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CEREBELLAR CORTEX, PURKINJE CELL LAYER (CBXpu)

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Introduction

This report contains a summary of expression patterns for genes that are enriched in the Purkinje cell layer (CBXpu) of the cerebellum. All data is derived from the Allen Brain Atlas (ABA) in situ hybridization mouse project. The structure's location and morphological characteristics in the mouse brain are described using the Nissl data found in the Allen Reference Atlas. Using an established algorithm, the expression values of the CBXpu were compared to the values of its larger parent structure, in this case the cerebellar cortex, for the purpose of extracting regionally selective gene expression data. The highest ranking genes were manually curated and verified. 50 genes were then selected and compiled for expression analysis. The experimental data for each gene may be accessed via the links provided; additional data in the sagittal plane may also be accessed using the ABA. A gene ontology table (derived from DAVID Bioinformatics Resources 2007) is also included, highlighting possible functions of the 50 genes selected for this report.

To read more about how our 50 Select Genes list is derived, please refer to the Fine Structure Annotation white paper.

Allen Reference Atlas Coronal Levels: 101-131
Allen Reference Atlas Sagittal Levels: 1-21
Shown below is a plate from the Allen Reference Atlas, depicting the Purkinje cell layer (118):


1
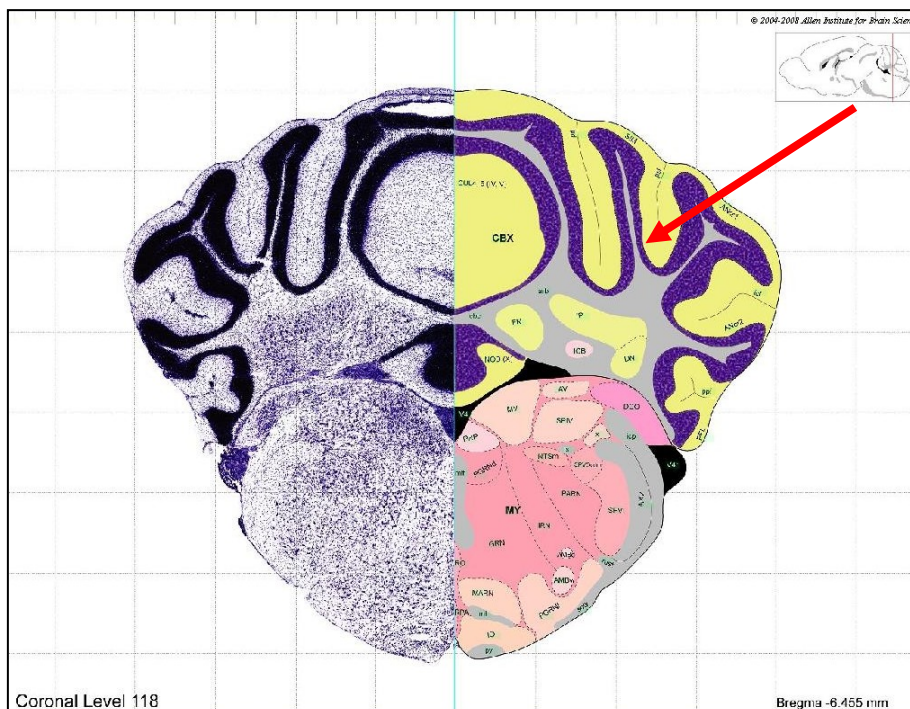
Description of Structure:

The Purkinje cell layer is situated between the molecular and granule cell layers of the cerebellar cortex. It contains a monolayer of Purkinje cells, GABA-ergic projection neurons noted for their large cell bodies. In addition to Purkinje cells, this cell layer also contains Bergman glia, specialized astrocytes within the cerebellum that play a role in development. These specialized glia can be differentiated from Purkinje cells by their projections around the Purkinje cells, often radiating into the molecular cell layer of the cerebellar cortex.

The Purkinje cell layer is present throughout all subdivisions of the cerebellar cortex. In the Allen Reference Atlas (ARA), the Purkinje cell layer is depicted as a dotted line superimposed between the molecular and granule cell layer. However, on the Nissl-stained sections within the ARA, individual Purkinje cells are difficult to identify due to their close apposition with the darkly staining, cell-dense granular cell layer.

The appearance and location of the Purkinje cell layer can be appreciated on the following two pages. Nissl-stained sections and Allen Reference Atlas plates reveal the cytoarchitecture and extent of the Purkinje cell layer, and its location in relation to surrounding structures.
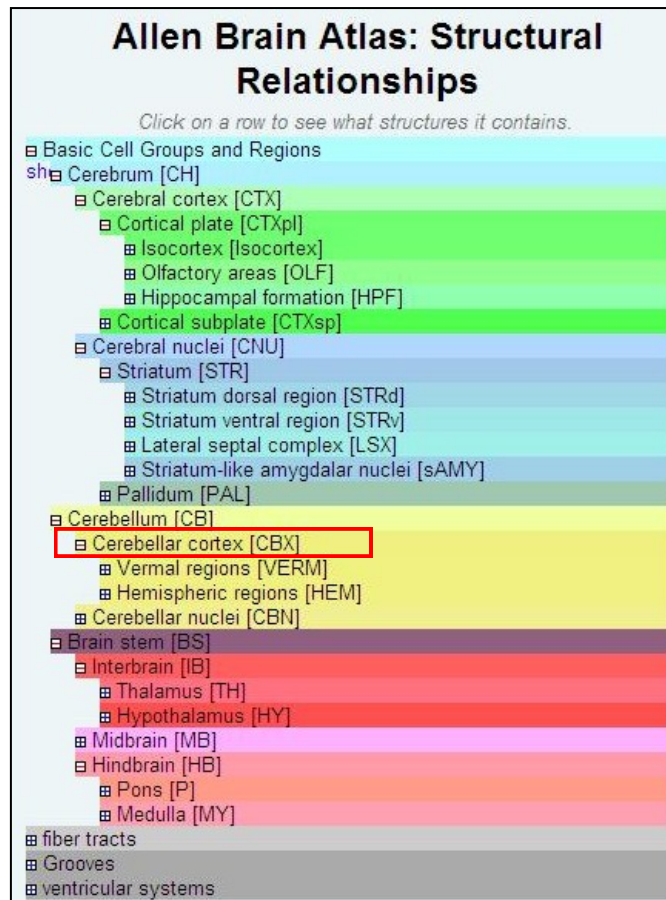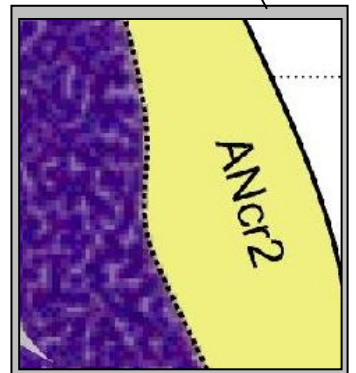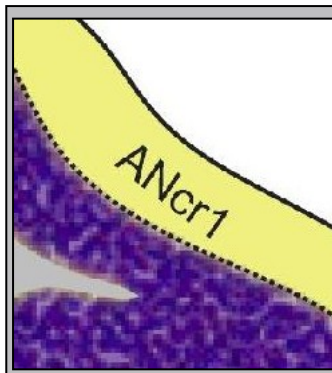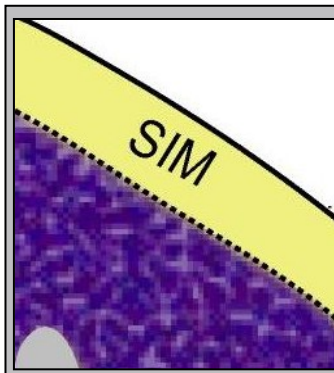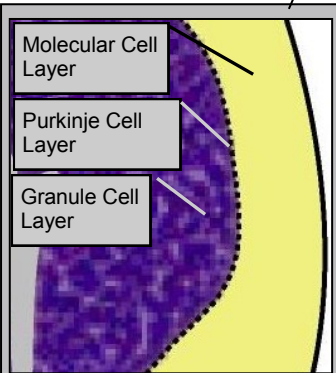
Atlas and Nissl:
Coronal:

Rostral   **Caudal**

Reference Atlas

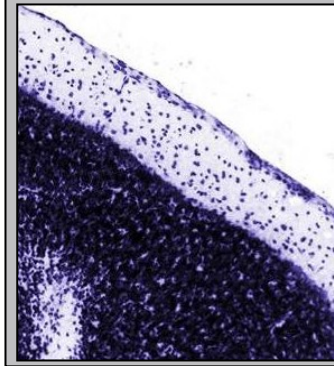Molecular Cell Layer
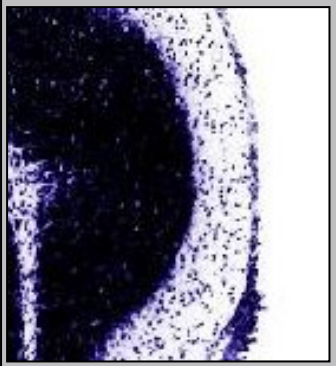
Purkinje Cell Layer

Granule Cell Layer

SIM

ANcr1

ANcr2
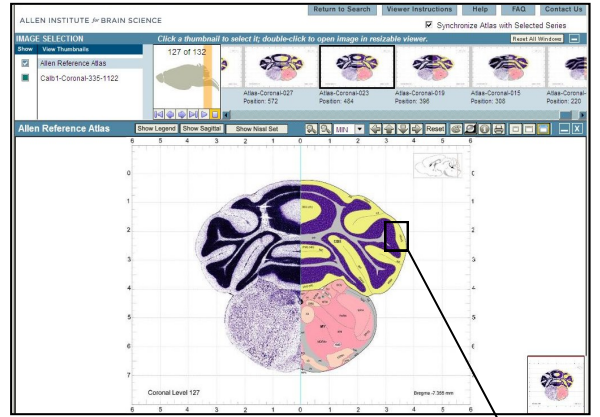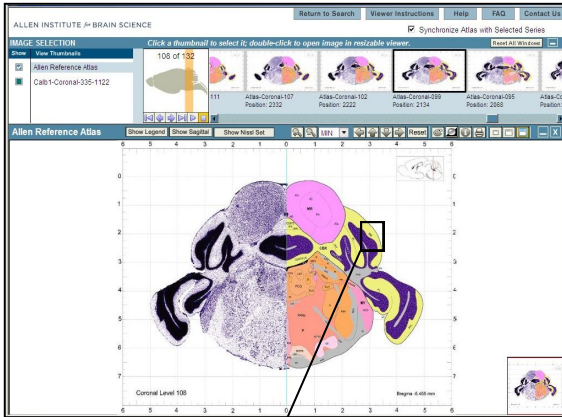
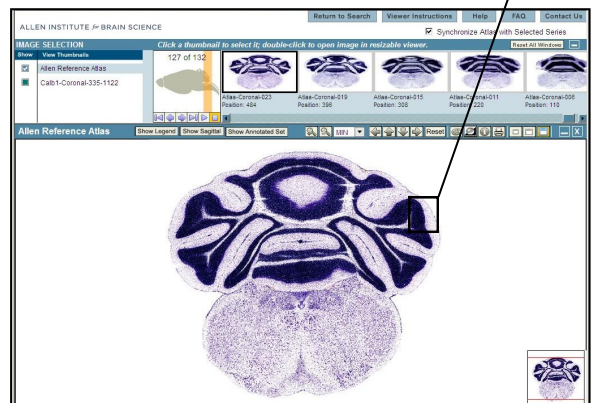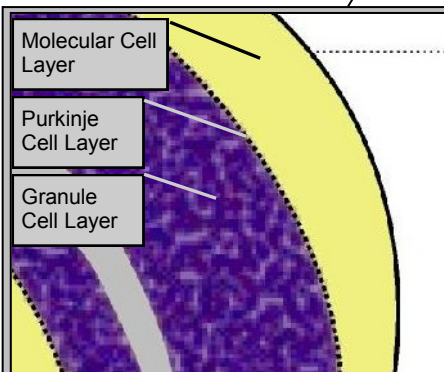Level 108 → Level 113 → Level 120 → Level 127
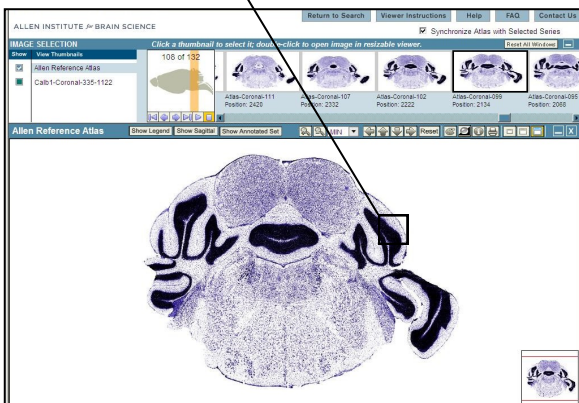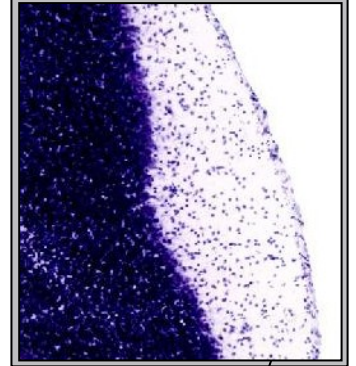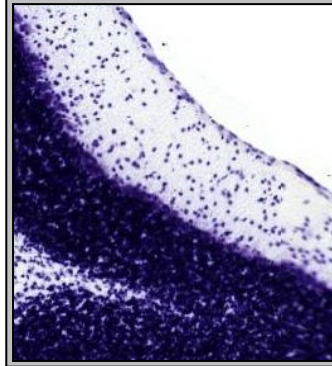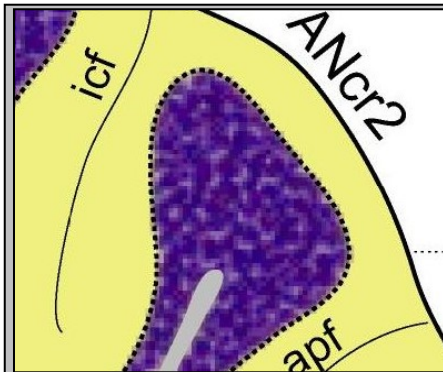
Nissl

Atlas and Nissl:
Sagittal:

Lateral

Medial

left side, Nature Precedings vertical text

Reference Atlas

Molecular Cell Layer

Purkinje Cell Layer

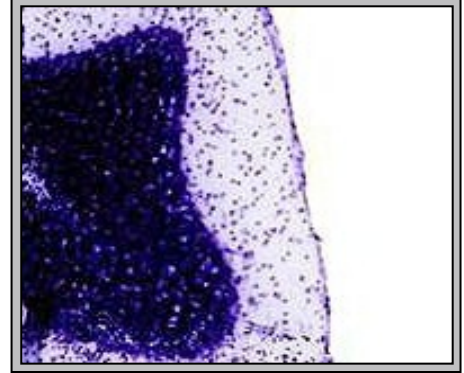Granule Cell Layer

icf   ANcr2

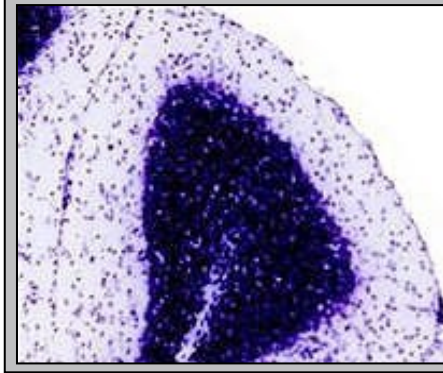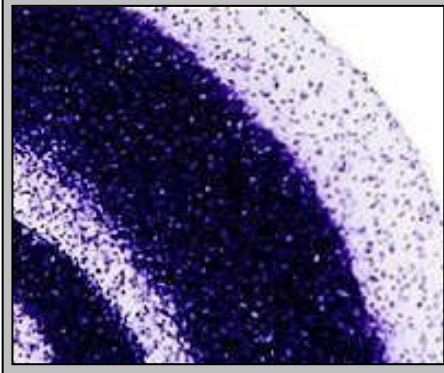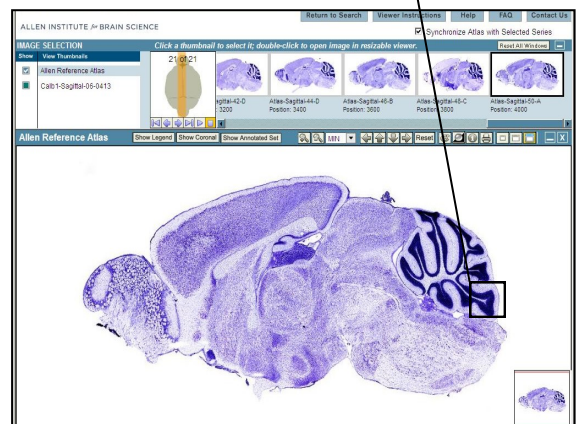apf

PYR (VIII)
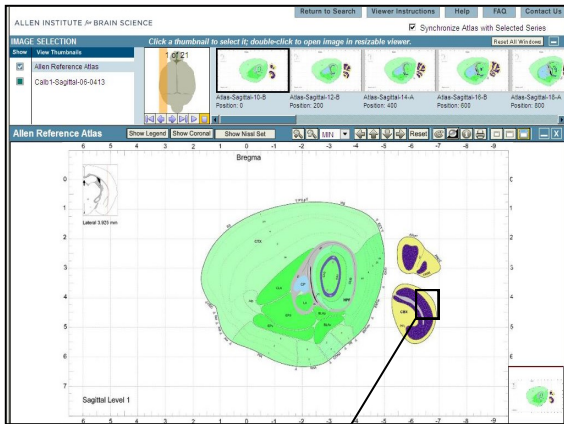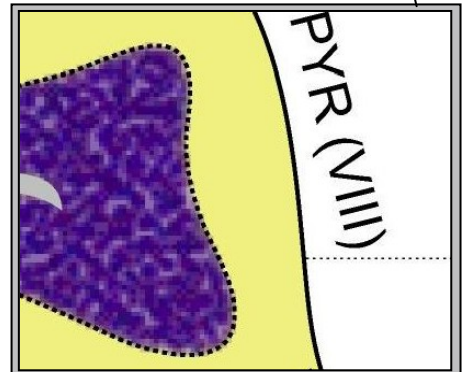
Level 1   →   Level 11   →   Level 21
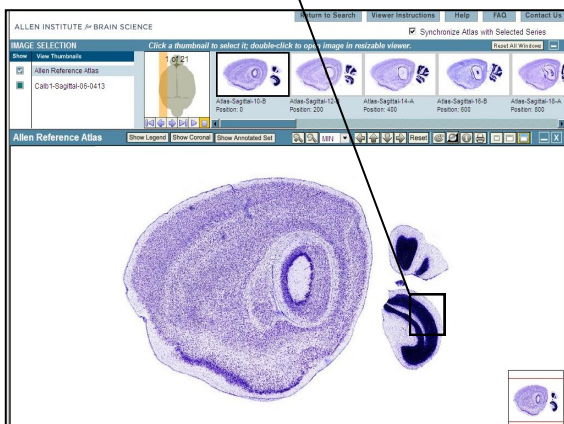
Nissl

In Situ Hybridization Expression Patterns of 50 Select Genes:

The in situ hybridization (ISH) data below presents the Purkinje cell layer's anatomical and cytoarchitectural characteristics in the context of actual gene expression. In addition to presenting molecularly defined borders, ISH gene expression patterns also aid in phenotyping cell populations that otherwise can not be differentiated on purely morphological grounds. The 50 genes in this section were selected based on a mathematical algorithm to identify gene expression patterns that allow selective identification of the Purkinje cell layer. The gene expression patterns were then verified manually. As such, these genes do not represent the only genes found in this structure, genes specific to this structure, or genes expressing at the highest level within this structure.

Please refer to our protocol in the Data Production Processes white paper.
To read about heat map conversion, refer to the Informatics Data Processing white paper.
The expression data presented can be further explored, in coronal and sagittal planes, at brain-map.org.

The expression patterns found with these 50 Select Genes have been divided into three categories. The majority of genes examined were expressed in the Purkinje cells; some of these genes labeled only the Purkinje cells (the first category), while others labeled additional cells in the cerebellar cortex (the second category). Within the second category, these genes most often labeled golgi cells within the granule layer, although a few genes labeled interneurons within the molecular cell layer. Finally, a third group of six genes is presented that label the Bergman glia but not the Purkinje cells.

While the Purkinje cells are hard to identify on the Nissl images presented in the ABA, they are clearly demarcated with these genes. Bergman glia, which are interdigitated between Purkinje cells and difficult to distinguish from the Purkinje cells on Nissl stained sections, were easily identified in the third category, as the Purkinje cells themselves did not label, leaving a negative impression of these cells within the Purkinje cell layer.

Cellular density expression key		Cellular intensity expression key	
None	No expression	No color	Very low intensity
Sparse	Very few cells expressing	Blue	Low intensity
Scattered	Less than 10% of cells expressing in scattered pattern	Green	Medium intensity
Medium	10-80% of cells expressing	Yellow	High intensity
High	Greater than 80% of cells expressing	Red	Very high intensity

To view the heat map at brain-map.org, right click on the ISH image and select "Show Expression Analysis."

ISH DATA *The images below were selected to highlight various expression patterns of the Purkinje cell layer.*

ISH
Gng13
Coronal:
Gng13 shows expression that is fairly restricted to Purkinje cells, but no labeling in the other layers of the cerebellar cortex.
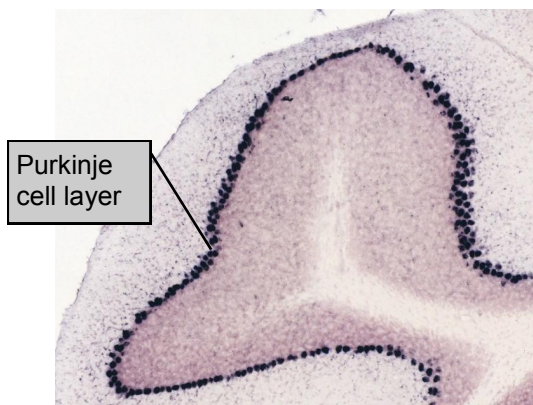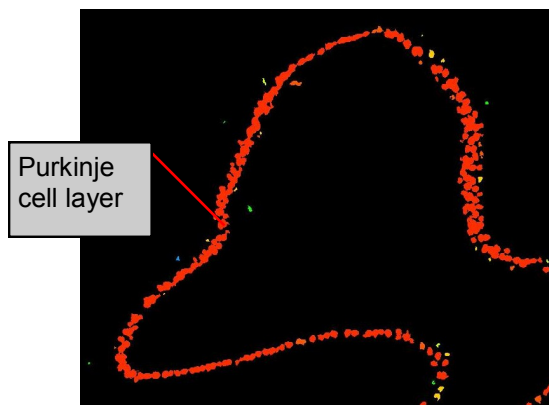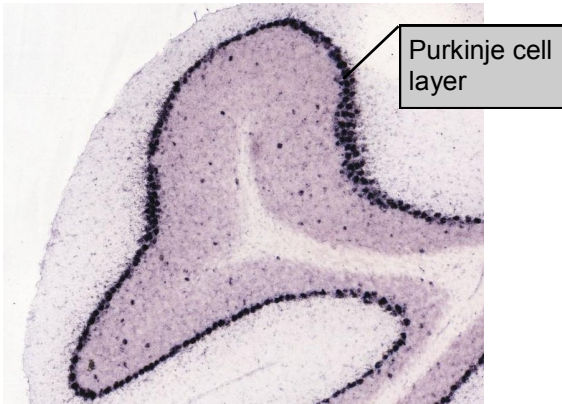

Heat Map
Gng13
Coronal:
The high density and very high intensity of *Gng13* expression, restricted to the Purkinje cells, is highlighted by the heat map image.
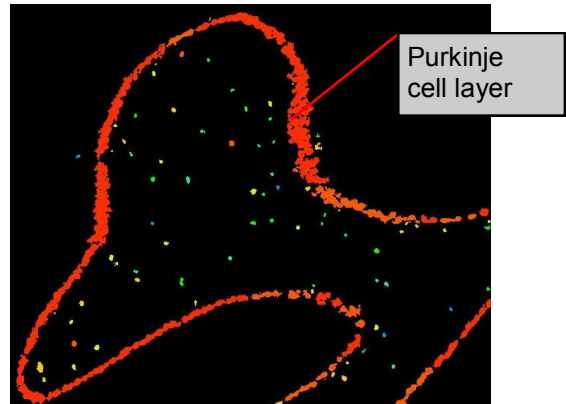
ISH
Abhd3
Coronal: *Abhd3* is expressed in both the Purkinje cells as well as Golgi II cells in the granule cell layer.

Purkinje cell layer

Heat map
Abhd3
Coronal: The heat map image reveals that *Abhd3* expression in the granule cell layer is less intense than the expression in the Purkinje cells.

Purkinje cell layer

ISH
Ppap2b
Coronal: *Ppap2b* is expressed in Bergman glia, here identified by their location within the Purkinje cell layer coupled with a lack of expression in Purkinje cells.

Purkinje cell layer
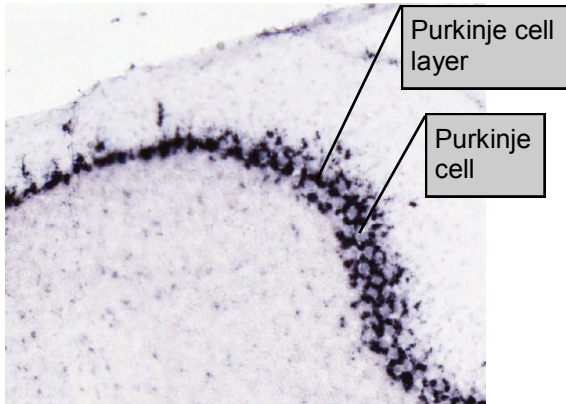
Purkinje cell

Heat map
Ppap2b
Coronal: The absence of *Ppap2b* expression in Purkinje cells is evident in the heat map image.

Purkinje cell layer

Purkinje cell

alleninstitute.org

50 SELECT GENES:

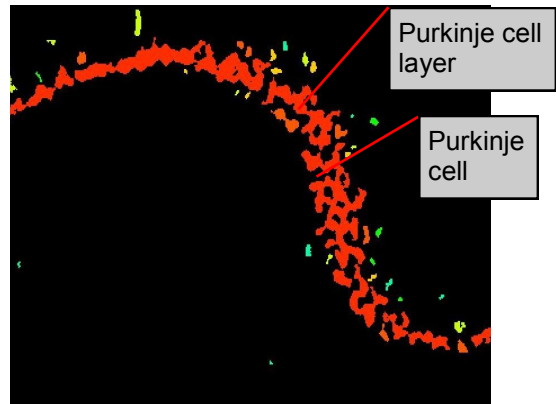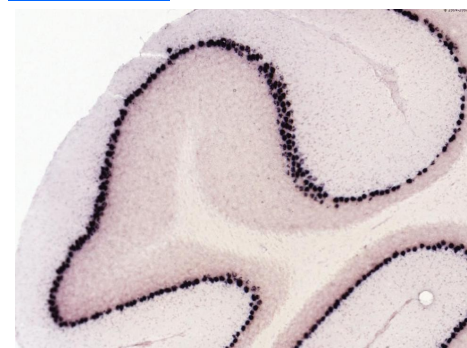*This gene list was generated by manual curation of an [algorithmically](#) derived list that compared gene expression values of the Purkinje cell layer to those of the cerebellum. Categories of expression are subjectively grouped by relative expression characteristics.*

Curation of 50 Select Genes List: June 2007

Expression Restricted to Purkinje cells

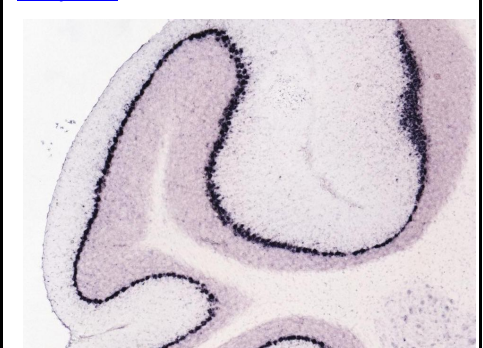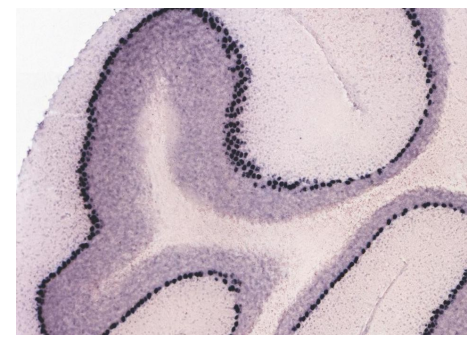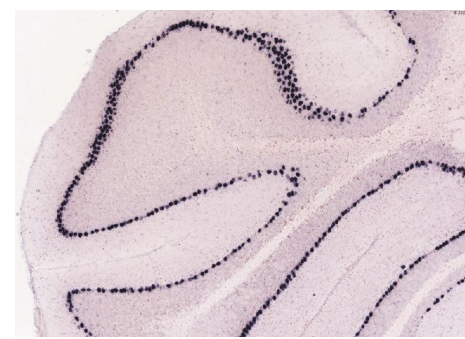| Number	Gene Symbol	Gene Name	Expression Pattern
1	TC1568100	TIGR MGI TC1568100	High density, very high intensity
2	Id2	inhibitor of DNA binding 2	High density, very high intensity
3	Cpne2	copine II	High density, very high intensity
4	Gsbs	G substrate	High density, very high intensity
5	3110001A13Rik	RIKEN cDNA 3110001A13 gene	High density, very high intensity
6	Pcsk6	proprotein convertase subtilisin/kexin type 6	High density, very high intensity
7	0610007P14Rik	RIKEN cDNA 0610007P14 gene	High density, very high intensity
8	Grid2ip	glutamate receptor, ionotropic, delta 2 (Grid2) interacting protein 1	High density, very high intensity
9	Gng13	guanine nucleotide binding protein 13, gamma	High density, very high intensity
10	Slc35b1	solute carrier family 35, member B1	High density, very high intensity
11	Itpr1	inositol 1,4,5-triphosphate receptor 1	High density, very high intensity
12	Baiap2	brain-specific angiogenesis inhibitor 1-associated protein 2	High density, very high intensity
13	Dgkh	diacylglycerol kinase, eta	High density, very high intensity
14	Grik1	glutamate receptor, ionotropic, kainate 1	High density, very high intensity
15	Creg1	cellular repressor of E1A-stimulated genes 1	High density, very high intensity

1.TC1568100

2.Id2

3.Cpne2

4.Gsbs

5.3110001A13Rik

6.Pcsk6

7

Expression Restricted to Purkinje Cells

7.0610007P14Rik	8.Grid2ip	9.Gng13
10.Slc35b1	11.Itpr1	12.Baiap2
13.Dgkh	14.Grik1	15.Creg1
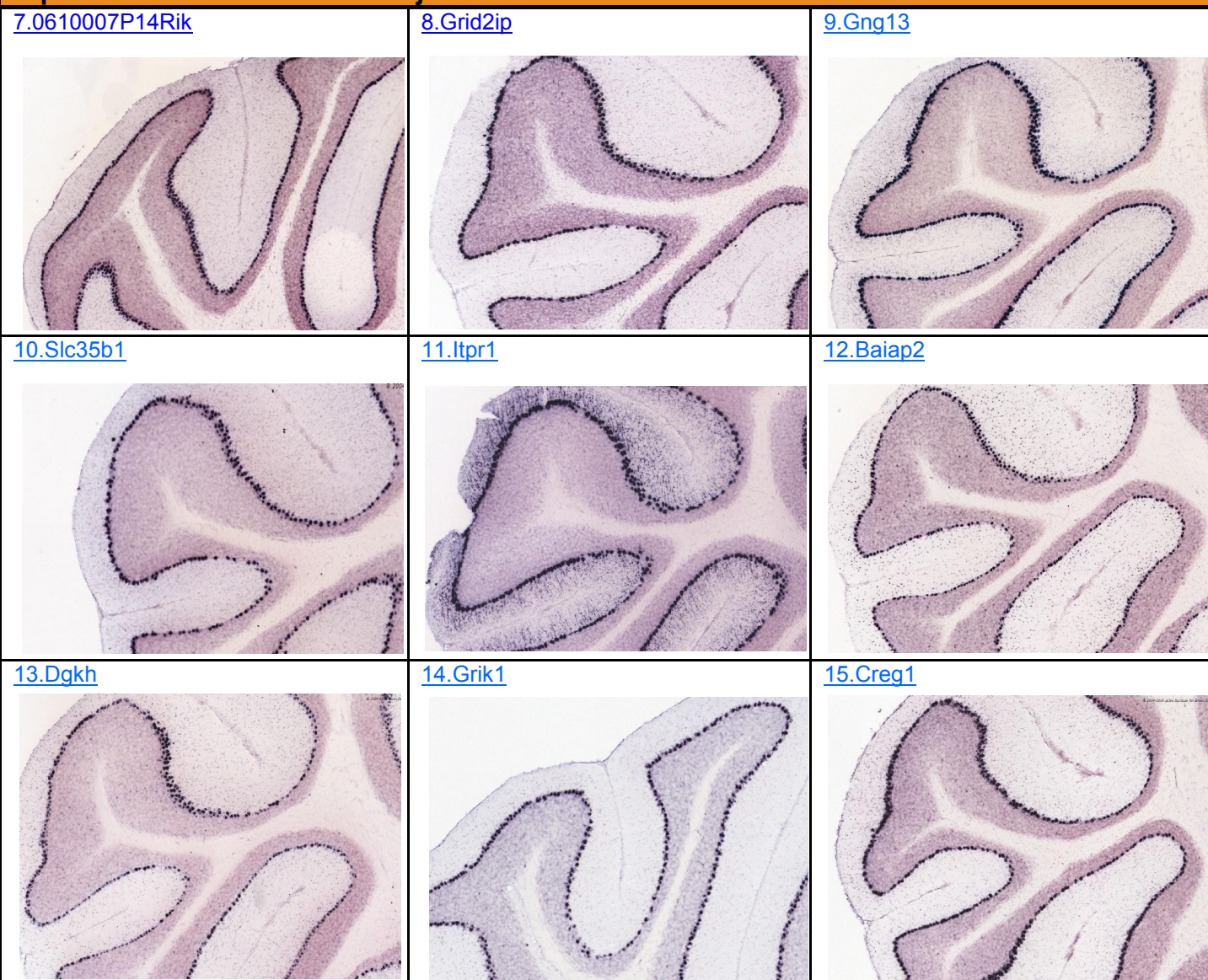
Enriched Expression in Purkinje Cells and Interneurons

|			
16	Abhd3	abhydrolase domain containing 3	High density, very high intensity
17	Arhgap26	Rho GTPase activating protein 26	High density, very high intensity
18	Dpp10	dipeptidylpeptidase 10	High density, very high intensity
19	Ryr1	ryanodine receptor 1, skeletal muscle	High density, very high intensity
20	Steap2	six transmembrane epithelial antigen of prostate 2	High density, very high intensity
21	Gabbr2	gamma-aminobutyric acid (GABA) B receptor 2	High density, very high intensity
22	Cerk	ceramide kinase	High density, very high intensity
23	Lrrn2	leucine rich repeat protein 2, neuronal	High density, very high intensity
24	Nefh	neurofilament, heavy polypeptide	High density, very high intensity
25	Atp2a2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	High density, very high intensity
26	Tm6sf1	transmembrane 6 superfamily member 1	High density, very high intensity
27	Nell2	NEL-like 2 (chicken)	High density, very high intensity
28	Hapln4	hyaluronan and proteoglycan link protein 4	High density, very high intensity
29	Hpca	hippocalcin	High density, very high intensity
30	Setd7	SET domain containing (lysine methyltransferase) 7	High density, very high intensity
31	Lrp8	low density lipoprotein receptor-related protein 8, apolipoprotein E receptor	High density, very high intensity
32	Grid2	glutamate receptor, ionotropic, delta 2	High density, very high intensity

8

Enriched Expression in Purkinje Cells and Interneurons

|			
33	Rgs8	regulator of G-protein signaling 8	High density, very high intensity
34	Erp29	endoplasmic reticulum protein 29	High density, very high intensity
35	Inpp5a	inositol polyphosphate-5-phosphatase A	High density, very high intensity
36	Hpcal1	hippocalcin-like 1	High density, very high intensity
37	Cacna2d2	calcium channel, voltage-dependent, alpha 2/delta subunit 2	High density, very high intensity
38	Ptpn4	protein tyrosine phosphatase, non-receptor type 4	High density, very high intensity
39	Cds1	CDP-diacylglycerol synthase 1	High density, very high intensity
40	LOC270764	similar to E2a-Pbx1-associated protein; amyloid-beta precursor protein intracellular domain associated protein 1; cajalin 2	High density, very high intensity
41	Cacna1g	calcium channel, voltage-dependent, T type, alpha 1G subunit	High density, very high intensity
42	Prkcc	protein kinase C, gamma	High density, very high intensity
43	Plcb4	phospholipase C, beta 4	High density, very high intensity
44	Kcnc3	potassium voltage gated channel, Shaw-related sub-	High density, very high intensity

16.Abhd3
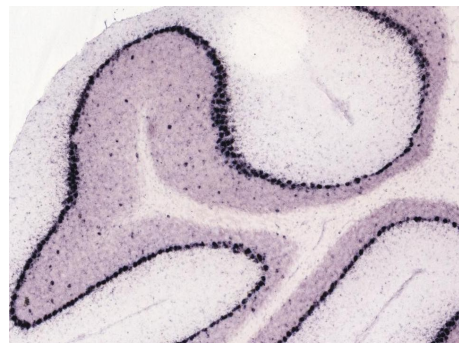
17.Arhgap26
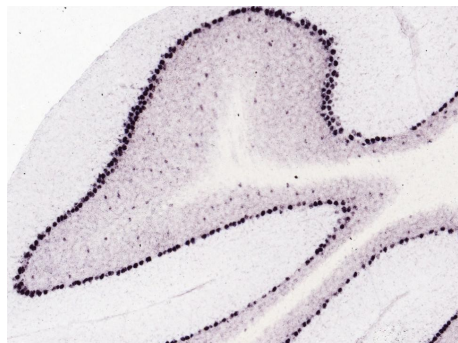
18.Dpp10
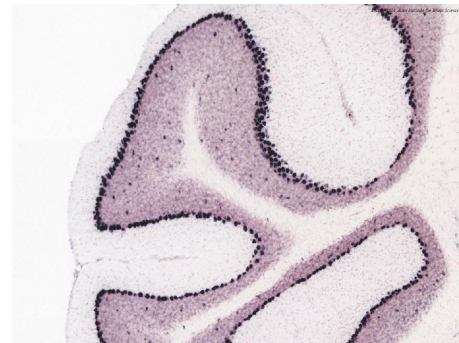
19.Ryr1
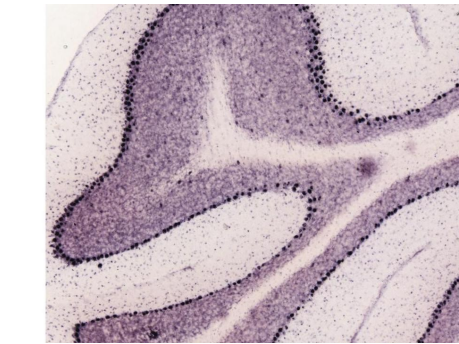
20.Steap2
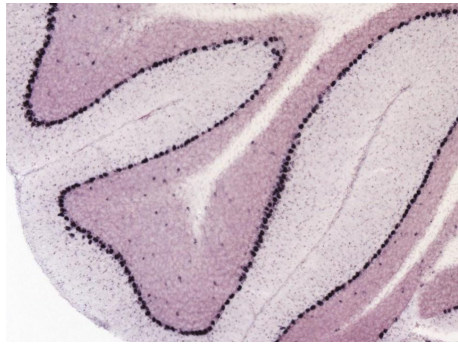
21.Gabbr2
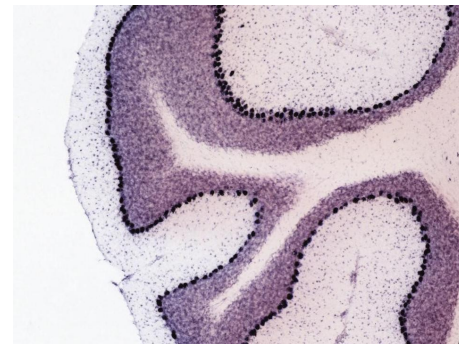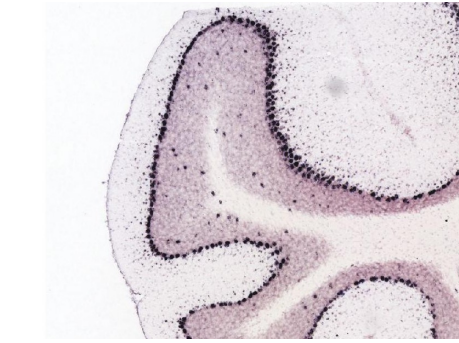

22.Cerk
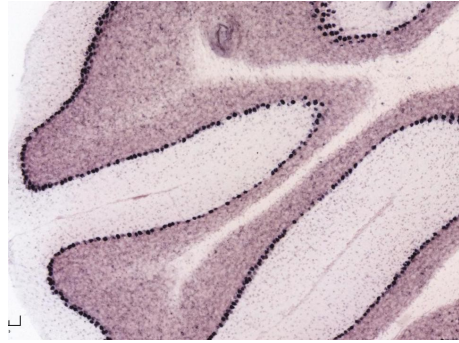
23.Lrrn2
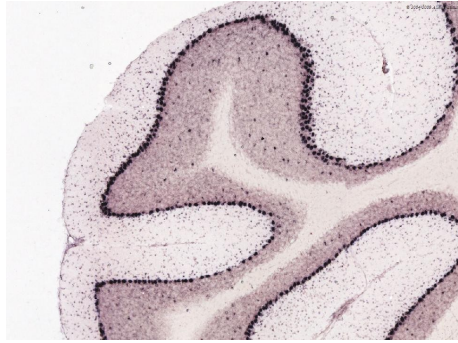
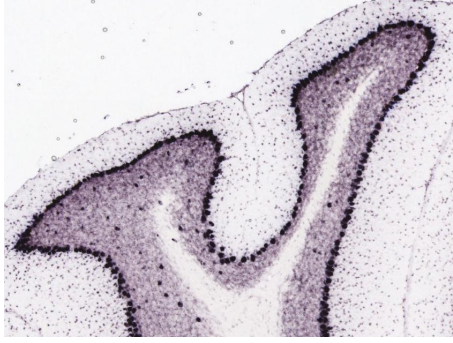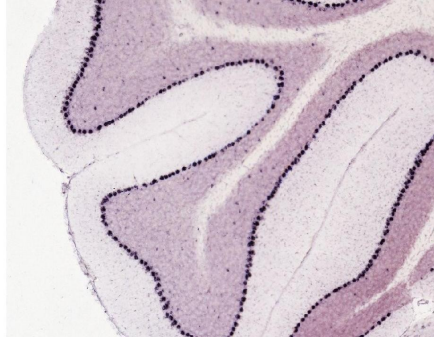24.Nefh

Enriched Expression in Purkinje Cells and Interneurons

25.Atp2a2	26.Tm6sf1	27.Nell2
28.Hapln4	29.Hpca	30.Setd7
31.Lrp8	32.Grid2	33.Rgs8
34.Erp29	35.Inpp5a	36.Hpcal1
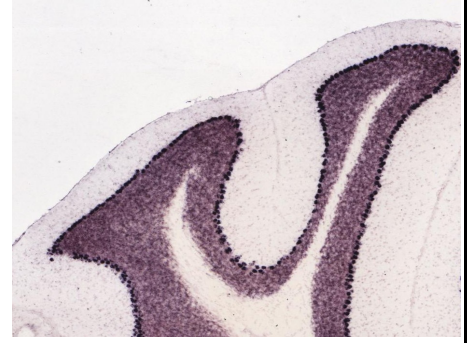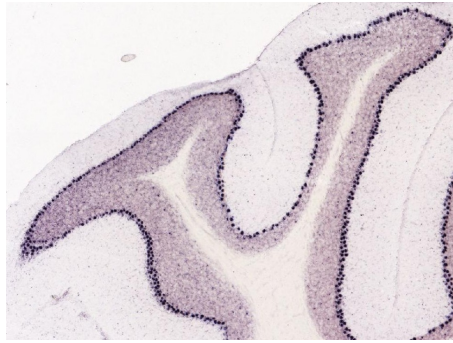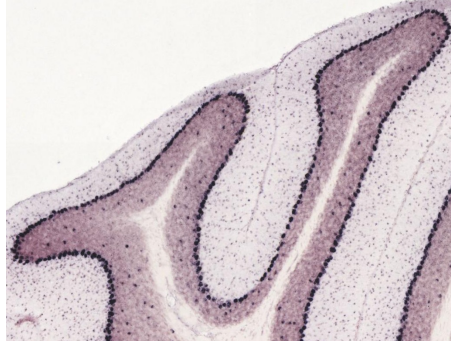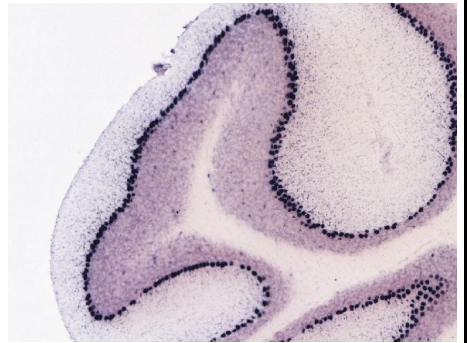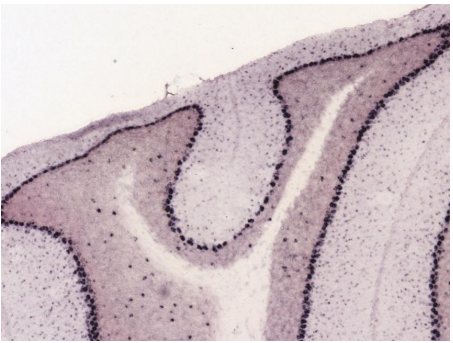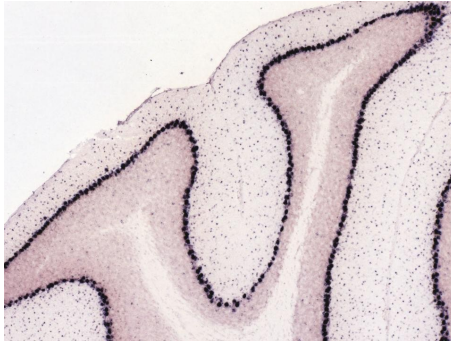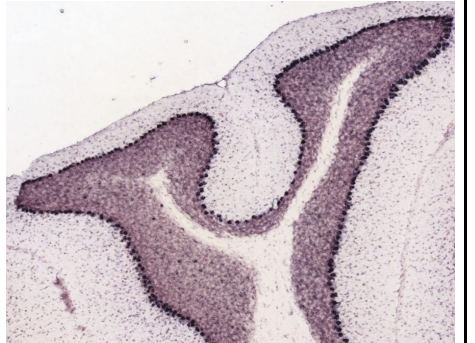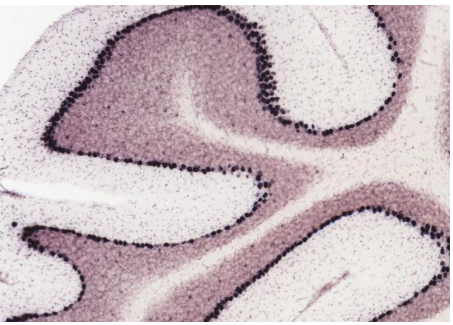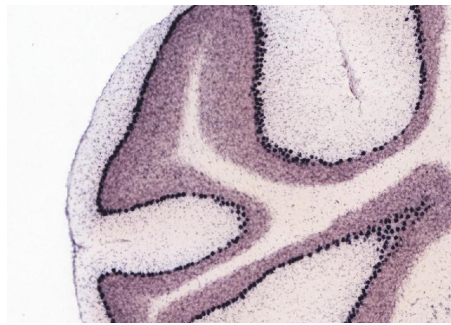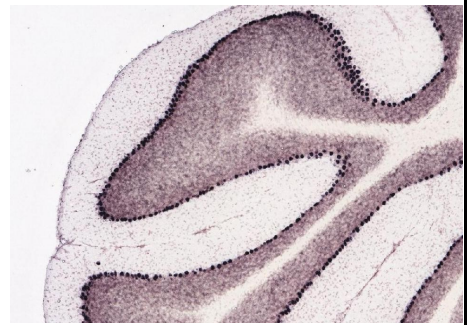
Enriched Expression in Purkinje Cells and Interneurons
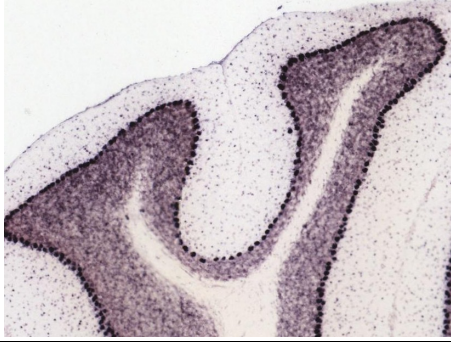
37.Cacna2d2	38.Ptpn4	39.Cds1
40.LOC270764	41.Cacna1g	42.Prkcc
43.Plcb4	44.Kcnc3	
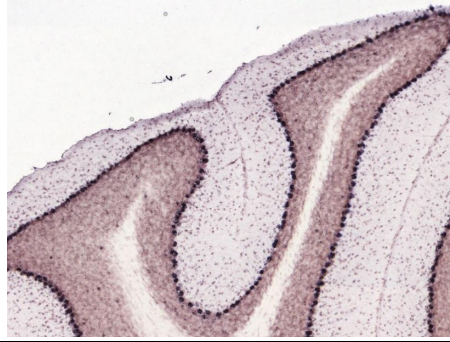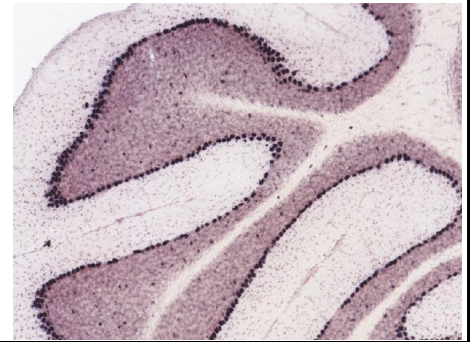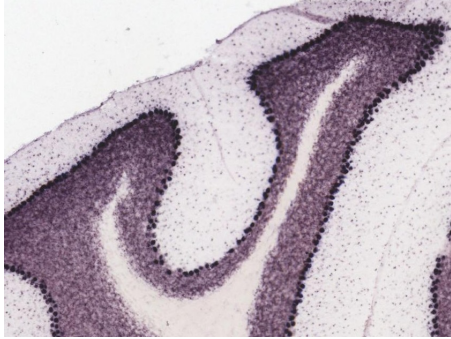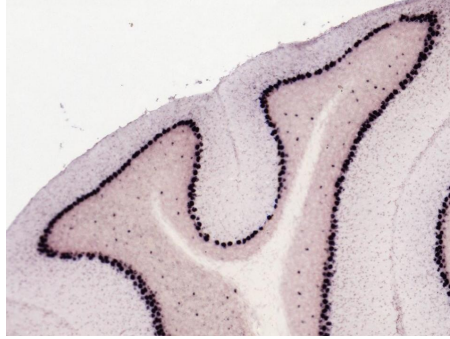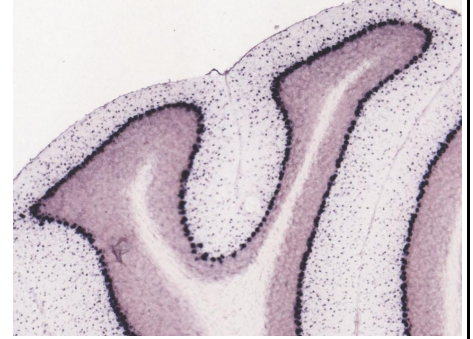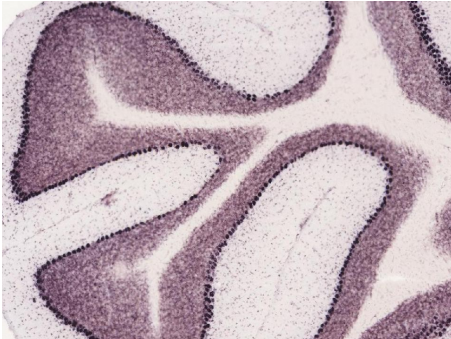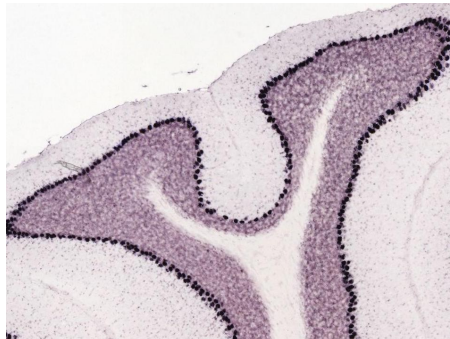
Expression in Bergmann Glia cells within Purkinje Cell Layer

45	Ppap2b	phosphatidic acid phosphatase type 2B	High density, very high intensity
46	Ptprz1	protein tyrosine phosphatase, receptor type Z, polypeptide 1	High density, very high intensity
47	Slc1a2	solute carrier family 1 (glial high affinity glutamate transporter), member 2	High density, very high intensity
48	Lcat	lecithin cholesterol acyltransferase	High density, very high intensity
49	Ednrb	endothelin receptor type B	High density and intensity
50	Dbi	diazepam binding inhibitor	High density, very high intensity
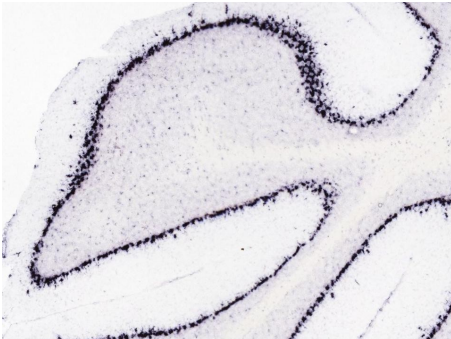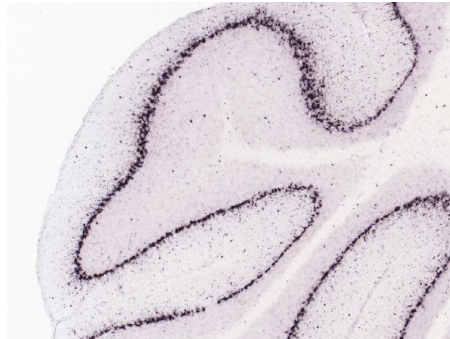
45.Ppap2b	46.Ptprz1	47.Slc1a2
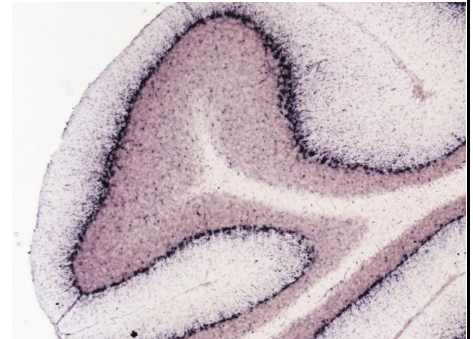
Expression in Bergmann Glia cells within Purkinje Cell Layer

48.Lcat
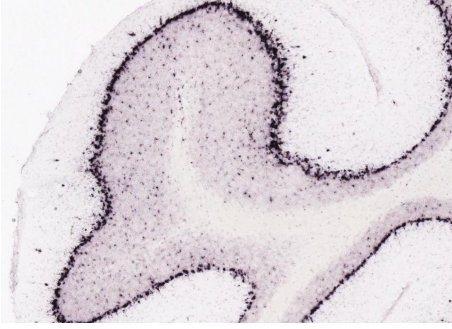
49.Ednrb
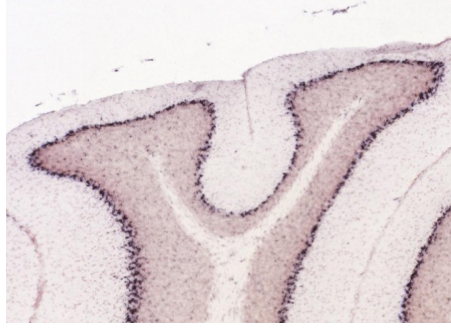
50.Dbi
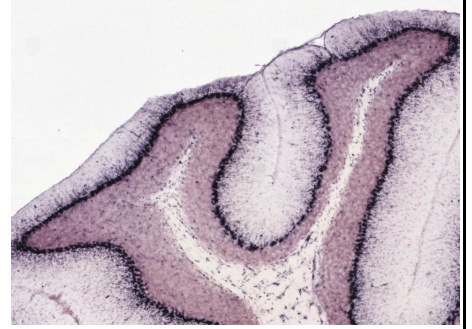
Gene Ontology (GO) Analysis:

GO TABLE:

Below is an ontological analysis of the 50 Select Genes, using DAVID Bioinformatics Resources. The functional terms that follow were returned using these constraints:

Category	Definition	Constraints
P-value	Probability that the term is over-represented in this 50 Select Genes list relative to the mouse genome	when p ≤ 0.05
Gene Count	The minimum number of genes that must fall into an onto-logical category to be considered a group	5 genes per term group
GO Level	The level of functional specificity for GO functional cate-gories: Molecular Function (mf), Biological Process (bp) and Cellular Components (cc)	Level GO_All
# of DAVID IDs	Number of unique DAVID gene IDs from user's input list	*48 DAVID gene IDs*/ 50 input genes

Date of table completion: June 2008

| GO Category	GO Term	Gene Count	% of Genes	p-value
GOTERM_MF_ALL	calcium ion binding	12	25.00%	4.33E-06
GOTERM_BP_ALL	di-, tri-valent inorganic cation transport	6	12.50%	3.24E-05
GOTERM_MF_ALL	gated channel activity	7	14.58%	4.62E-05
GOTERM_MF_ALL	metal ion transmembrane transporter activity	7	14.58%	7.45E-05
GOTERM_CC_ALL	membrane fraction	8	16.67%	1.78E-04
GOTERM_BP_ALL	calcium ion transport	5	10.42%	1.89E-04
GOTERM_MF_ALL	ion channel activity	7	14.58%	1.97E-04
GOTERM_MF_ALL	substrate specific channel activity	7	14.58%	2.32E-04
GOTERM_MF_ALL	cation channel activity	6	12.50%	3.59E-04
GOTERM_MF_ALL	passive transmembrane transporter activity	7	14.58%	3.75E-04
GOTERM_MF_ALL	channel activity	7	14.58%	3.75E-04
GOTERM_CC_ALL	cell fraction	8	16.67%	3.89E-04
GOTERM_BP_ALL	ion transport	9	18.75%	4.45E-04
GOTERM_MF_ALL	substrate-specific transmembrane transporter activity	9	18.75%	5.29E-04
GOTERM_BP_ALL	metal ion transport	7	14.58%	5.59E-04
GOTERM_MF_ALL	ion transmembrane transporter activity	8	16.67%	0.001158
GOTERM_MF_ALL	transmembrane transporter activity	9	18.75%	0.001375
GOTERM_MF_ALL	cation transmembrane transporter activity	7	14.58%	0.001413
GOTERM_BP_ALL	cation transport	7	14.58%	0.001656
GOTERM_CC_ALL	endoplasmic reticulum	8	16.67%	0.002352
GOTERM_MF_ALL	substrate-specific transporter activity	9	18.75%	0.002402
GOTERM_MF_ALL	transporter activity	10	20.83%	0.004188
GOTERM_BP_ALL	lipid metabolic process	7	14.58%	0.004372
GOTERM_BP_ALL	establishment of localization	13	27.08%	0.019275
GOTERM_BP_ALL	intracellular signaling cascade	8	16.67%	0.023088
GOTERM_MF_ALL	metal ion binding	16	33.33%	0.024839
GOTERM_MF_ALL	cation binding	15	31.25%	0.029425
GOTERM_MF_ALL	ion binding	16	33.33%	0.029561
GOTERM_BP_ALL	cell communication	17	35.42%	0.029929
GOTERM_CC_ALL	plasma membrane	11	22.92%	0.035826
GOTERM_BP_ALL	transport	12	25.00%	0.038328
GOTERM_MF_ALL	transferase activity, transferring phosphorus-containing groups	7	14.58%	0.042668
GOTERM_BP_ALL	localization	13	27.08%	0.044765
GOTERM_CC_ALL	cytoplasm	21	43.75%	0.045585
GOTERM_BP_ALL	cellular lipid metabolic process	5	10.42%	0.046498

Glynn Dennis Jr., Brad T. Sherman, Douglas A. Hosack, Jun Yang, Michael W. Baseler, H. Clifford Lane, Richard A. Lem-picki. "DAVID: Database for Annotation, Visualization, and Integrated Discovery." *Genome Biology.* 2003 **4**(5): P3.

CEREBELLAR CORTEX, PURKINJE LAYER (CBXpu):

Anatomy
- The Purkinje layer, situated between the molecular and granule cell layers of the cerebellar cortex, contains two types of cells: Purkinje cells, a monolayer of large neurons, and Bergman glia, a specialized type of astrocyte.
- The Purkinje cell layer is present in all subdivisions of the cerebellum, including the vermis, the hemi-spheres, and the flocculonodular lobe.
- This layer is evident in both coronal and sagittal views, but is hard to discern on the images of Nissl-stained sections due to the high density and intensity of label in the granule cell layer.

Expression Patterns of the 50 Select Genes
- All 50 select genes have a widespread, high density and very high intensity expression pattern.
- A subset of genes examined showed expression restricted to the Purkinje cells.
- Another subset of genes showed expression that was enriched in the Purkinje cells and in multiple other cell types of the cerebellar cortex, primarily other GABA-ergic interneurons in the granule cell and the molecular cell layers.
- A final subset of the genes examined highlighted Bergman glia. In these cases, the ISH reaction product highlights the Purkinje cell layer, but the Purkinje cells themselves are devoid of label.

We encourage you to reply with any comments or questions by email to !Annotation@alleninstitute.org. To further explore the gene expression data and analytical tools referred to in this report, please access our genome-wide data set at brain-map.org.

Other Tools:

NEUROBLAST:
Many of the 50 genes listed in this report can be used to explore the NeuroBlast tool. This unique mining tool works seamlessly from within brain-map.org to produce a list of genes that share similar expression patterns to any gene in the coronal data set. Search for and select any gene, then select one of several brain regions from the NeuroBlast drop-tab to explore a ranked list of similarly expressed genes for that region.
To learn more about this function, please refer to the NeuroBlast white paper.

BRAIN EXPLORER:
To compare gene expression levels across anatomical structures in 3-D detail, download the Brain Explorer desktop application. This program is used to view gene expression in 3-D view (coronal, sagittal, horizontal and everywhere in between) across all brain structures and allows for simultaneous viewing of multiple expression profiles.

The NeuroBlast spatial homology function and an anatomic search tool are also available from within Brain Explorer to allow the user to search for and visualize genes with similar expression patterns.