



Centre for Molecular Medicine
and Therapeutics

RESEARCHING LIFE to CHANGE LIVES

CHILD & FAMILY RESEARCH INSTITUTE | THE UNIVERSITY OF BRITISH COLUMBIA



Celebrating CMMT 15

helicosCAGE Analysis for Mouse Cerebellum Developmental Time Series

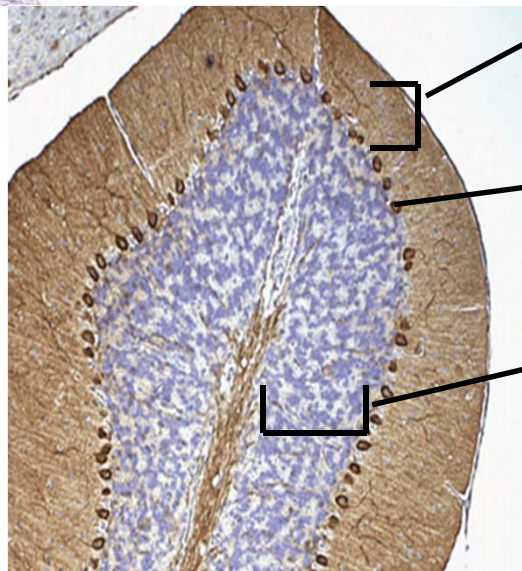
Thomas Ha, Peter Zhang, Doug Swanson, Matt Larouche,
Gloria Mak and Dan Goldowitz
University of British Columbia
Vancouver, Canada

Cerebellar Neuronal types



Cerebellum has been implicated in Autism-spectrum disorder, schizophrenia, Fragile X mental retardation, and Dyslexia.

- Granule cells – Glutamatergic (excitatory)
- Purkinje cells – GABAergic (inhibitory)
- Interneurons – Inhibitory (Basket cells, stellate cells, Golgi cells) and excitatory (Unipolar brush cells)
- Cerebellar nuclear neurons – glutamatergic and GABAergic

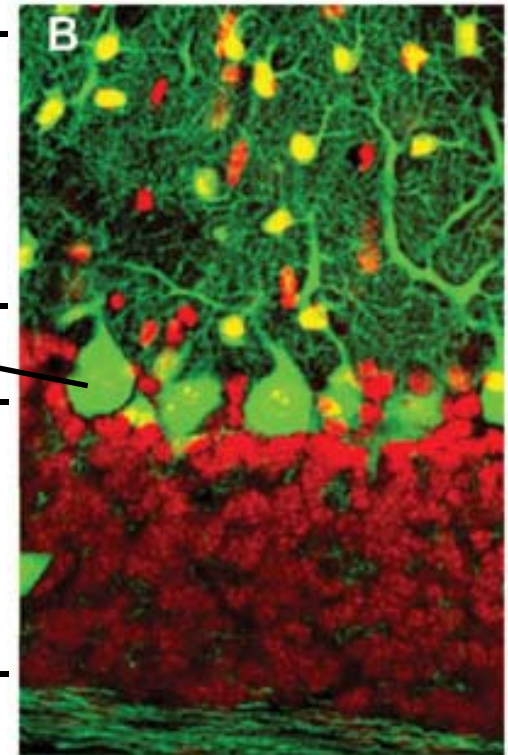


Simple Layer Architecture

Molecular Layer

Purkinje Cell

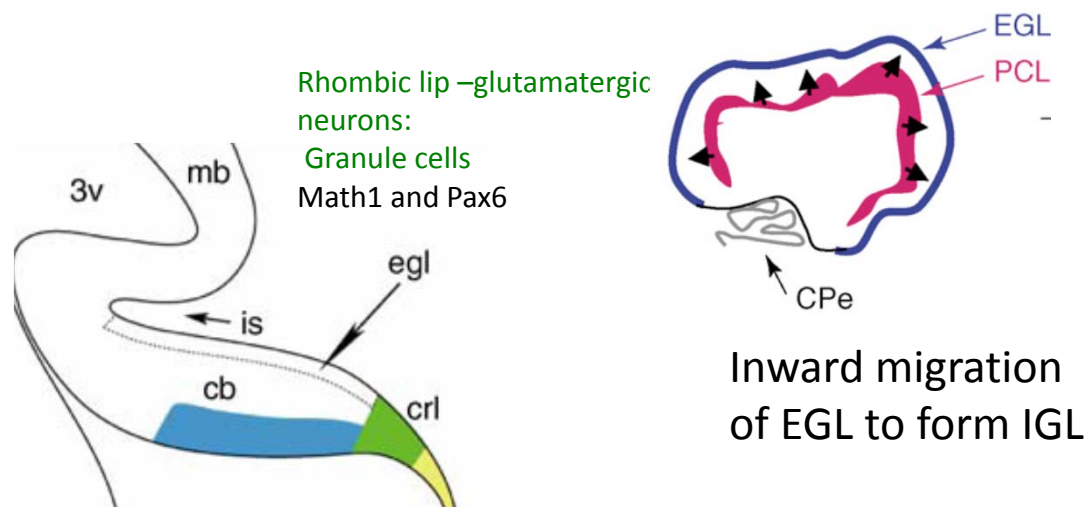
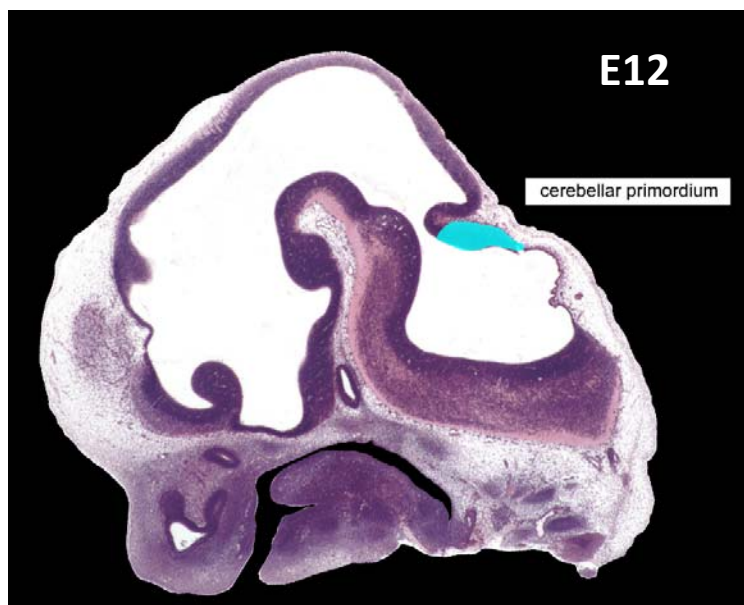
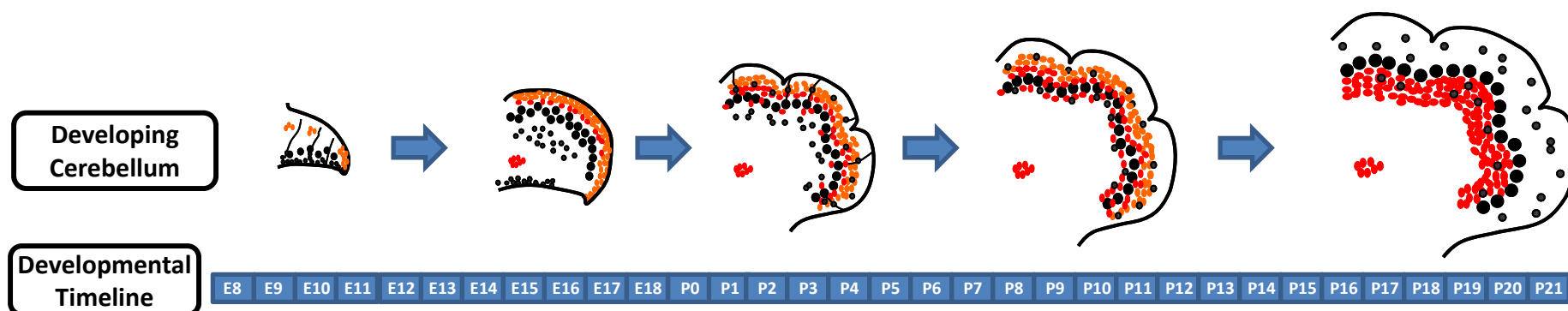
Granule Cell





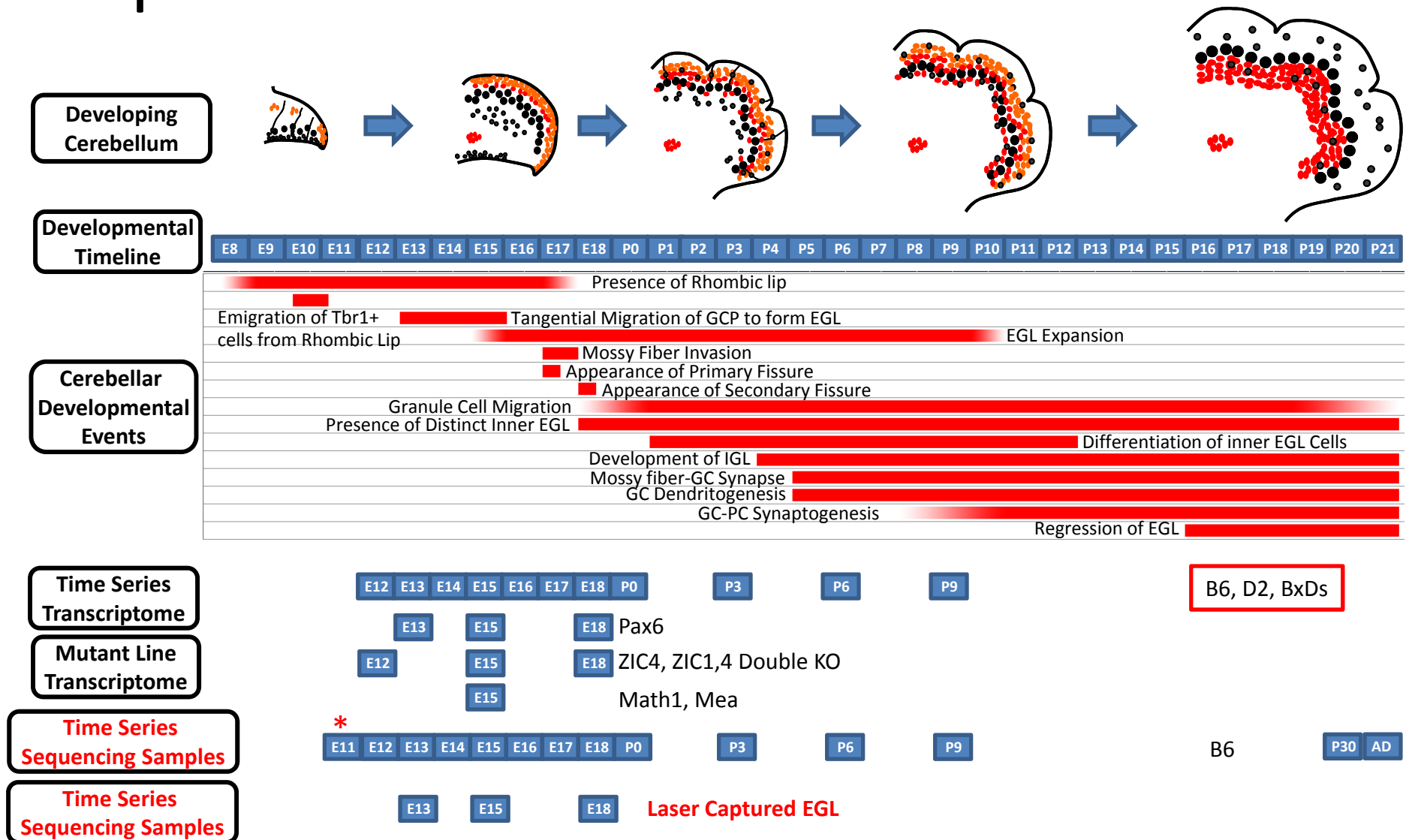
Cb_Dev_E12-P21_v29.swf

Cerebellar Development



Ventricular zone- GABAergic neurons:
Purkinje cells and inhibitory interneurons
Ptf1a

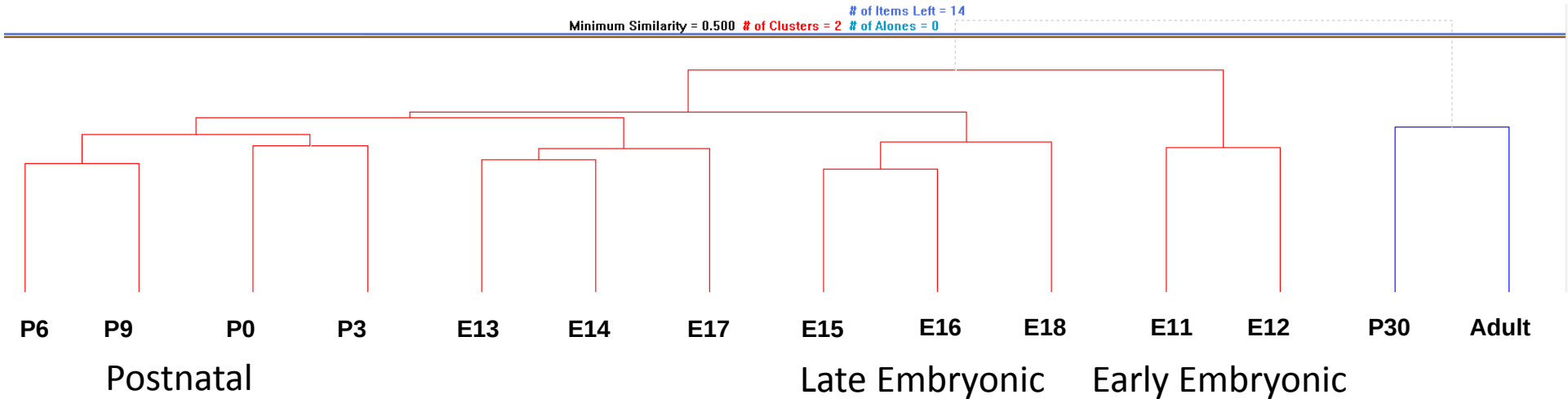
Expression data resource on cerebellum



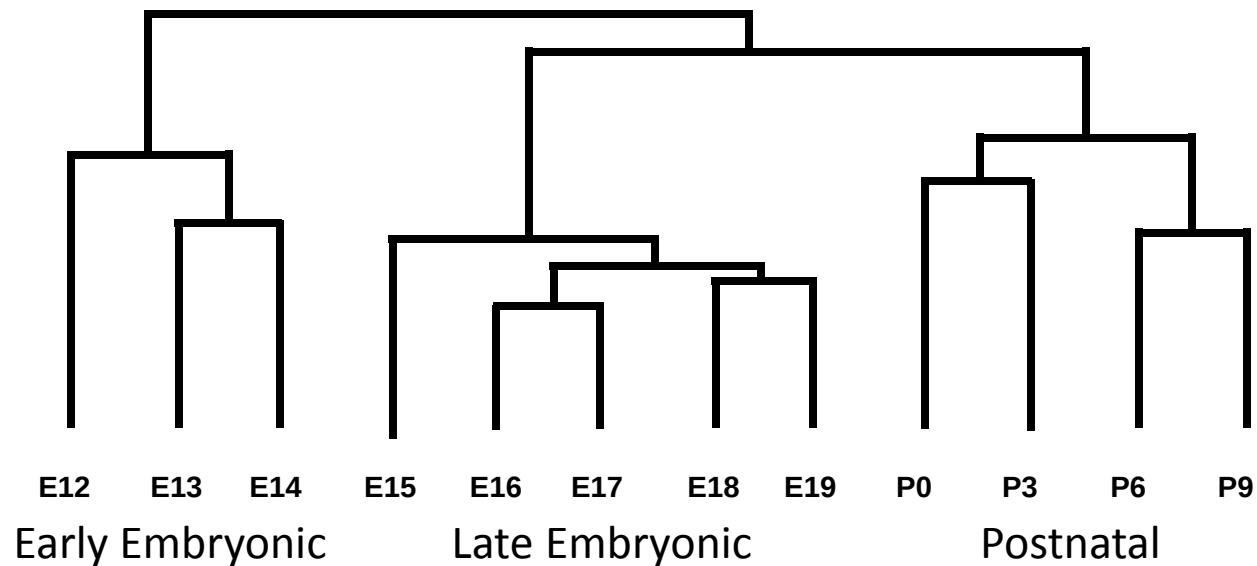
All the time series samples were prepared in triplicates with timed pregnancy with 4 hr time window to minimize sample variability. C57BL/6 strain is a recombinant inbred strain with the most genetic and genomic information available next to men.

Hierarchical Clustering

HelicoCAGE mouse cerebellum time series



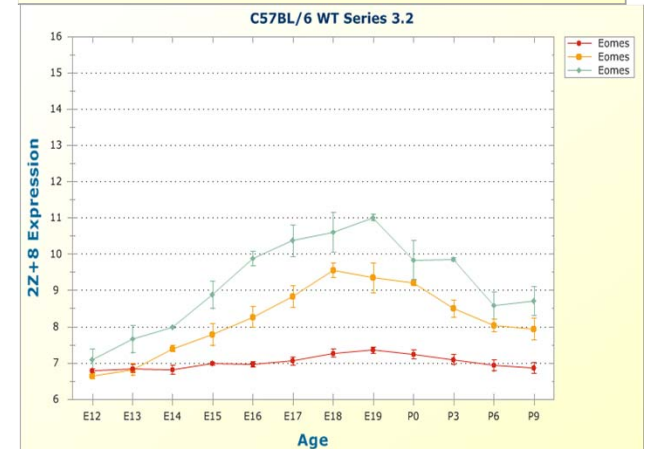
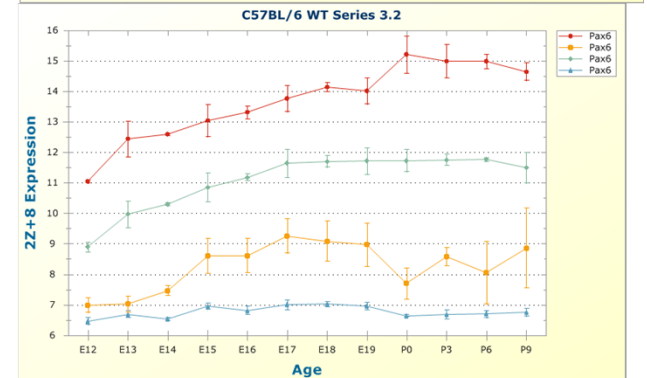
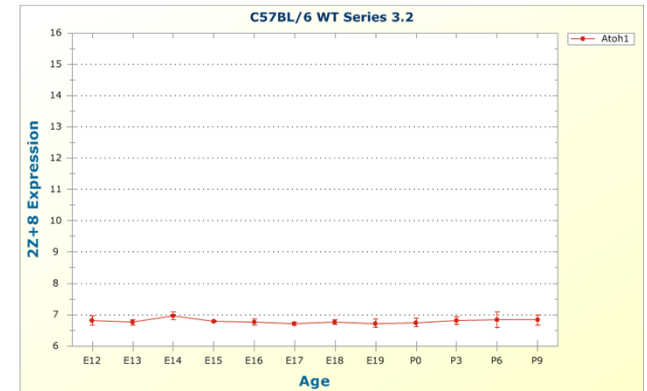
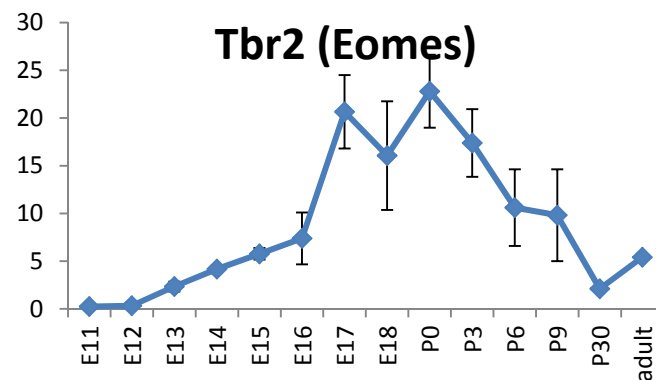
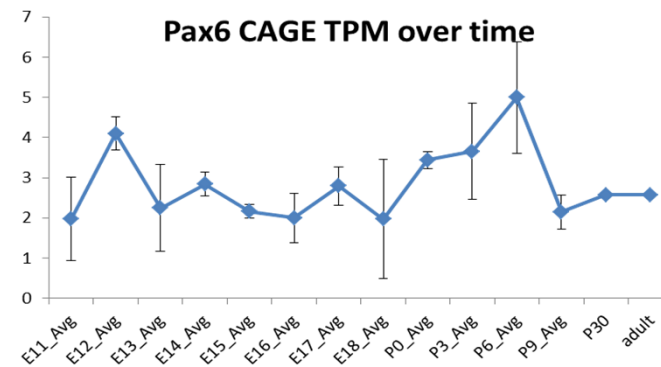
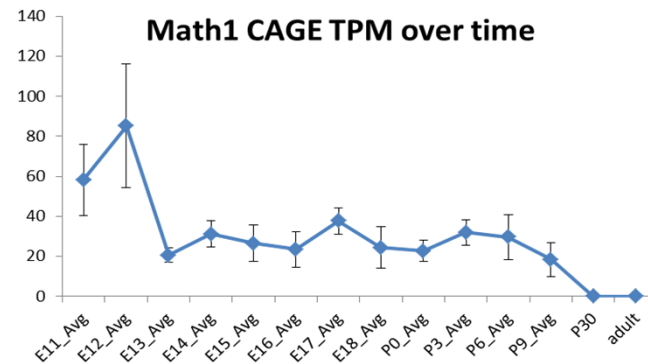
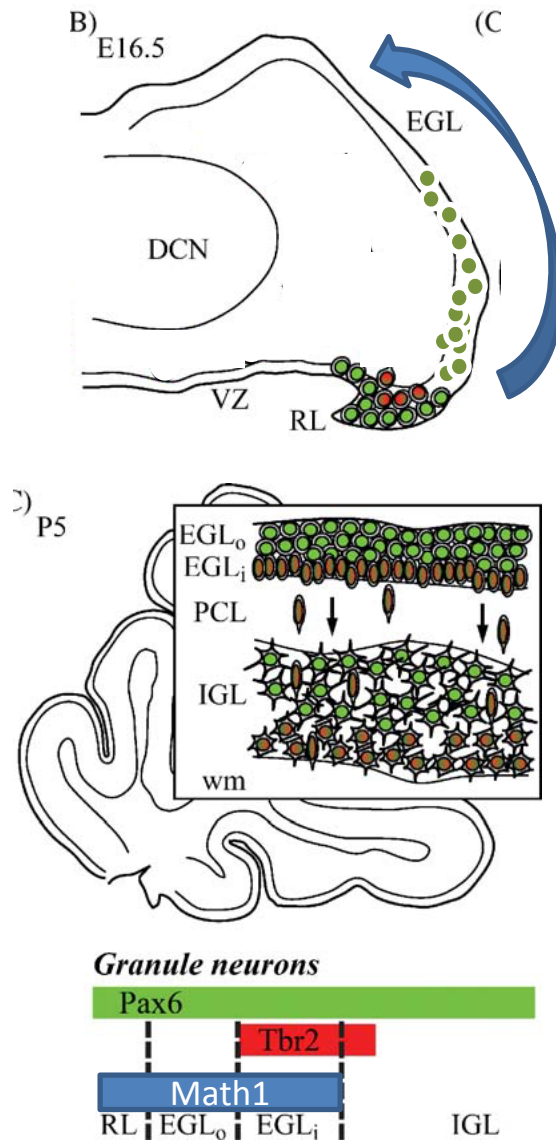
Illumina
microarray
time series



Glutamatergic Rhombic Lip Lineage – Granule cell

hCAGE

Illumina microarray

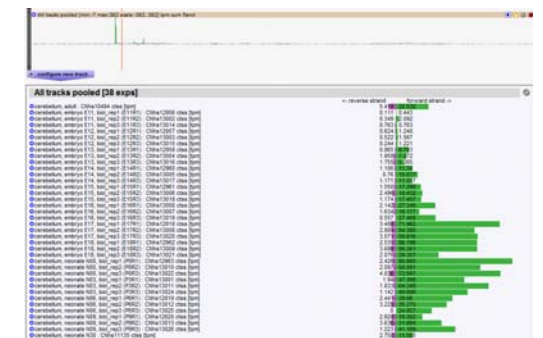
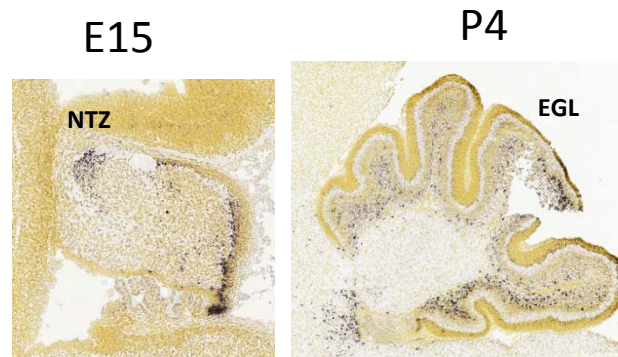
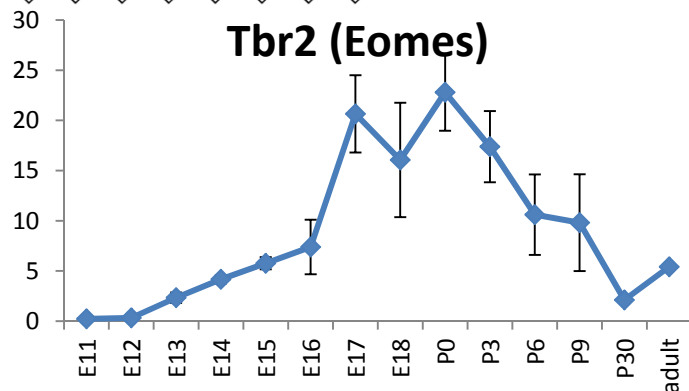
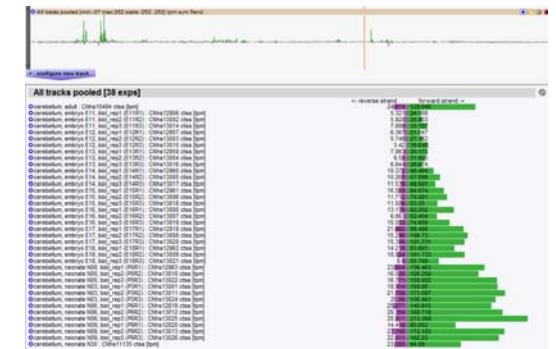
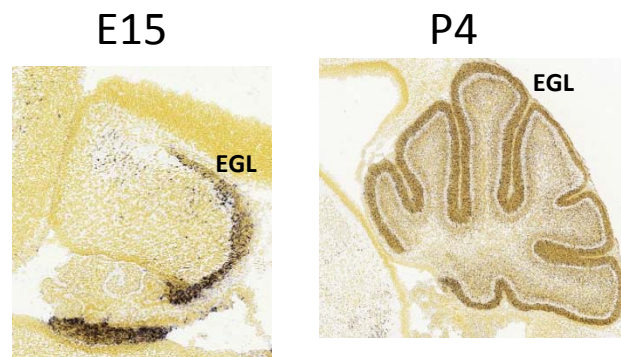
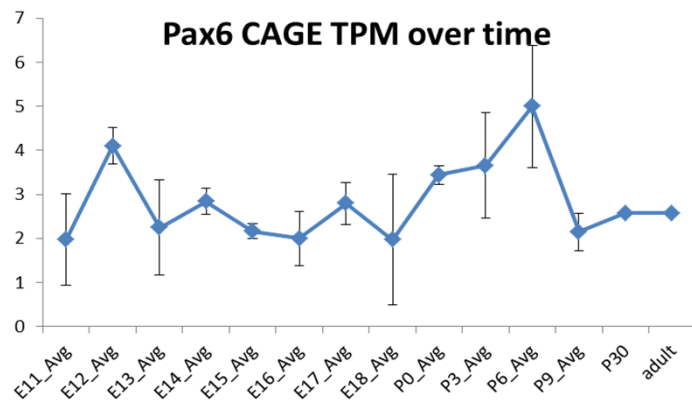
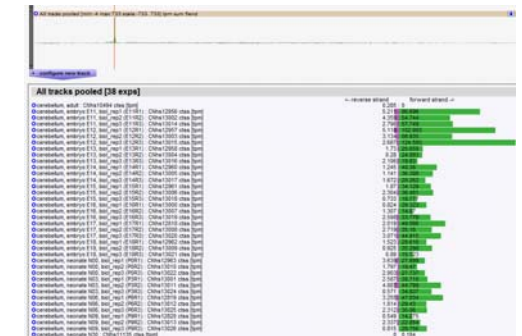
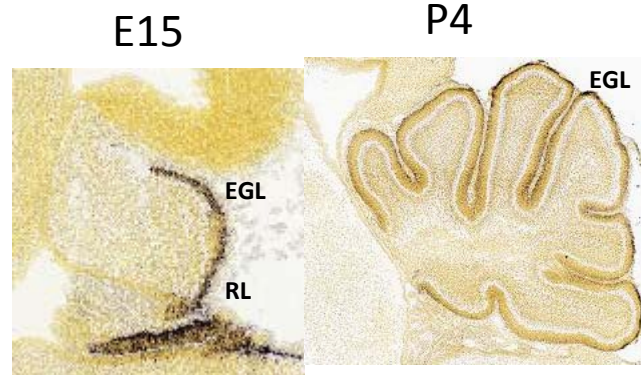
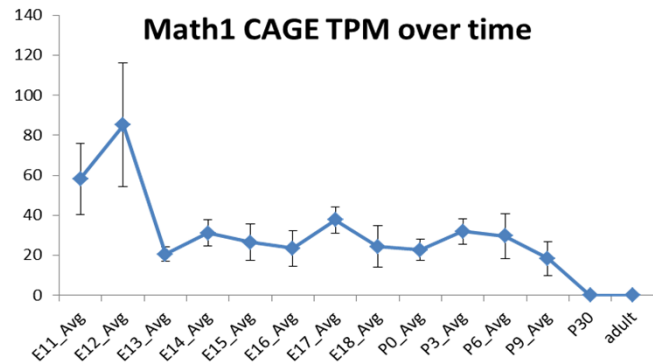


Glutamatergic Rhombic Lip Lineage – Granule cell

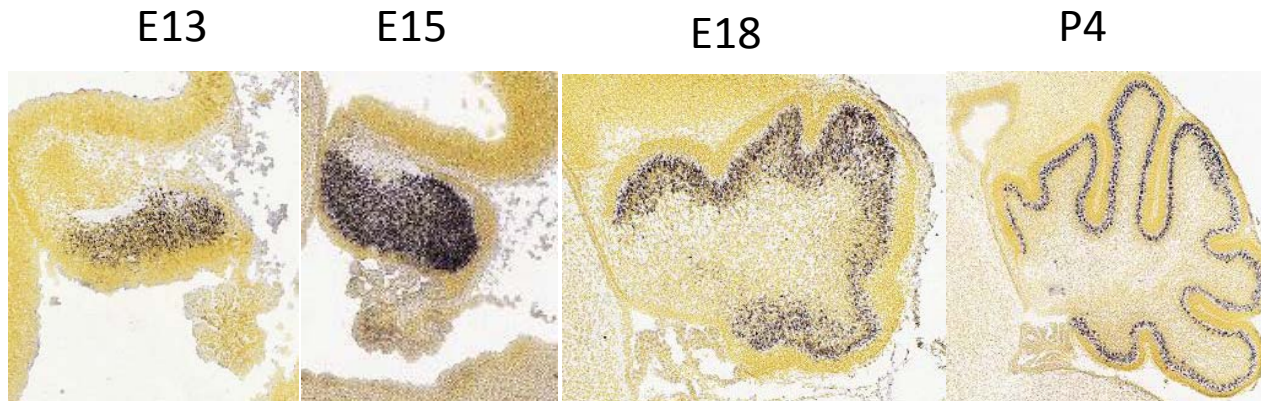
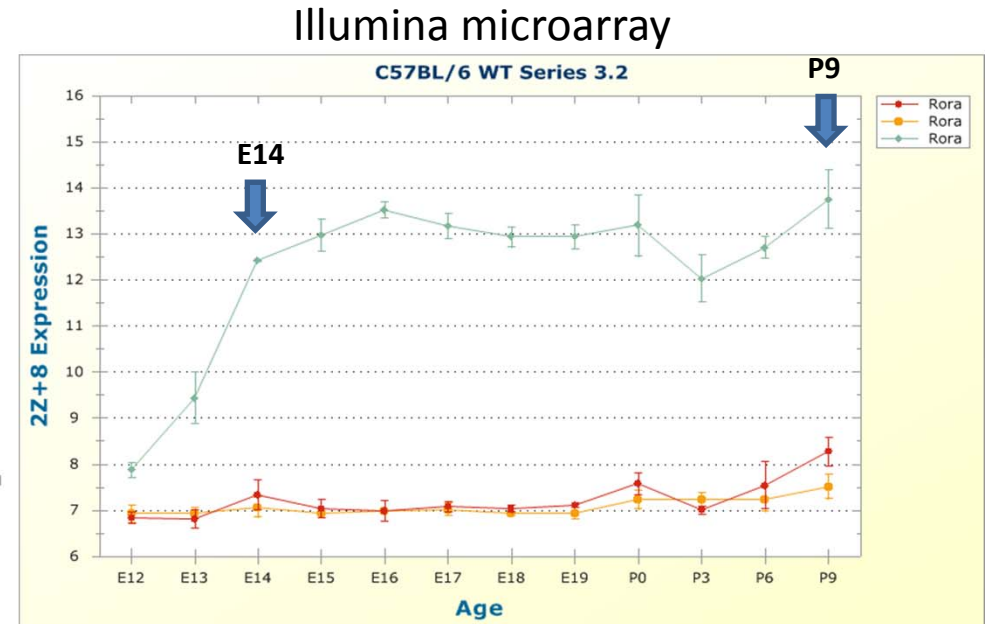
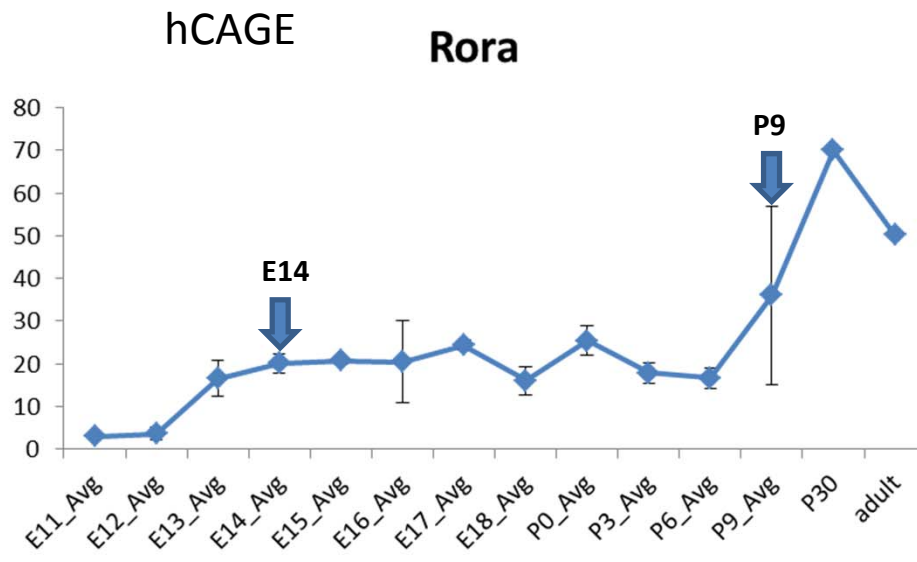
hCAGE

Allen Atlas in situ data

ZENBU tracks

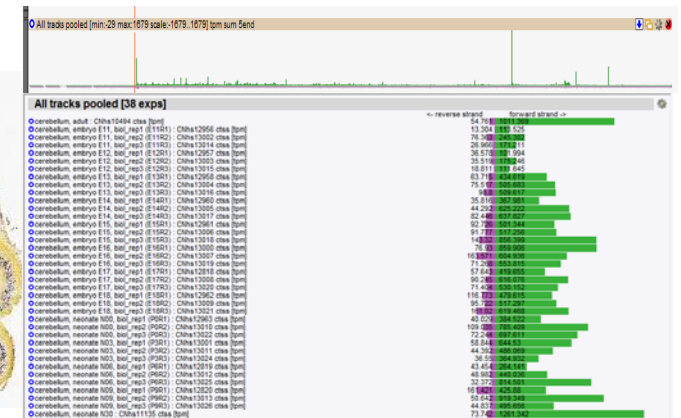


Rora as a Purkinje cell marker



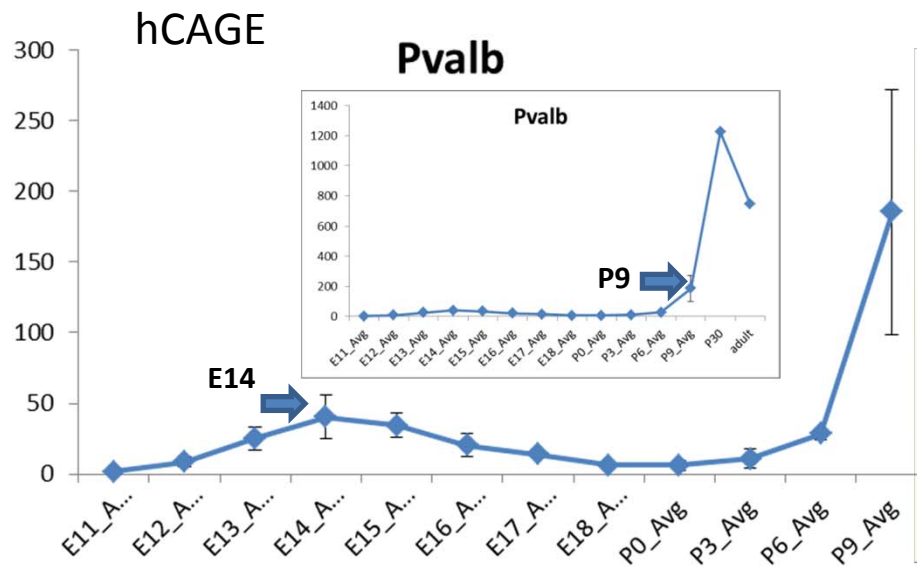
Allen Atlas in situ data

ZENBU tracks

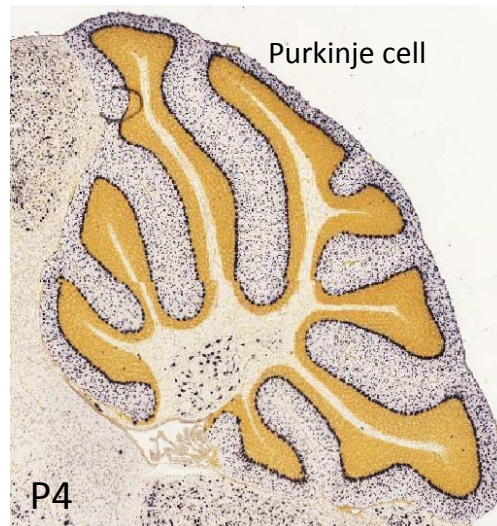
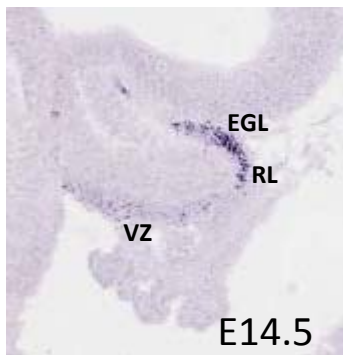
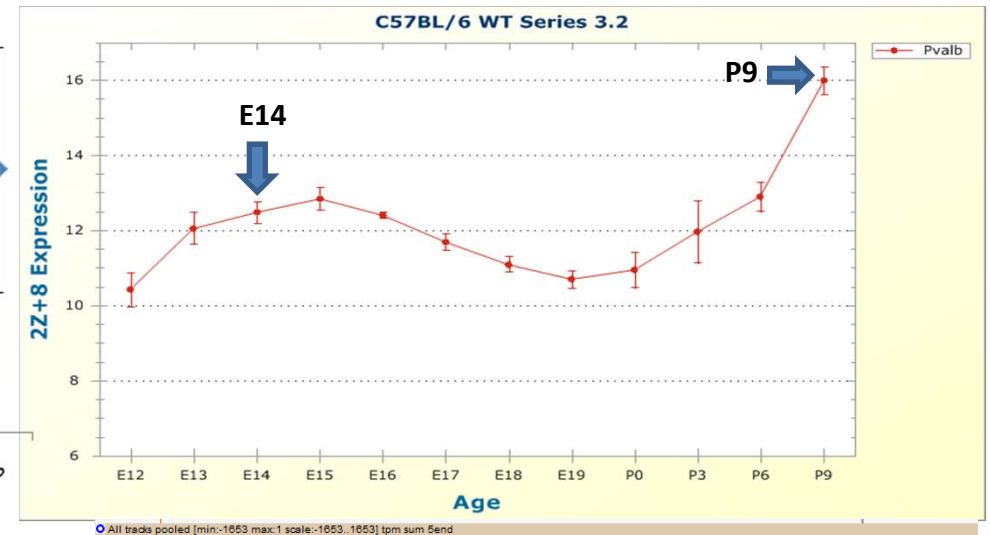


ZENBU tracks

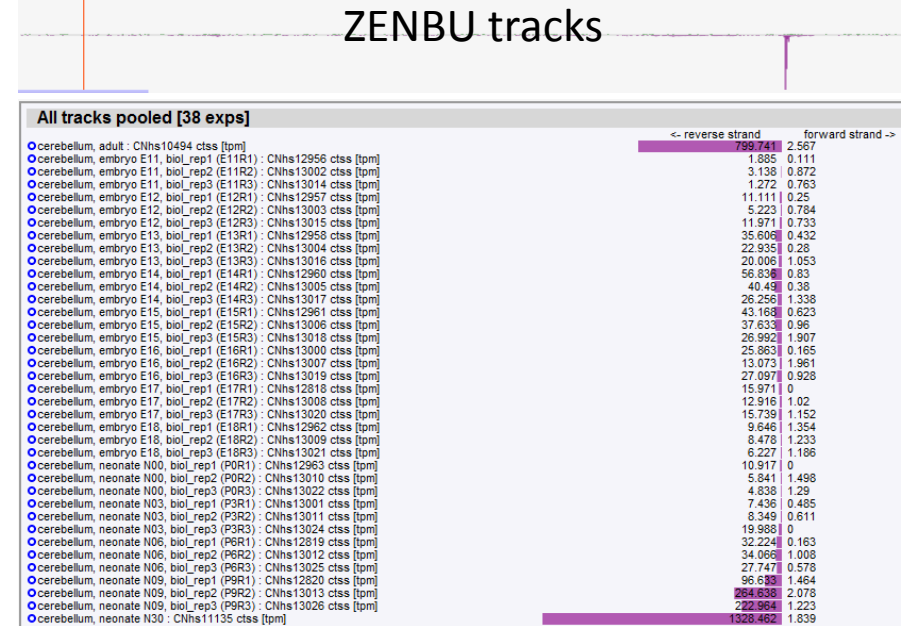
Pvalb is expressed in both cerebellar lineages



Illumina microarray



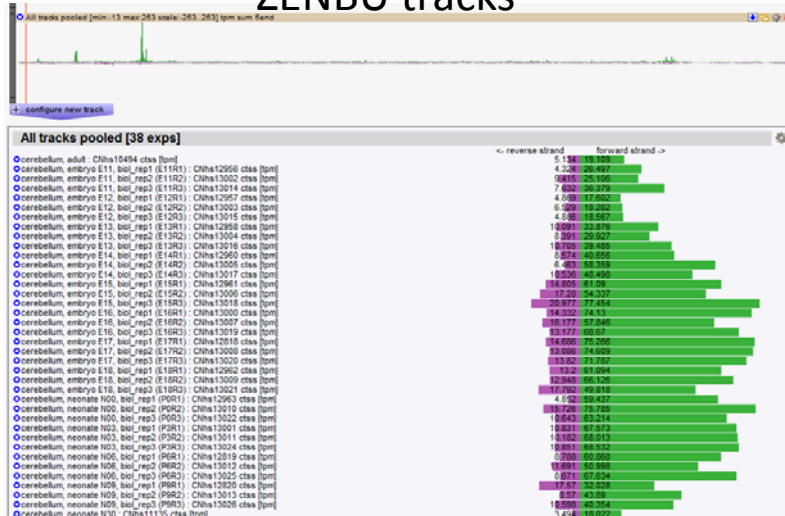
Allen Atlas in situ data



Single prominent start site exists.

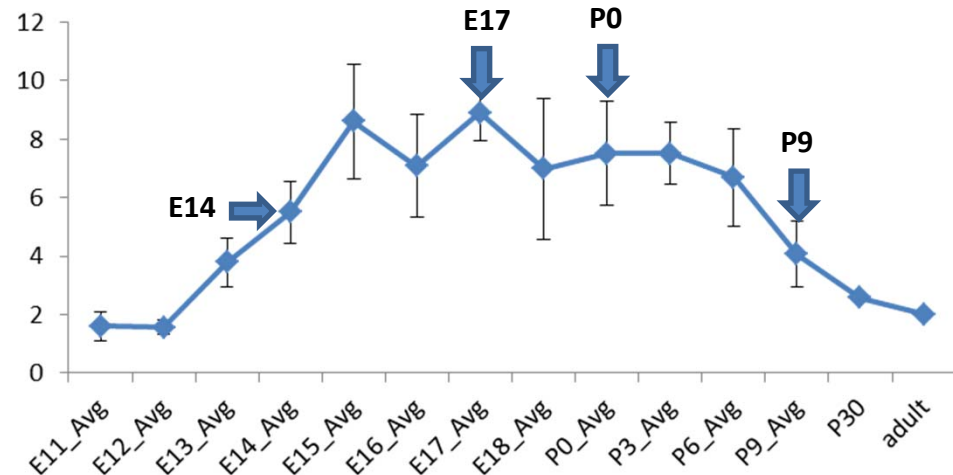
Cerebellar Interneuron marker Pax2

ZENBU tracks



Pax2 CAGE TPM over time

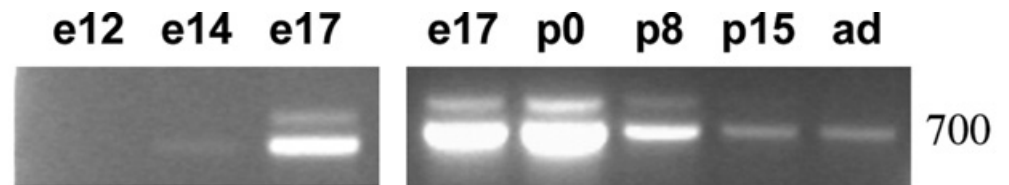
hCAGE



There is a good correlation with published RT-PCR data on Pax2 expression in cerebellum.

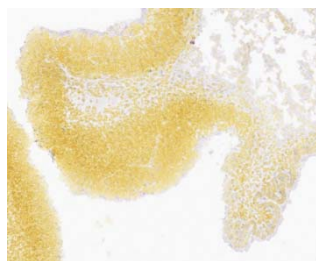
RT-PCR

Pax2 Ex2/3-8

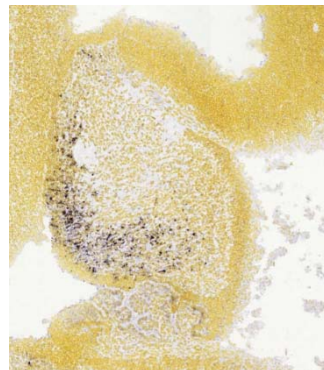


Glassmann 2009

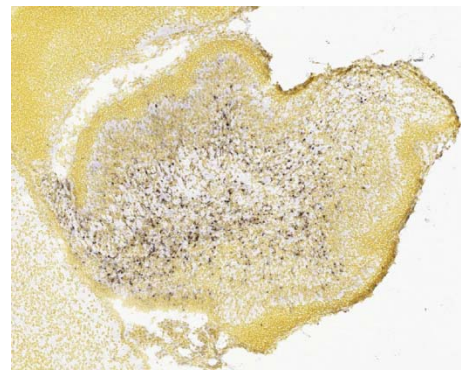
Allen Atlas in situ data



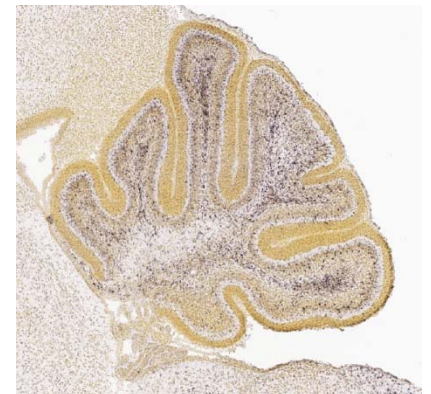
E13



E15



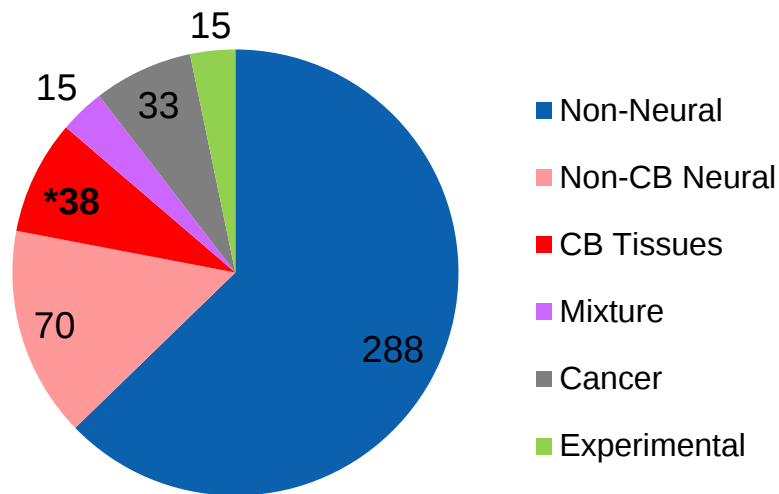
E18



P4

Allen Atlas

FANTOM5 mouse tissue collection as of Oct 2011 (total N=451)



Currently the cerebellum time course data comprises about 8% of the total mouse CAGE dataset.

2 fold higher in both maximum and median expression and max is at least 10 TPM

Group of interest	Compared to	Specific TSSs
Cerebellar tissues (N=38)	Other neural tissues (N=70)	4716
Neural tissues (N=108)	Non-neural tissues (288)	766
Cerebellar tissues (N=38)	All other tissues (N=413)	91

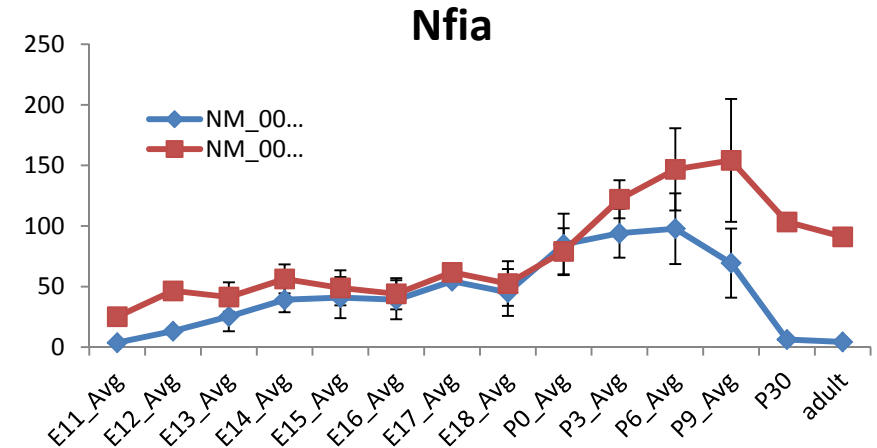
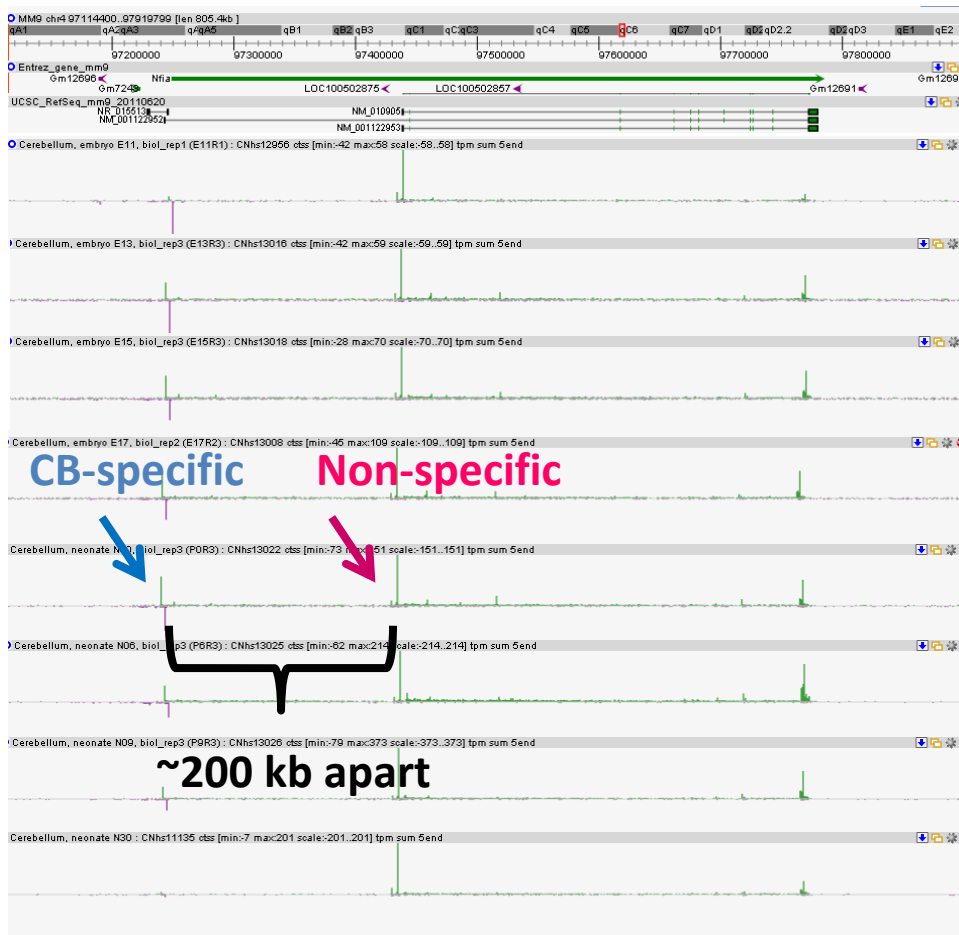
→ Pcp2, Rora, Pvalb (Purkinje cells)
Zic1, Nfia, Neurod1, Cbln1 (granule cells).

Parameter	2 fold Max(CB) > Med(ALL)	2 fold and Max(CB) is at least 10 tags	2 fold, +10 tags and 2 fold Med(CB) > Med(ALL)
CB development enriched Refseq TSS	9941 (39.4%)	7442 (29.5%)	2586 (10.2%)
CB development specific Refseq TSS	161 (0.64%)	115 (0.46%)	91 (0.36%)

17 Genes with at least one cerebellar enriched transcription start site (TSS) and one non-enriched TSS

Gene symbol	Gene name	Cerebellar Specific TSS	Non-specific TSSs	Cerebellar Expression (Allen Brain Atlas)
Anks1b	Ankyrin 1b	1	2	Purkinje cells
Chn2	chimerin 2	1	1	Granule cells
Dpp6	dipeptidylpeptidase 6	1	3	Granule cells
Etv1	ets variant 1	1	1	Granule cells
Foxp2	forkhead box P2	1	1	Granule cells
Inadl	InaD-like	1	1	Granule cells
Kcnp1	Kv channel-interacting protein 1	1	1	Purkinje cells
Kcnp4	Kv channel interacting protein 4	1	4	Purkinje cells, granule cells
Myt1	myelin transcription factor 1	1	2	Granule cells
Nfia	nuclear factor I/A	1	1	Granule cells
Olfm3	olfactomedin 3	1	1	Data not available
Phactr3	phosphatase and actin regulator 3	1	3	Purkinje cells, granule cells
Pld5	phospholipase D family, member 5	1	1	Granule cells
Ptprr	protein tyrosine phosphatase, receptor type, R	2	2	Purkinje cells, granule cells
Skor1	SKI family transcriptional corepressor 1	1	2	Data not available
Slc25a22	solute carrier family 25, member 22	1	1	Purkinje cells, granule cells
Tcfap2b	transcription factor AP-2 beta	1	1	Interneurons

Nuclear factor I/A and its two transcription start sites



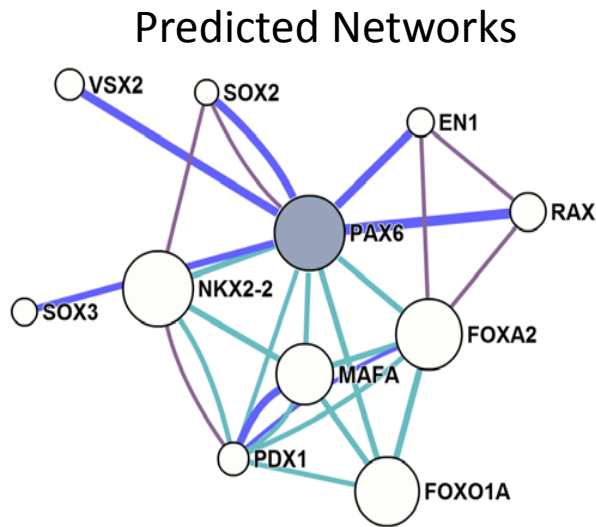
Meandertail mutant cerebellum
Only anterior granule cells are affected.



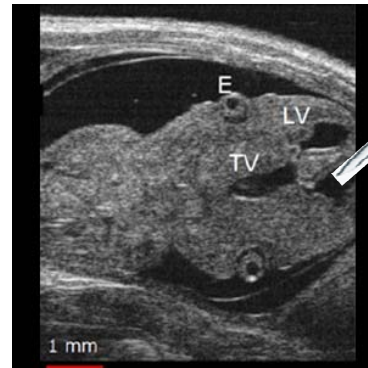
More information is presented at the Poster Session
by Peter Zhang, Thomas Ha and Dan Goldowitz.

Hamre and Goldowitz, 1997

Biological Validation



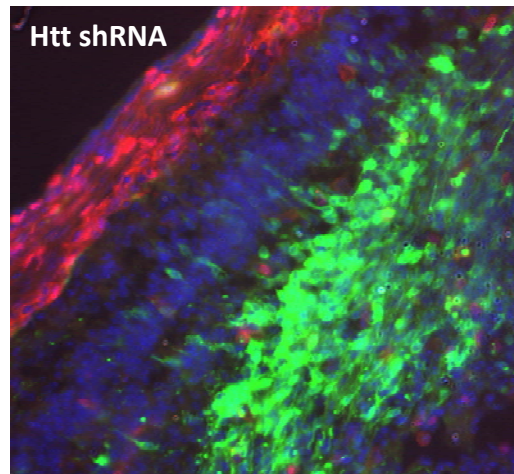
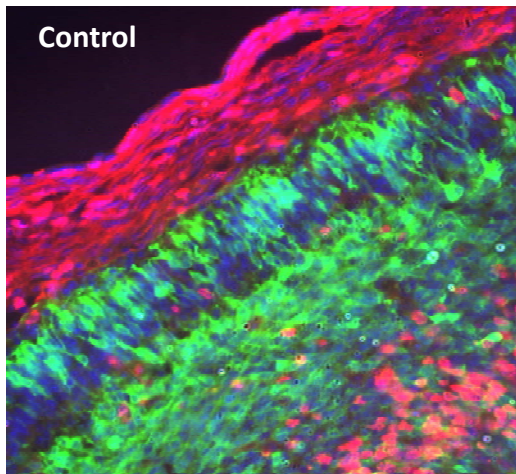
siRNA against TFs



Vevo 770 Ultrasound imaging system



in vivo transfection



Once we have MARA predictions on the transcriptional network of cerebellar development, we will perform series of validation experiments with shRNA targeting the members of the network.

Knockdown of Htt leads to disrupted migration of cortical neurons during development.

Summary and Future Directions

- The Cerebellar Time Course CAGE TPM values are in agreement with the current knowledge of gene expression during cerebellar development.
- We have observed high correlations between the Cerebellar Time Course CAGE TPM, microarray expression data, and *in situ* data.
- The next step is the identification of transcriptional networks in cerebellar time course data with MARA analysis.
- We will then perform biological validation experiments including *in utero* injections of siRNA into the developing cerebellum.



Acknowledgements

- Dan Goldowitz
- Peter Zhang
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- Matt Larouche
- Gloria Mak
- Kate Campbell
- Alistair Forrest
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