

Validation of Gene Expression in Postmortem Brain Following Single Nuclei Sequencing Data

UW Neurological Surgery Summer Program

August 11, 2017

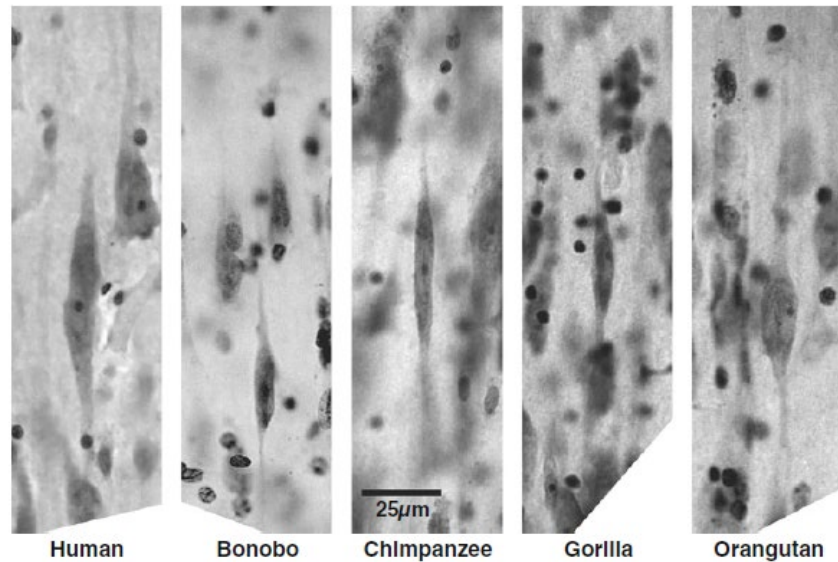
Maddy Berkowitz-Cerasano



ALLEN INSTITUTE *for*
BRAIN SCIENCE

Transcriptomic Signature of von Economo Neurons (VEN)

von Economo neurons are large, spindle-shaped neurons found in layer 5b



Allman ... Hof, 2010 (Brain Structure Function)

Species	Frontal pole		ACC		Anterior insula		Occipital pole	
	% VEN	% Fork cells	% VEN	% Fork cells	% VEN	% Fork cells	% VEN	% Fork cells
Bowhead whale	24	10	21	6	14	1	3	3
Rock hyrax	0	0	0	0	0	0	0	0
Cow	9	2	6	1	8	6	3	0.4
Pig	6	1	8	2	3	1	1	0
Deer	5	1	5	1	2	0.4	0.5	0.5
Sheep	7	2	7	2	4	2	0.4	1
Horse	11	3	15	2	5	5	2	2
Human	0	0	13	1	11	4	0	0

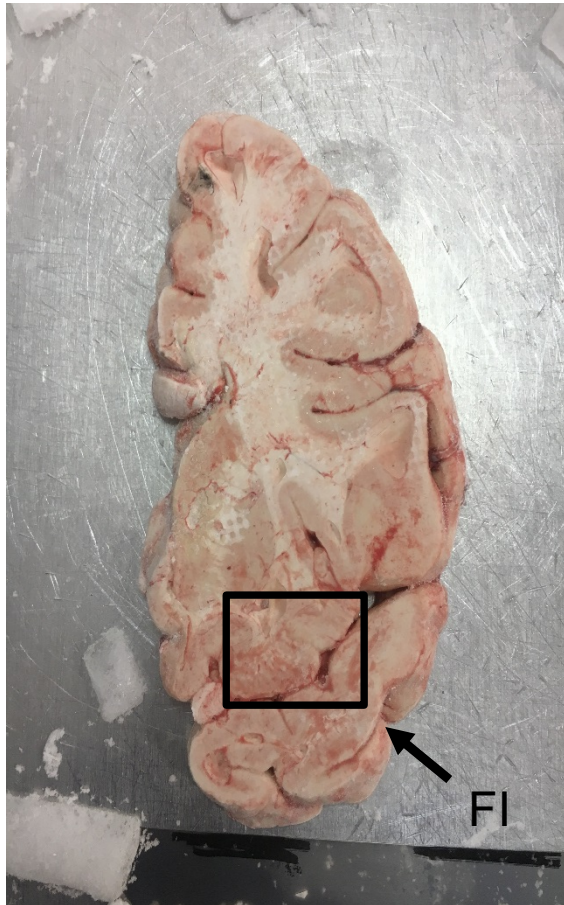
Raghanti... Hof, 2015 (Brain Structure Function)

von Economo neurons are unique to humans, great apes, and a few other mammalian species. VENs are not present in rodent brain.

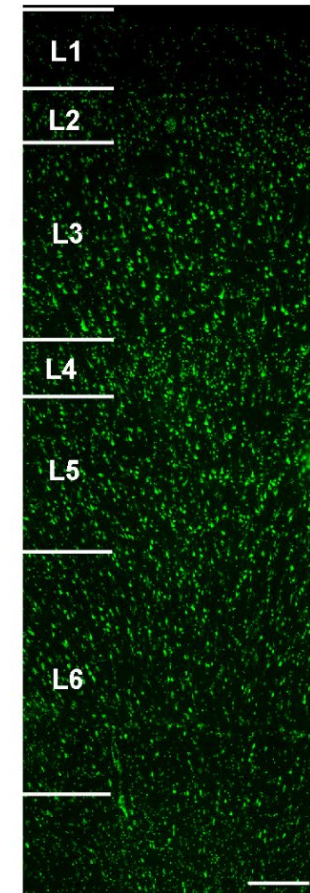


Isolation and Sequencing of Nuclei from Postmortem Brain

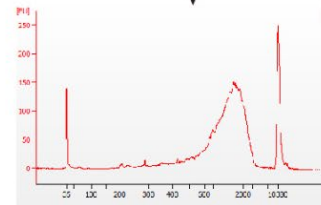
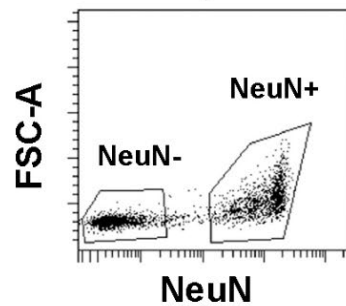
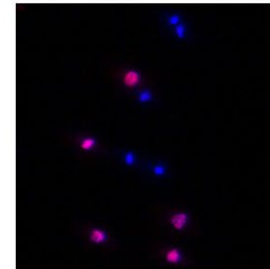
Collect single nuclei from postmortem specimens



Fluorescent Nissl



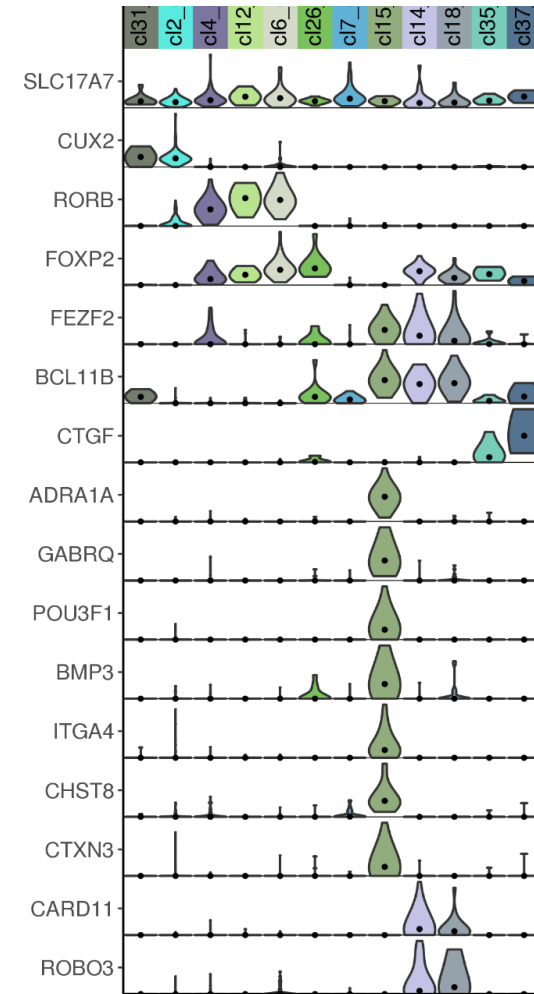
DAPI NeuN



Layer 5 Frontoinsula

~5% of L5 neurons

Two human specimens –
1000 single nuclei +
controls

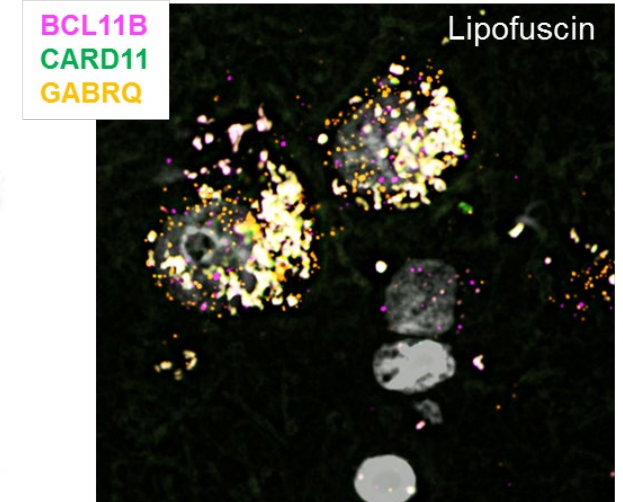
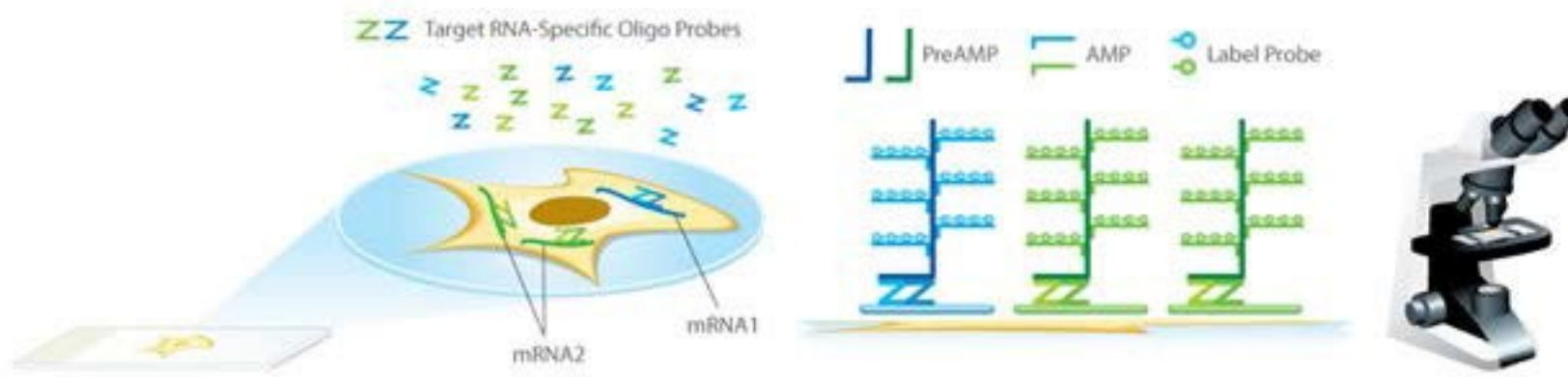


12 glutamatergic
neuron clusters

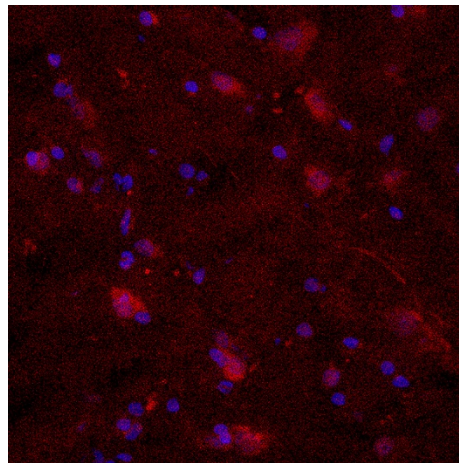


Limitations of RNAScope Multiplex Fluorescent In Situ Hybridization (ISH) Combined with Immunohistochemistry (IHC)

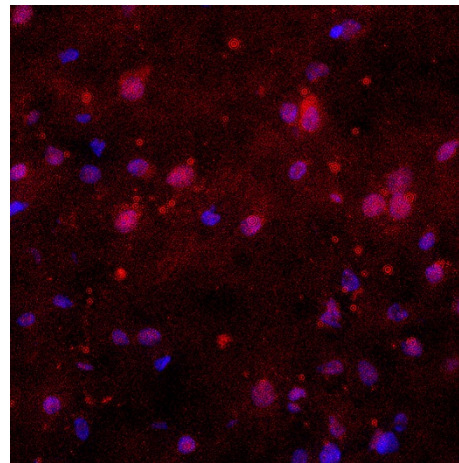
RNAScope Protocol (mRNA detection)



MAP2 DAPI (nuclei)



TUJ1 DAPI (nuclei)



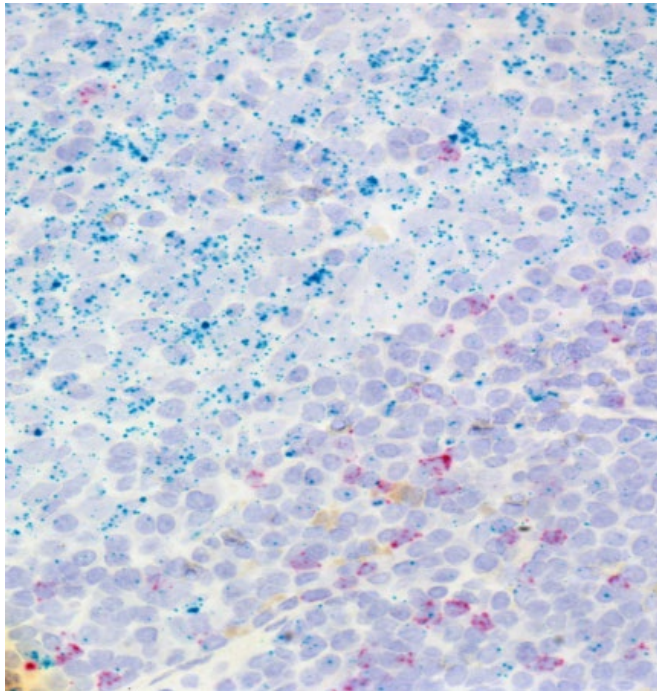
Slides processed with RNAScope reagents and antibodies for specific proteins

- Weak fluorescence signal
- Long exposure time
- Antibody labeling highlights cell body but not processes

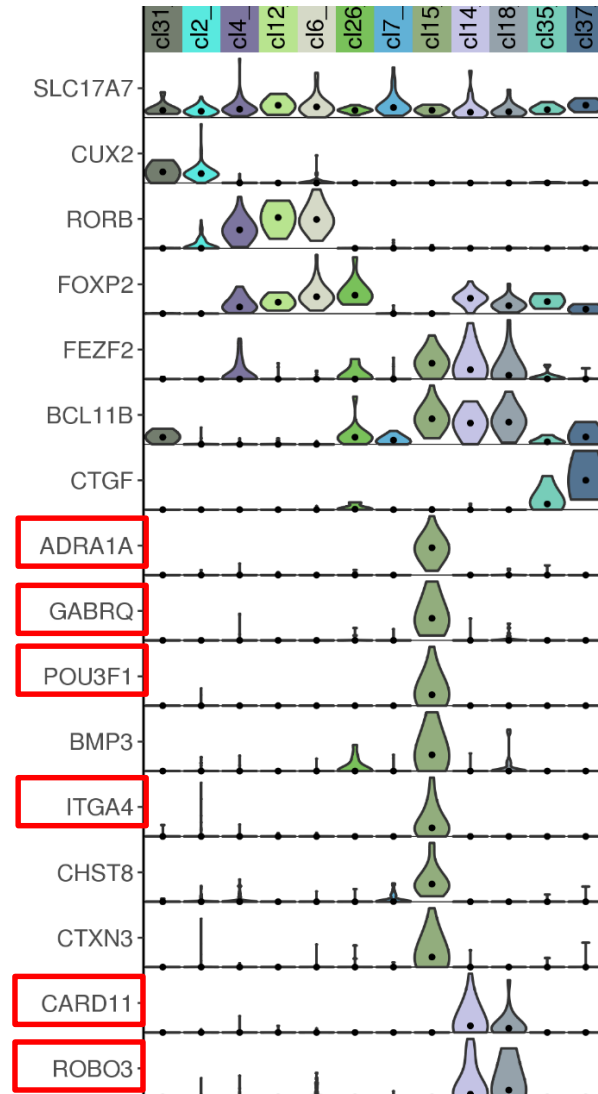


The Solution: RNAscope Double Chromogenic ISH Labeling

Gene 1 Gene 2 Cells



Sample image from
RNAscope protocol
2 color chromogenic labeling



Probe combinations tested:

ADRA1A/POU3F1

GABRQ/ITGA4

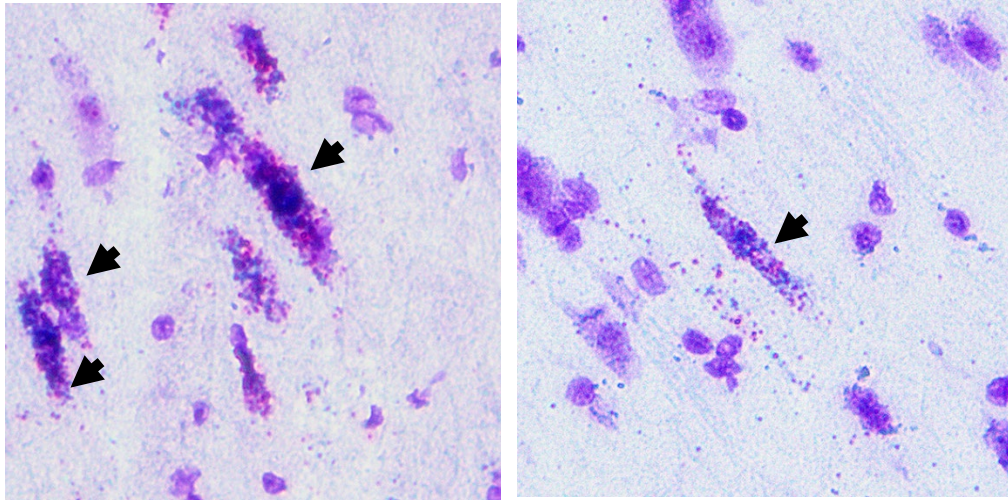
GABRQ/CARD11

ADRA1A/ROBO3

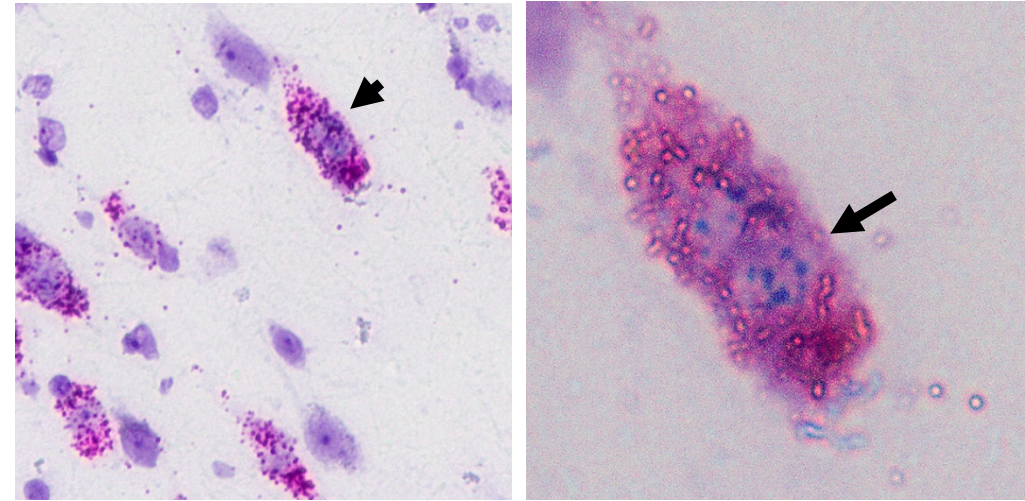


Validation of Transcriptomic Cell Types Using Double ISH

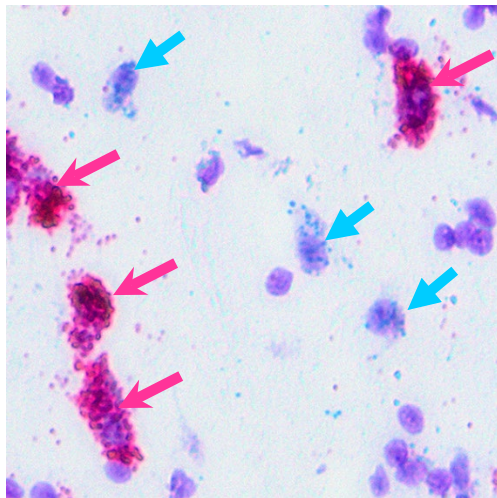
ADRA1A POU3F1 Cells



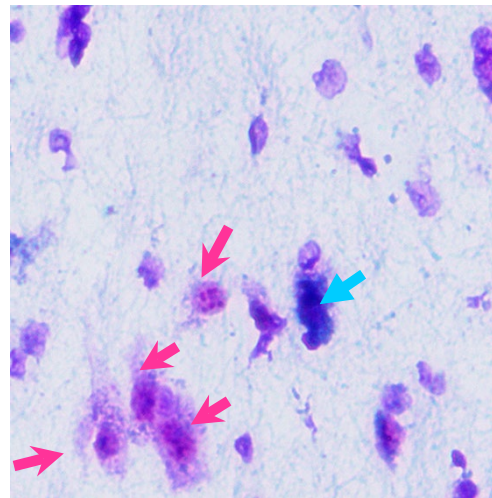
ITGA4 GABRQ Cells



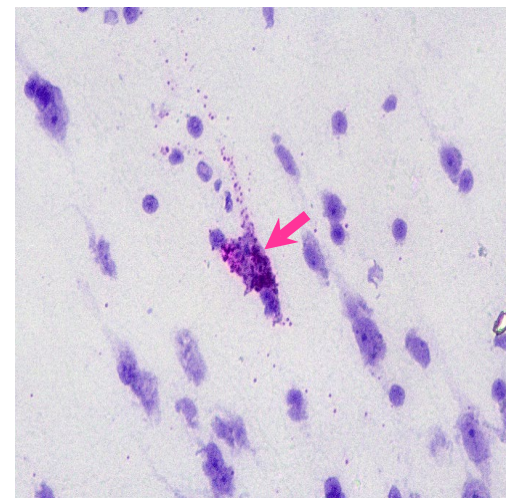
CARD11 GABRQ Cells



ADRA1A ROBO3 Cells

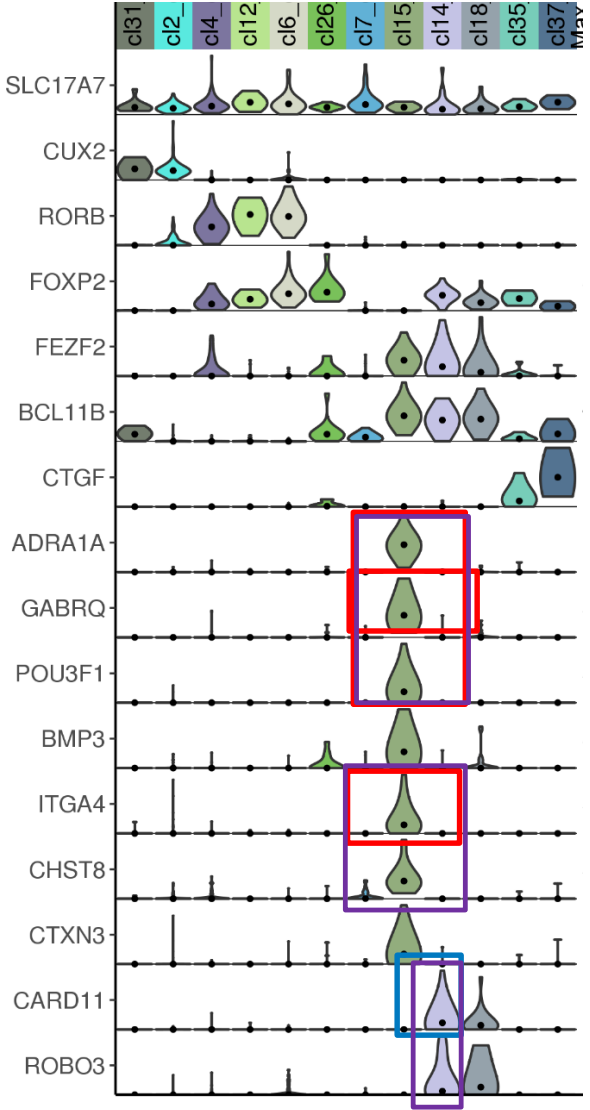


ITGA4 GABRQ Cells

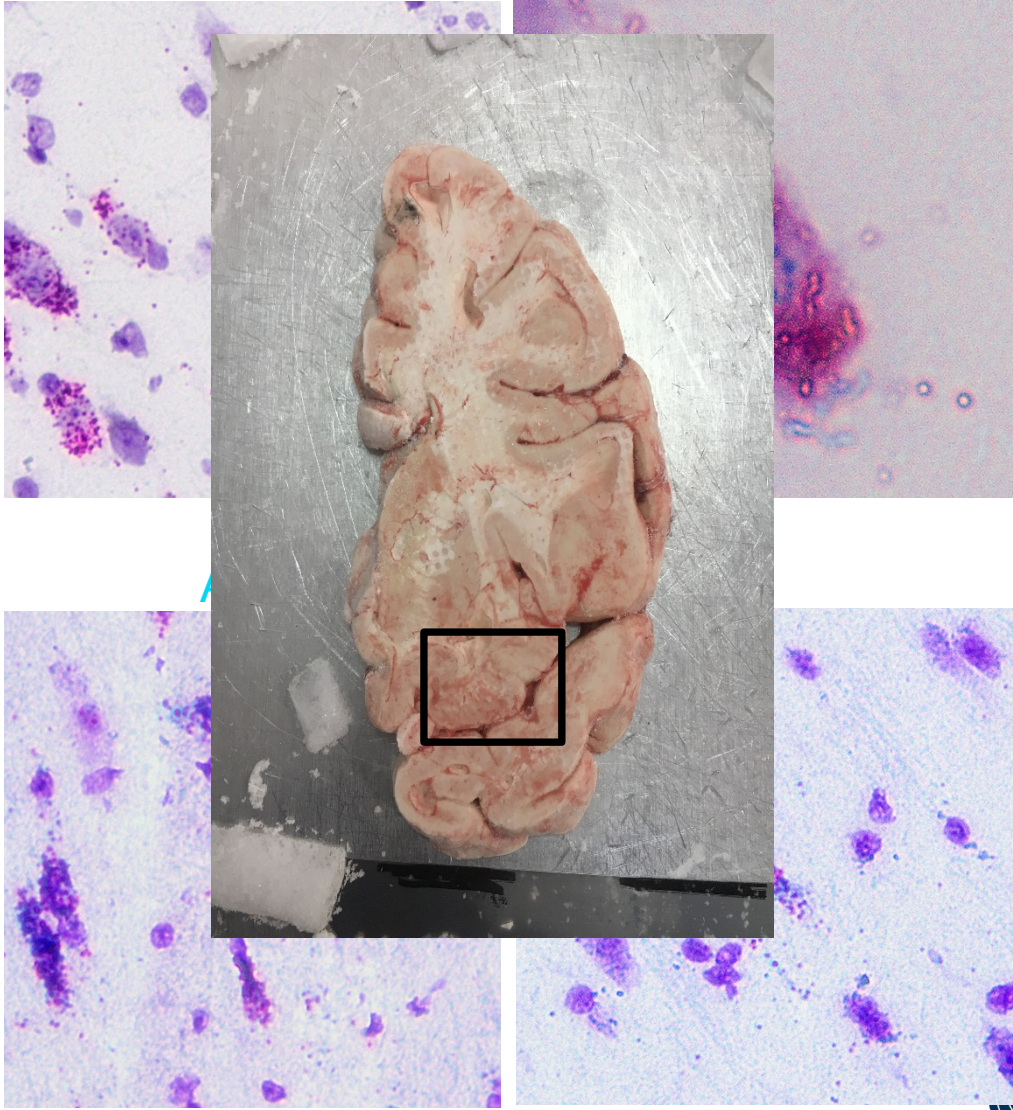




Summary and Conclusions



ITGA4 GABRQ Cells



Thank You!

Allen Institute for Brain Science

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Rebecca Hodge, PhD



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Richard Ellenbogen, MD

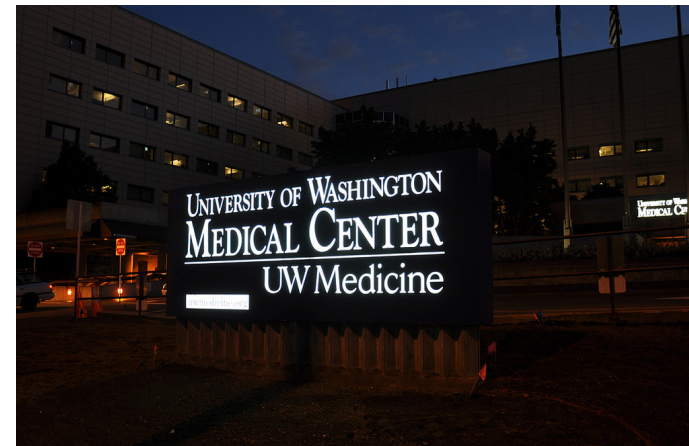
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