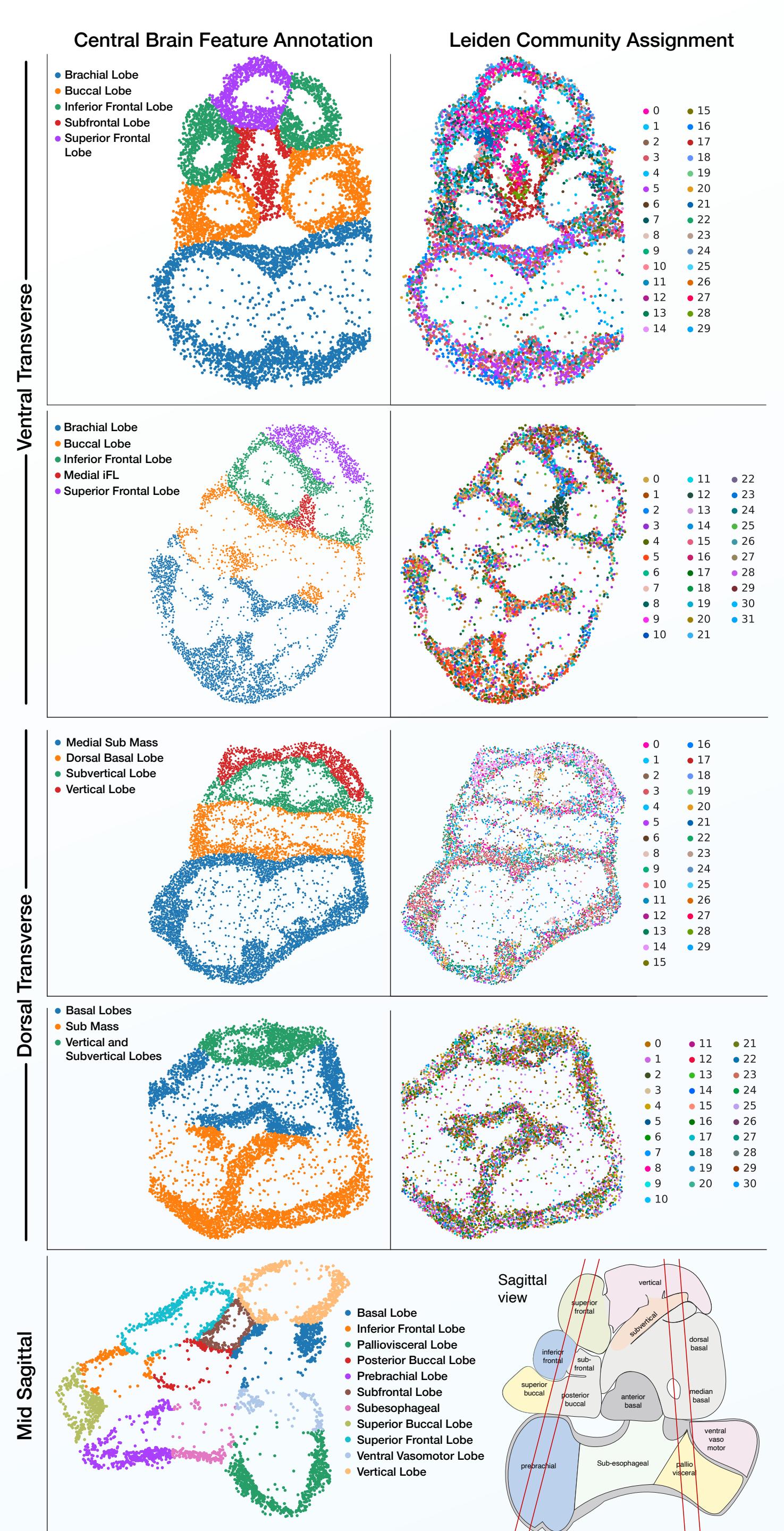
Regional Variation in Optic Lobe



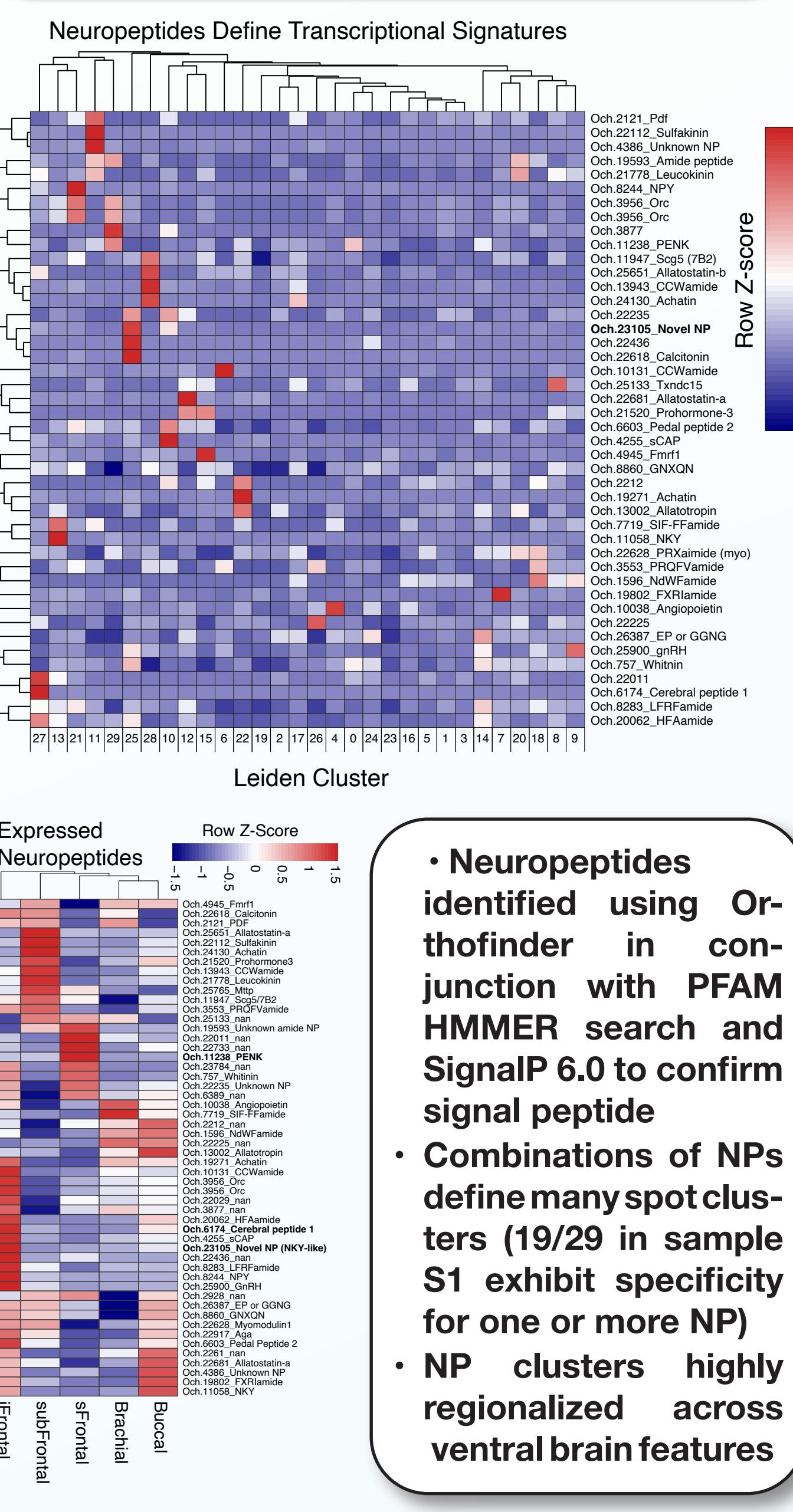
- Regional variation in Calmodulin-family gene expression: Calm1 is a marker for RZ, Calm3-like (Och.6472) is specific to IGL
- 7TM glutamate receptor Och.125 and FMRFamide Neuropeptide fmrf1 (Och.4945) specific to medulla
- R-spondin gene (Och.13458), involved in potentiating Wnt/β-catenin signaling, is a marker for OGL

- Scan to download poster
- While on our website, check out our available positions: <http://www.gofflab.org/>
- We're always on the lookout for fun collaborations and new scientific questions!

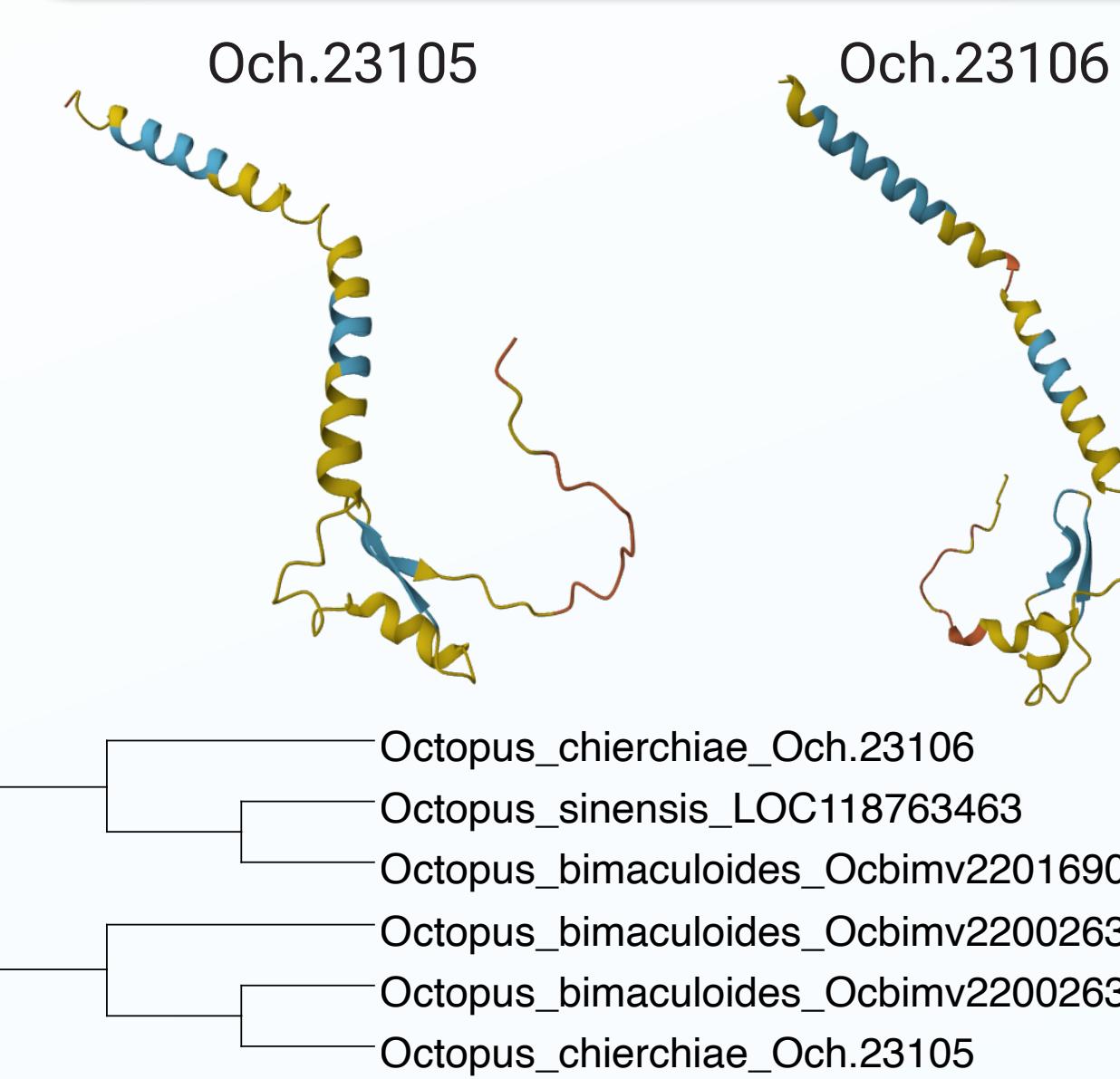
Brain Feature Annotation



Neuropeptides Drive Spatial Transcriptional Diversity

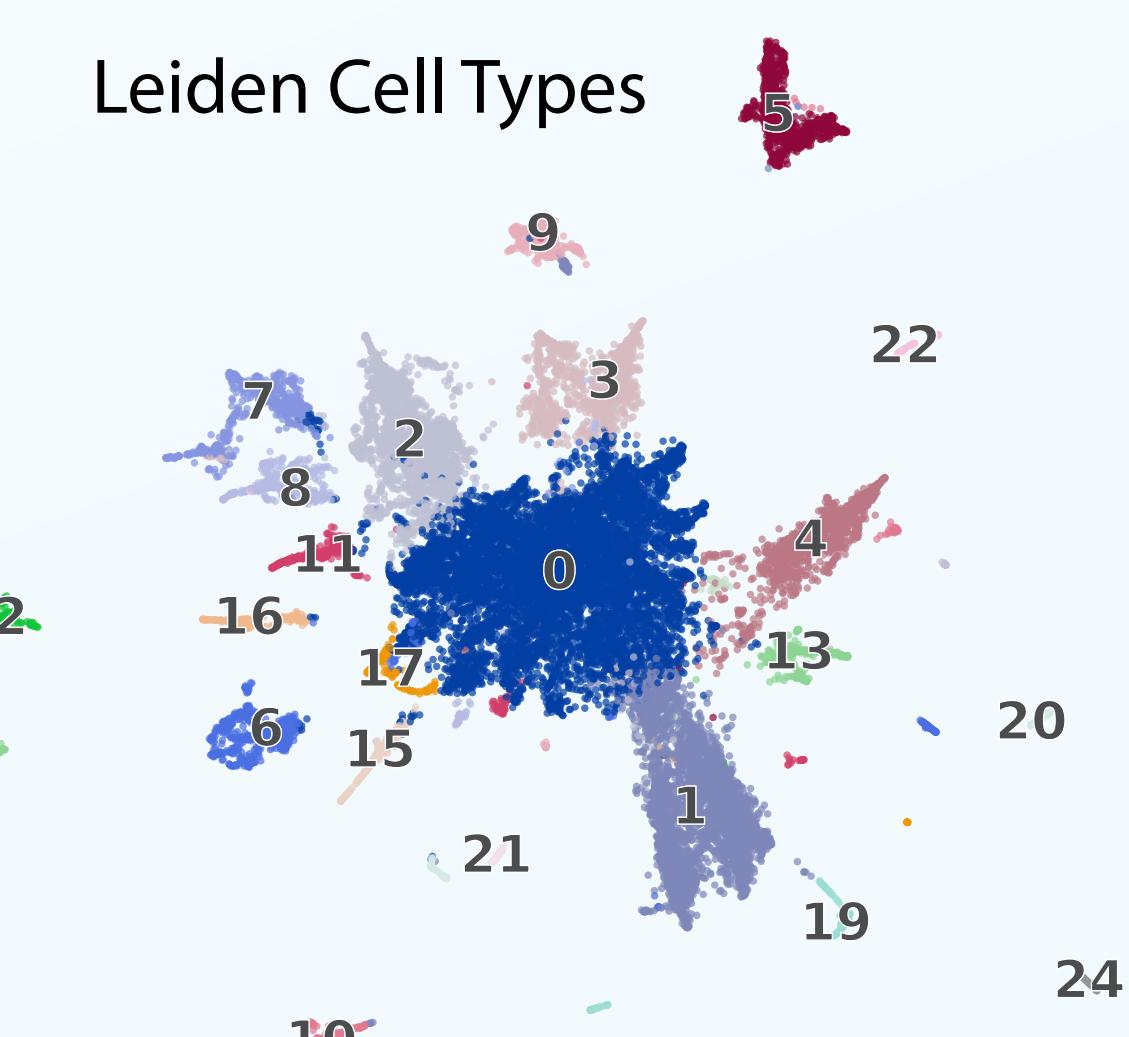


Octopus-Specific Novel NKY-like Neuropeptide

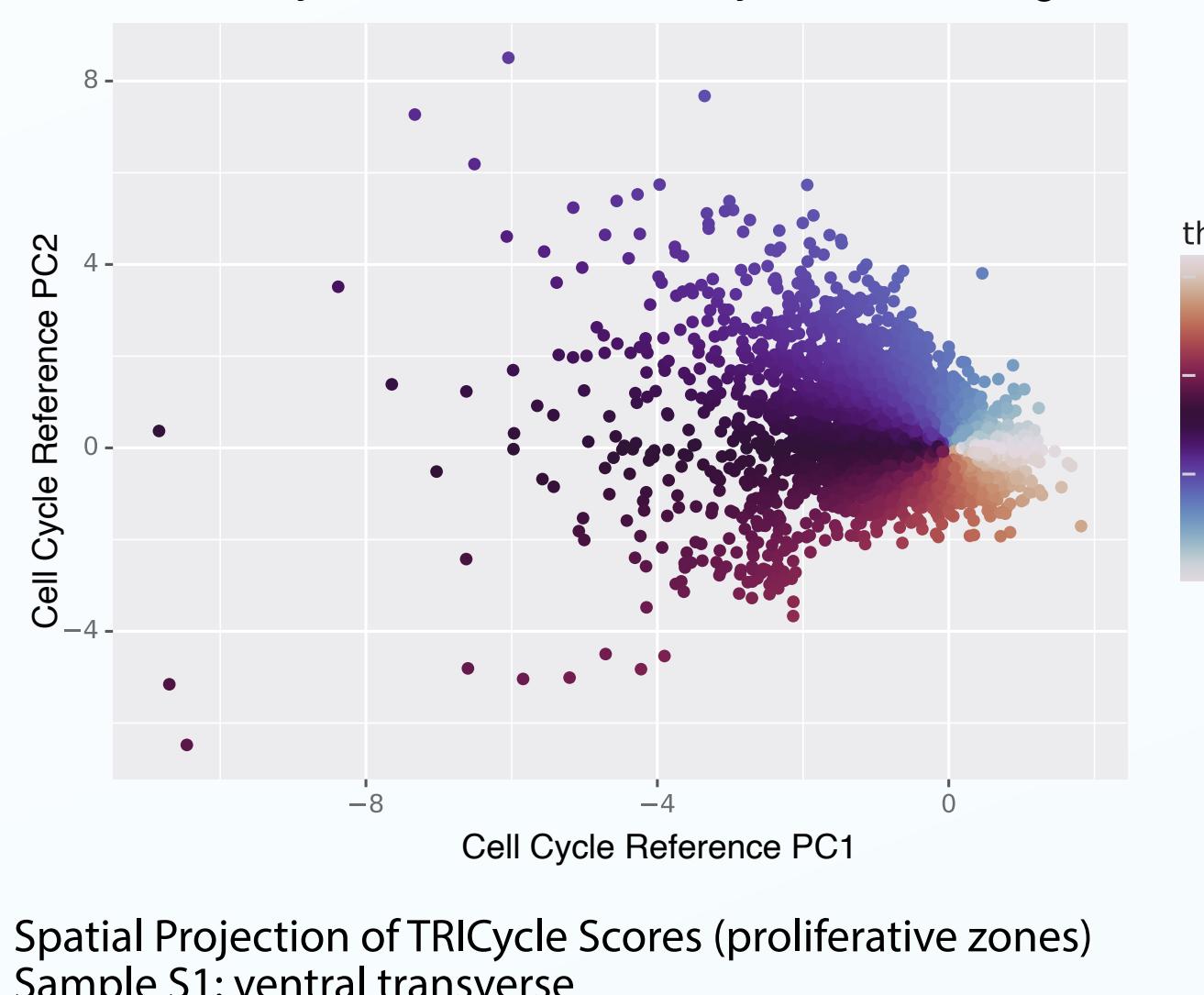


- Identification of octopus-specific neuropeptide with specific, regionalized expression in brain
- Genomic proximity suggests local duplication event

Optimized Ultra-High-Throughput snRNA-Seq



TRICycle analysis of cell proliferation



- TRICycle predicts continuous cell cycle position by projecting individual cells/nuclei into a universal reference cell cycle embedding space learned from mouse cortical progenitors.
- Radial position around the origin provides a proxy measure for pseudotemporal cell cycle state.
- Regions of cell proliferation are identified by visualizing TRICycle scores in spatial embedding.
- Distance from origin is used as a confidence measure for cell cycle state prediction.

Future Directions

- Establish atlas of cell types and states in adult *O. chierchiae* neural tissues.
- Validation and higher-resolution characterization of expression patterns via smFISH.
- Identify trajectories for neural cell fate specification in developmental and adult *O. chierchiae*.
- Integrative analysis of single cell and spatial data to define hierarchy of spatial and molecular organization within the central brain.
- Cross-sample identification of patterns of gene co-regulation via NMF.
- Comparative analysis across cephalopod clade

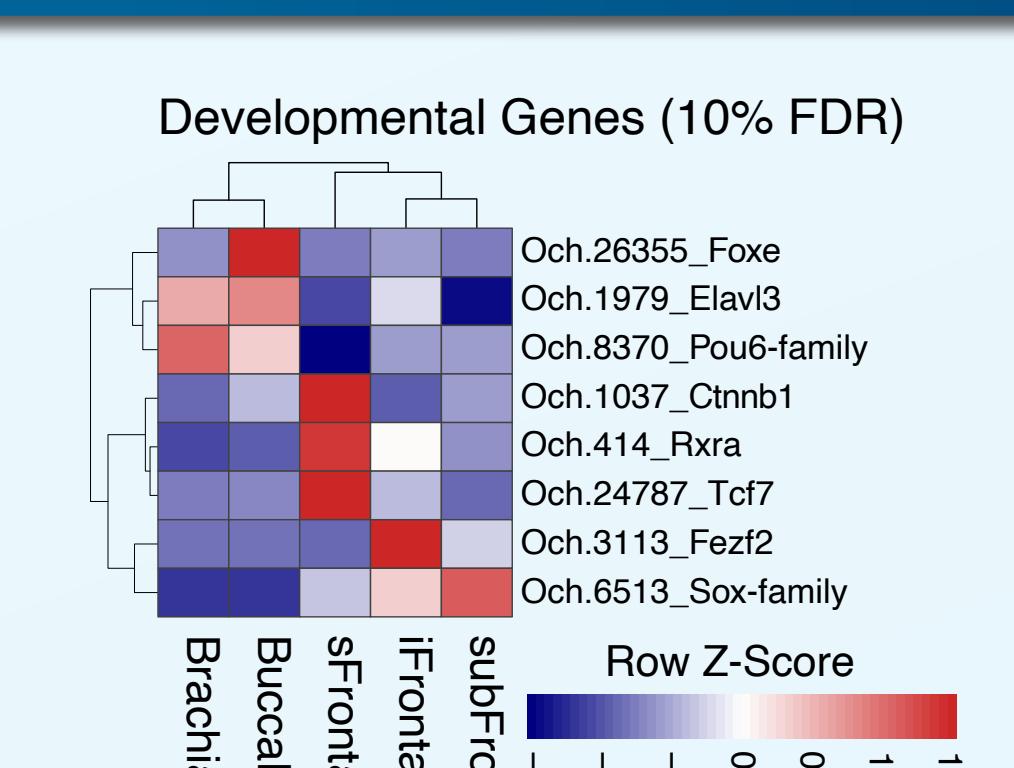
Conclusions

- Unbiased spatial transcriptomics reveals patterns of gene co-expression across the octopus central brain
- Identification of cell type/state neighborhoods with regional variation
- Several key 'development-associated' genes/pathways are differentially expressed across major brain lobes
- Cost-effective, optimized, ultra-high-throughput snRNA-Seq in *O. chierchiae* optic lobe identifies discrete transcriptionally-defined cell types
- TRICycle projection of spatial features suggests regional variation in proliferation

References

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Differential Expression of Developmental Genes



- 120 'development-associated' genes identified via OrthoFinder homology to Mouse, Human, Drosophila, or C. elegans
- 77/120 are expressed in sample S1 ventral brain
- 8/77 show significant differential expression w.r.t. brain region (GLM, Wald test, 10% FDR, BH-corrected)
- Pou5-family gene expression in subesophageal brachial lobe
- Greater variation in supraesophageal mass
- Wnt/β-catenin family members & RA receptor specifically expressed in superior frontal lobe

- 22,659 single nuclei from adult *O. chierchiae* optic lobe.
- $11,815 \pm 1,526 \mu$ UMLs per nucleus (min 500)
- $1,787 \pm 1,059 \mu$ detected genes per nucleus
- 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 FACS
- $>80\%$ cost reduction in library preparation

- 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. chierchiae* in culture.

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