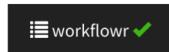


Main Steps for Hook dataset

Data Analysis

Yasin Kaymaz

2/07/2019



Main Steps for Hook dataset

Citation: Hook, Paul W., Sarah A. McClymont, Gabrielle H. Cannon, William D. Law, A. Jennifer Morton, Loyal A. Goff, and Andrew S. McCallion. 2018. “Single-Cell RNA-Seq of Mouse Dopaminergic Neurons Informs Candidate Gene Selection for Sporadic Parkinson Disease.” American Journal of Human Genetics 102 (3): 427–46.

1. Obtain the data

473 single cell RNA-Seq samples from sorted mouse Th-eGFP+ dopaminergic neurons collected at two timepoints from three distinct brain regions.

SRA raw fastq files:

Using sratoolkit, downloaded raw fastq files from [SRA](#)

Expression table deposited to GEO:

For sanity check and quality control.

```
#wget ftp://ftp.ncbi.nlm.nih.gov/geo/series/GSE108nnn/GSE108020/suppl/GSE108020_fpkm_table.txt.gz  
#unzip GSE108020_fpkm_table.txt.gz
```

After downloading the data, unzip the file of **FPKM** matrix for further analysis.

Human.ID	Transcript ID	Mouse.ID	Transcript ID	Biotype
GRIA1-201	ENST00000285900.9	Gria1-202	ENSMUST00000094179.10	hGluA1o Flop
GRIA1-202	ENST00000340592.9	Gria1-201	ENSMUST00000036315.15	hGluA1i Flip
GRIA2-201	ENST00000264426.13	Gria2-201	ENSMUST00000075316.9	hGluA2o Flop
GRIA2-202	ENST00000296526.11	Gria-202	ENSMUST00000107745.7	hGluA2i Q/R Mut Flip
GRIA3-209	ENST00000622768.4	Gria3-209	ENSMUST00000165288.1	hGluA3o Flop
GRIA3-207	ENST00000620443.1	Gria3-201	ENSMUST00000076349.11	hGluA3i Flip
GRIA4-203	ENST00000393127.6	Gria4-201	ENSMUST00000027020.12	hGluA4o Flop
GRIA4-201	ENST00000282499.9	Gria4-202	ENSMUST00000063508.14	hGluA4i Flip

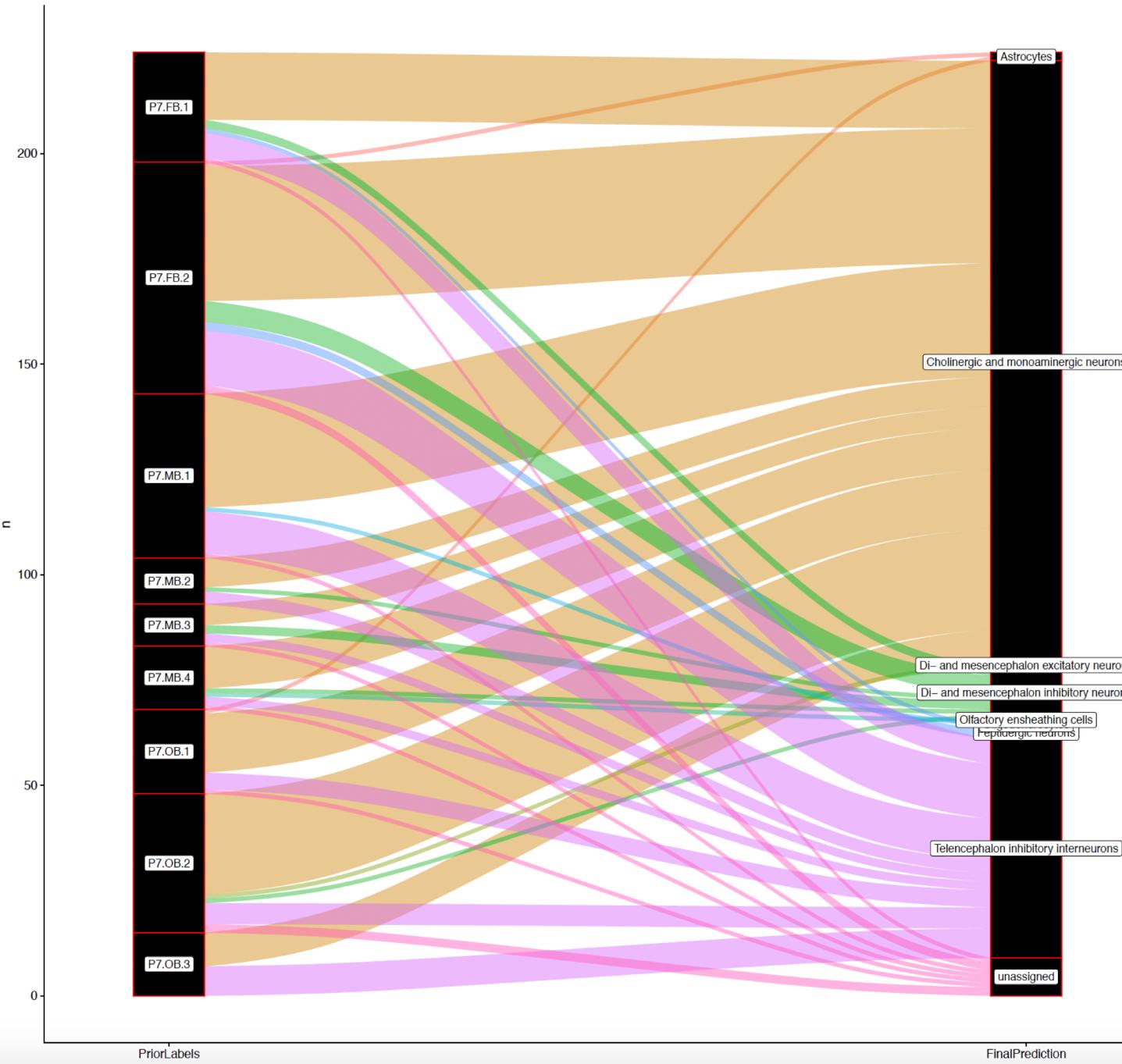
Table 1. Summary of Cell Population Identities

Age	Cluster	Identity
E15.5	FB.1	forebrain neuroblast
	FB.2	post-mitotic forebrain Th^+ neurons
	MB.1	midbrain neuroblast
	MB.2	post-mitotic midbrain DA neuron
P7	FB.1	arcuate nucleus neuroendocrine Th^+ neurons
	FB.2	mixture of arcuate nucleus Th^+ subtypes
	MB.1	ventral tegmental area (VTA)
	MB.2	postnatal neuroblast
	MB.3	periaqueductal gray area (PAG)
	MB.4	<i>substantia nigra</i> (SN)
	OB.1	least mature Th^+ neurons
	OB.2	progressively maturing Th^+ neurons
	OB.3	most mature Th^+ neurons

Summary of the identities of cell populations identified through recursive scRNA-seq analysis of E15.5 and P7 DA neurons. 13 cell populations are described, each with their age, cell cluster name, and biological identity. Additional information can be found in [Table S3](#).

In the analysis, I excluded E15.5 cells as requested by CNSDR and kept only P7 mice cells (224 cells).

Predictions Cross-Check



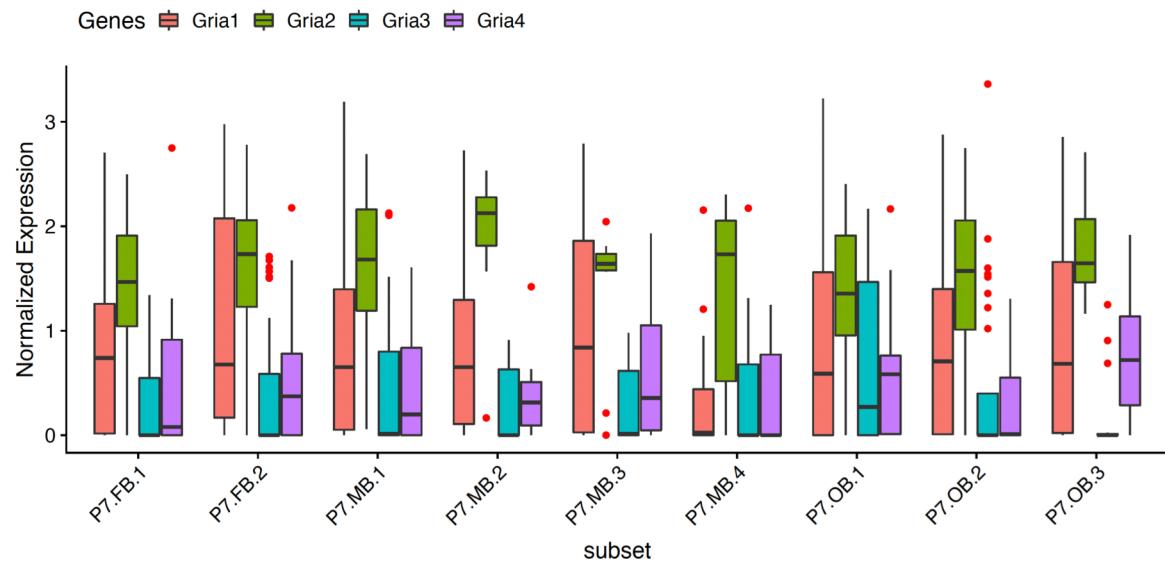
Cell types projection from KI-superset (Zeisel et al 2018, Taxonomy Rank 4)

Using scmap:

<https://www.nature.com/articles/nmeth.4644>

Prediction

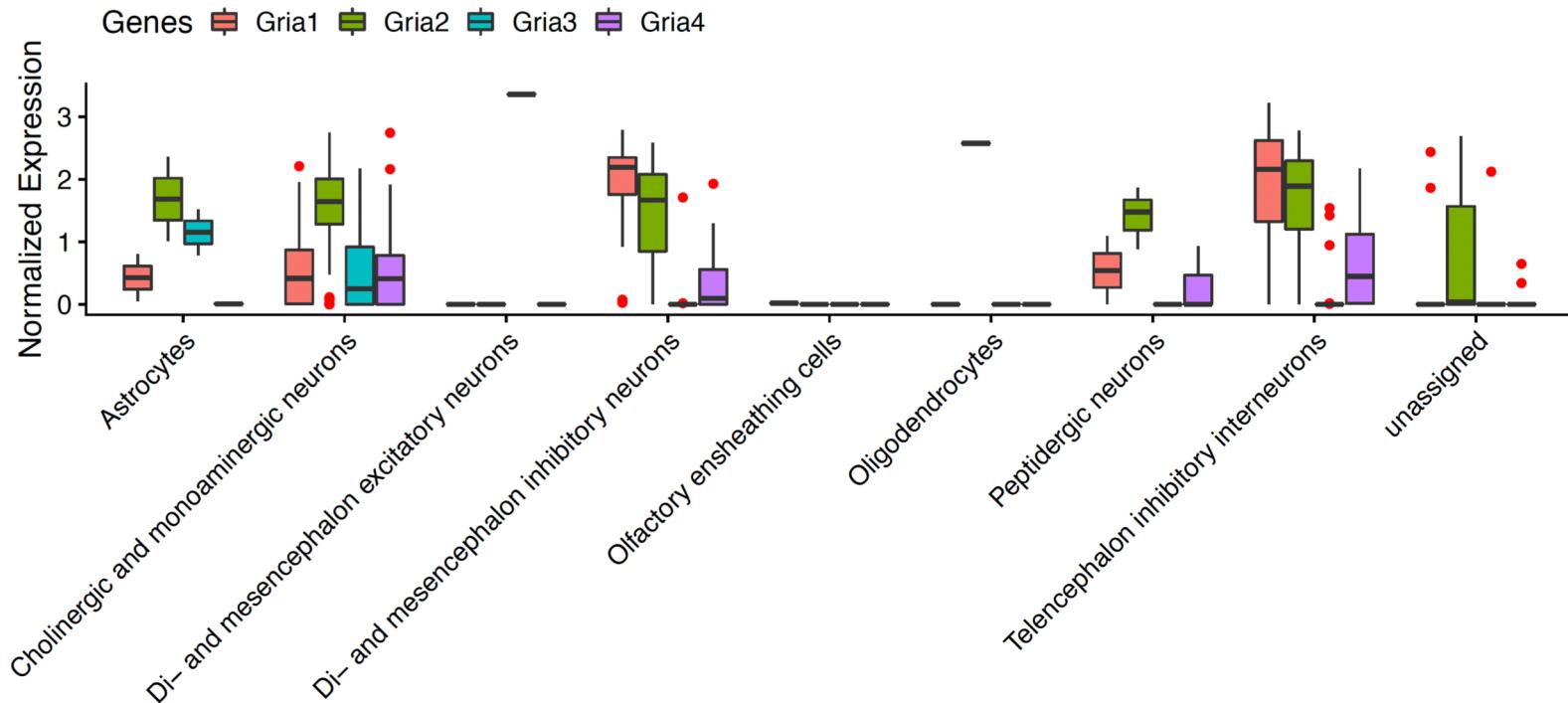
- Astrocytes
- Cholinergic and monoaminergic neurons
- Di- and mesencephalon excitatory neurons
- Di- and mesencephalon inhibitory neurons
- Olfactory ensheathing cells
- Oligodendrocytes
- Peptidergic neurons
- Telencephalon inhibitory interneurons
- unassigned

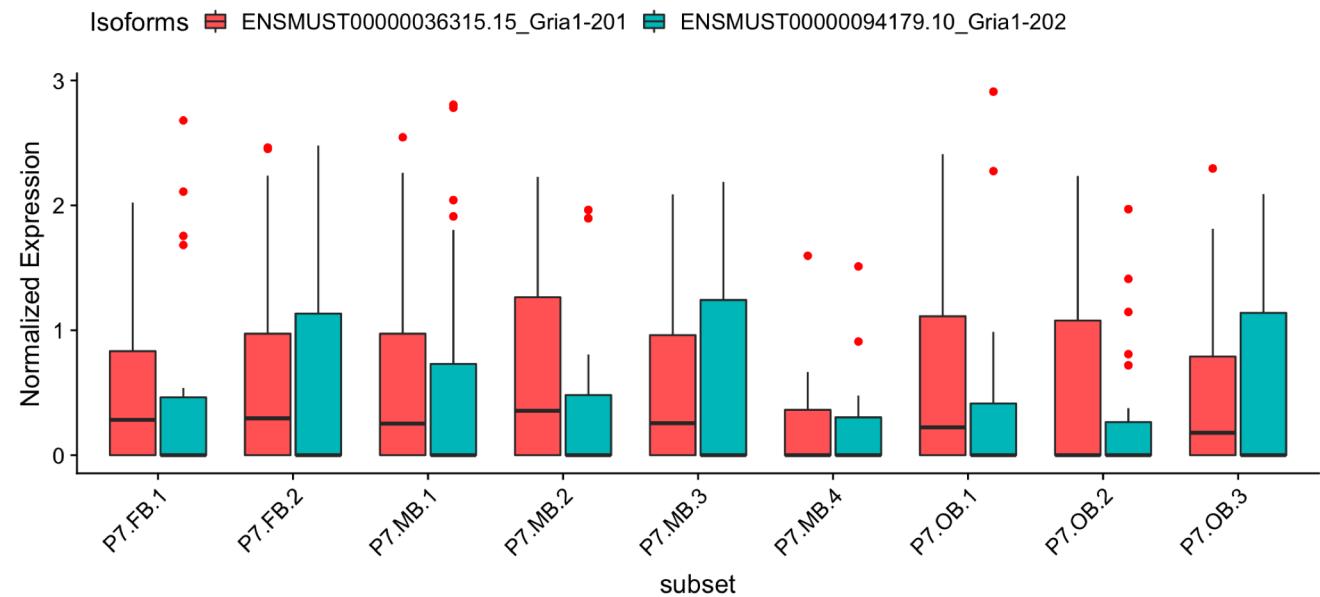


Gene level expression

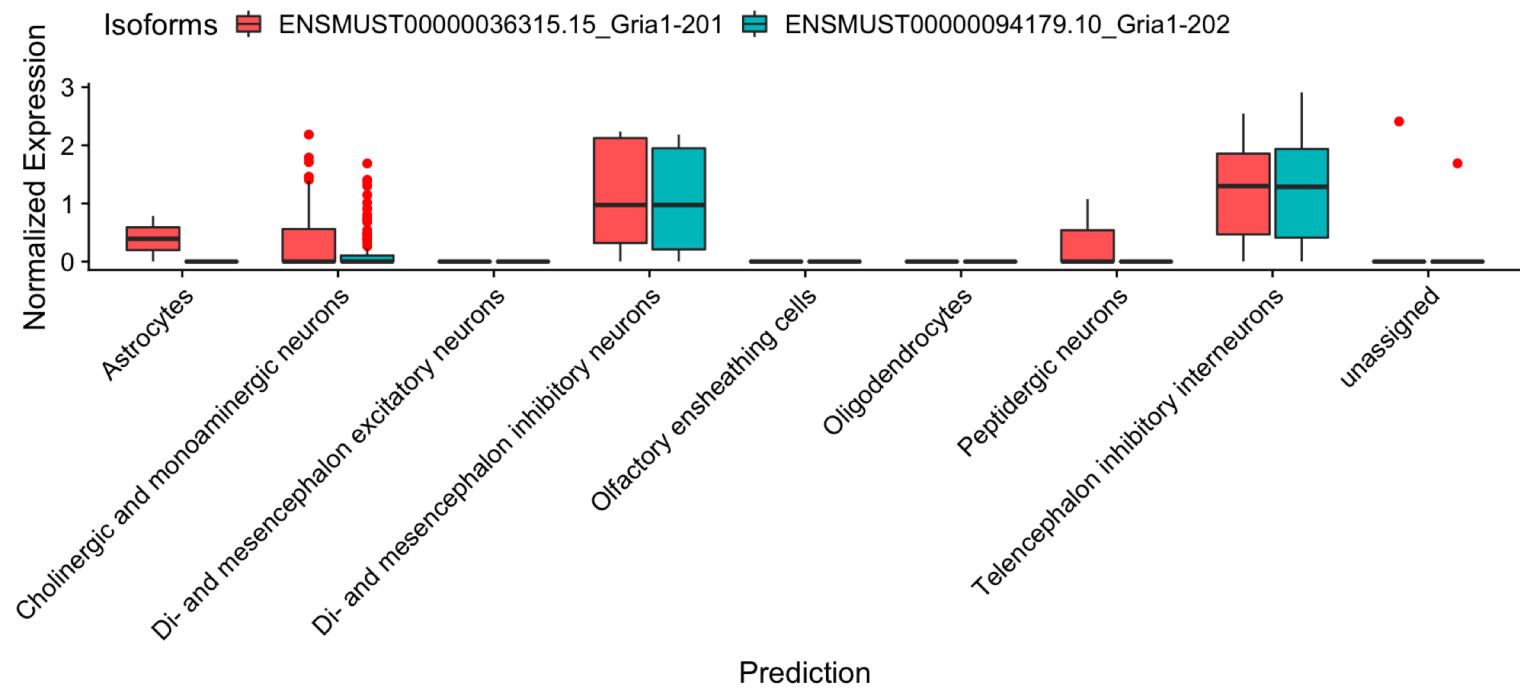
These figures show the distribution of normalized expression (log2) of each glutamate ionotropic receptor AMPA genes in each cell group identified by Hook et al (upper) and cell type annotations with KI-superset (right).

The horizontal lines in bars = Median, Red dots = outlier cells.

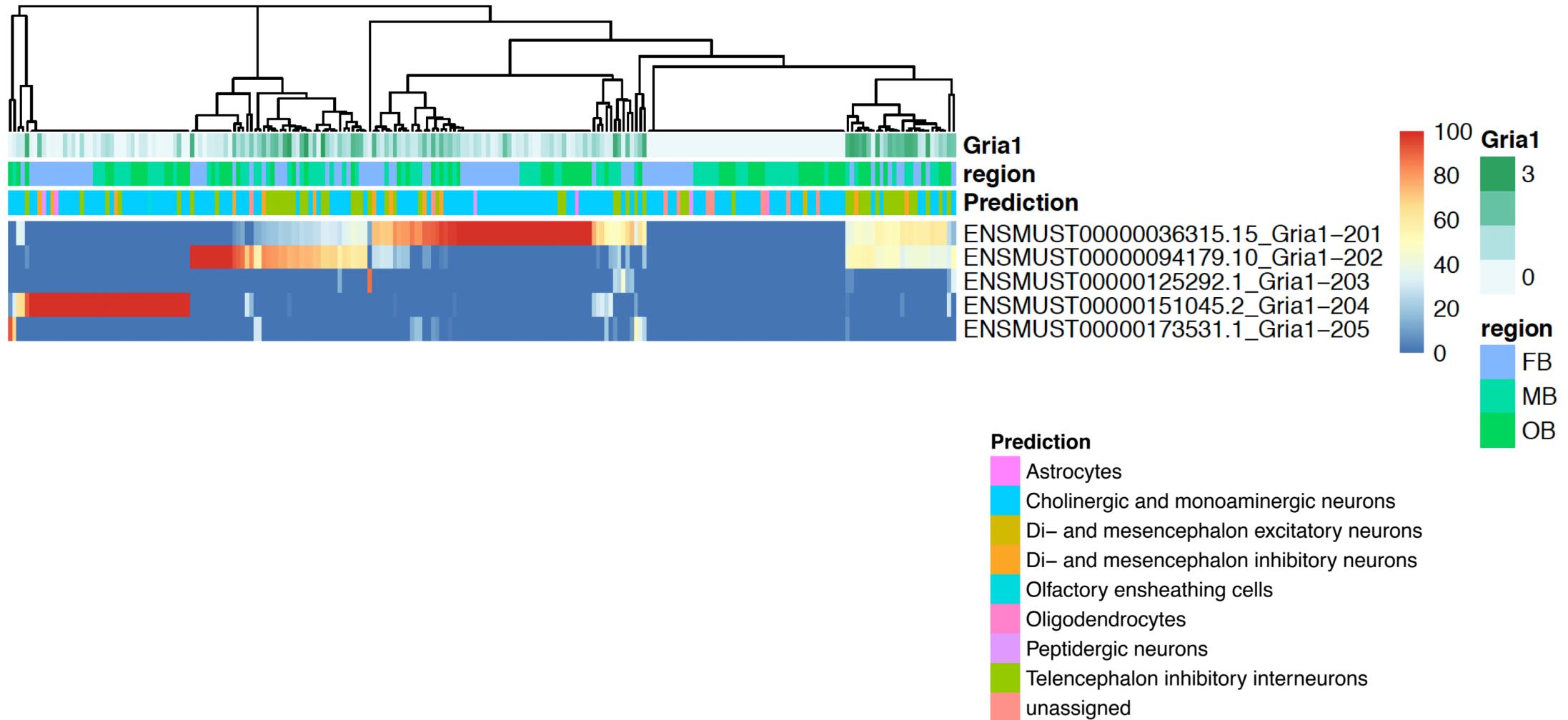




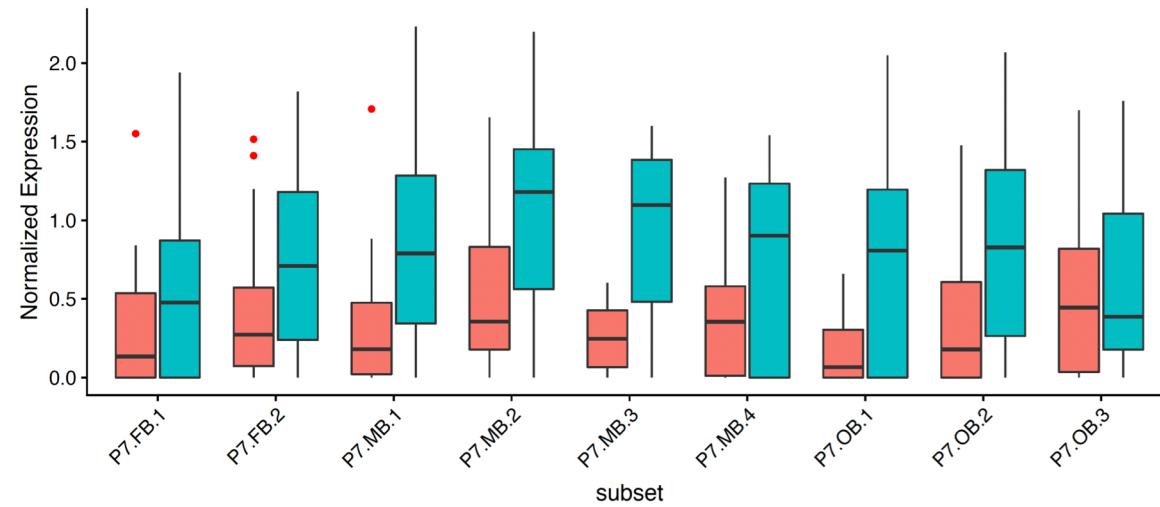
Gria1 Isoform level expression



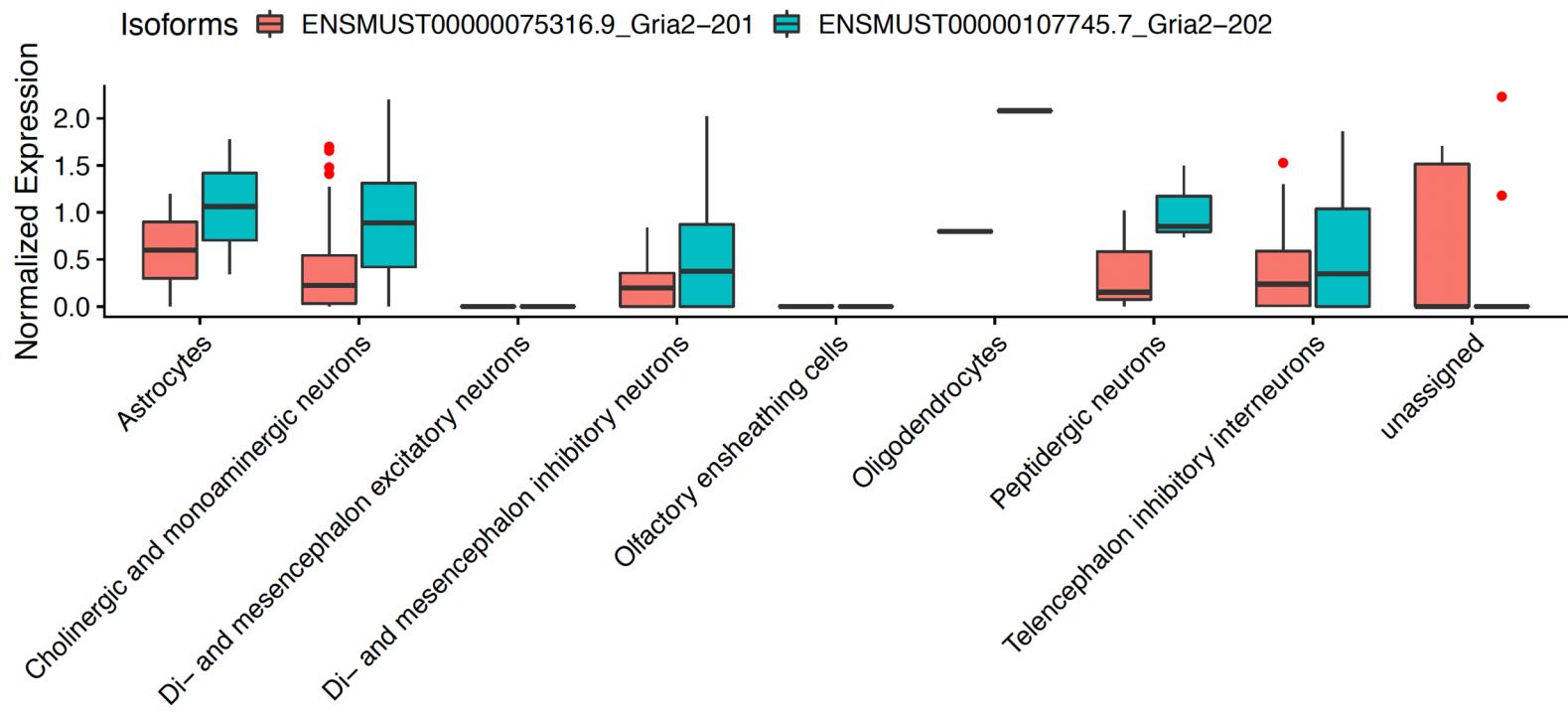
Gria1 Isoform level expression



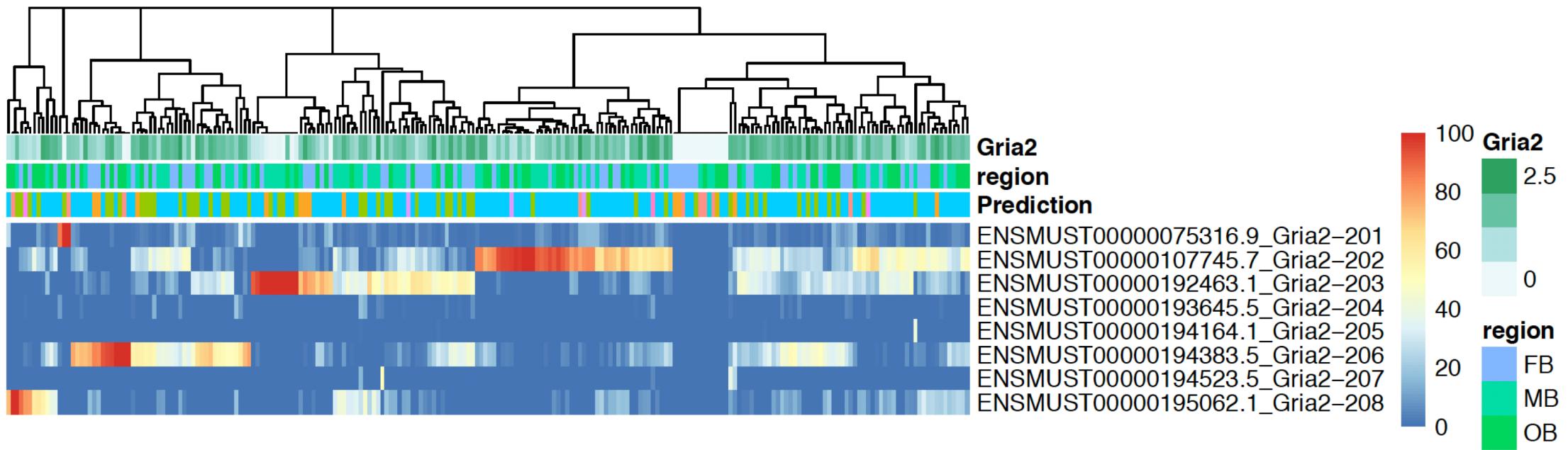
Isoforms ENSMUST0000075316.9_Gria2-201 ENSMUST00000107745.7_Gria2-202



Gria2 Isoform level expression



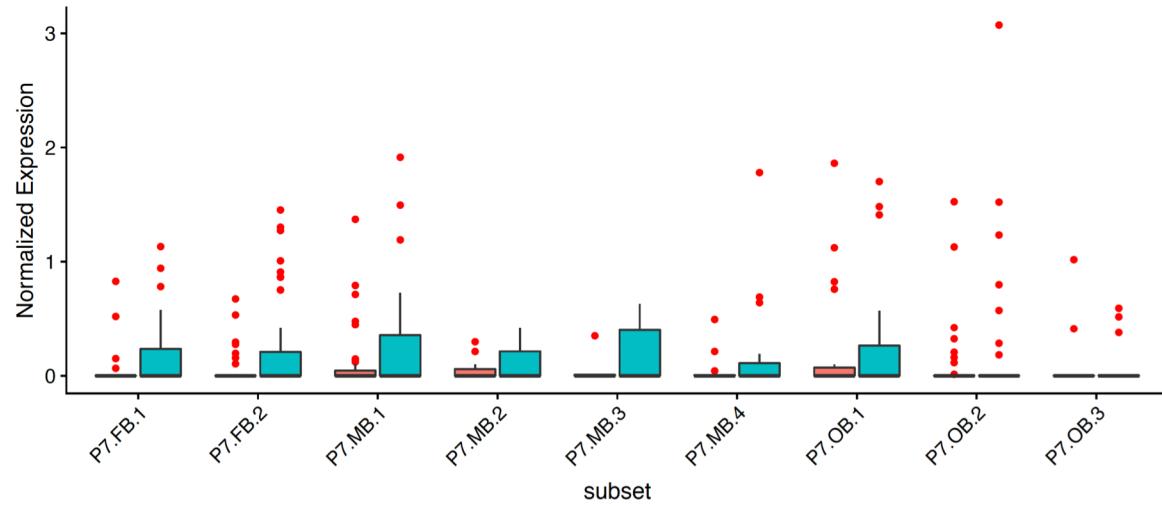
Gria2 Isoform level expression



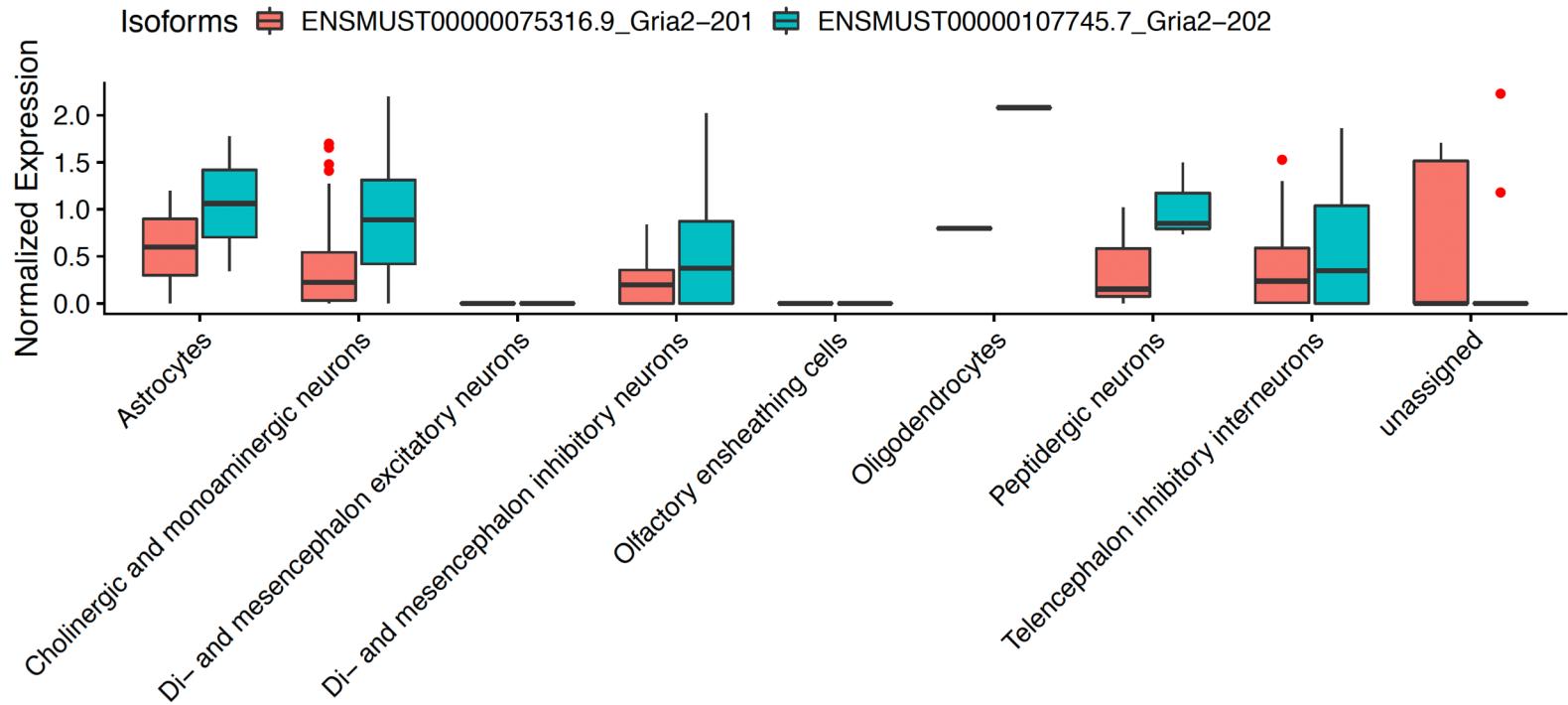
Prediction

- Astrocytes
- Cholinergic and monoaminergic neurons
- Di- and mesencephalon excitatory neurons
- Di- and mesencephalon inhibitory neurons
- Olfactory ensheathing cells
- Oligodendrocytes
- Peptidergic neurons
- Telencephalon inhibitory interneurons
- unassigned

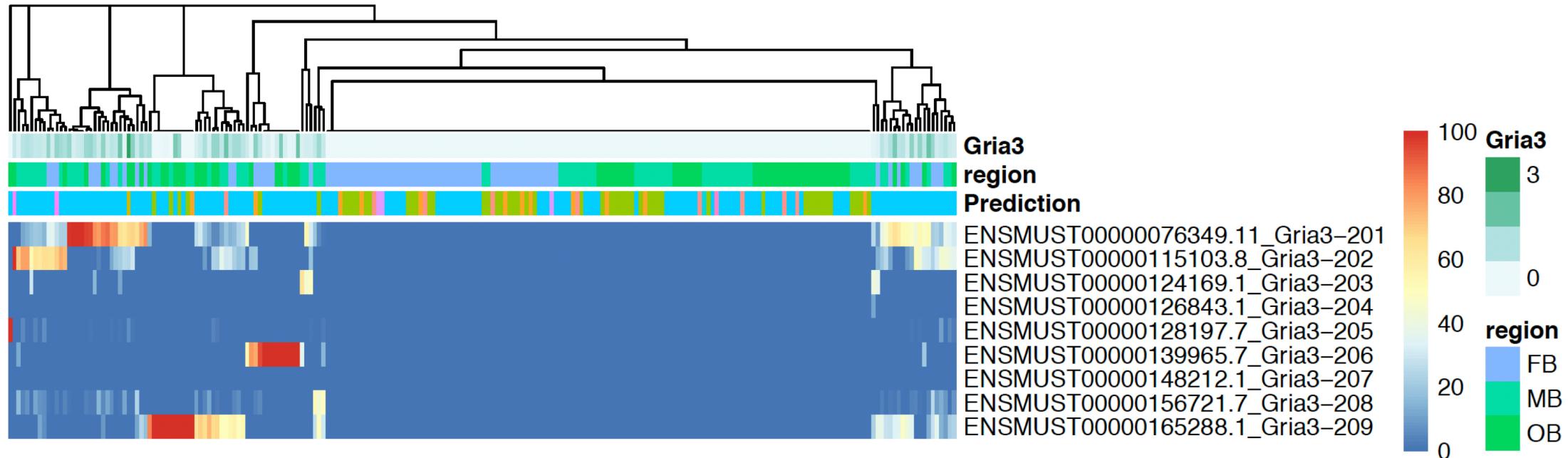
Isoforms ENSMUST00000165288.1_Gria3-209 ENSMUST0000076349.11_Gria3-201



Gria3 Isoform level expression



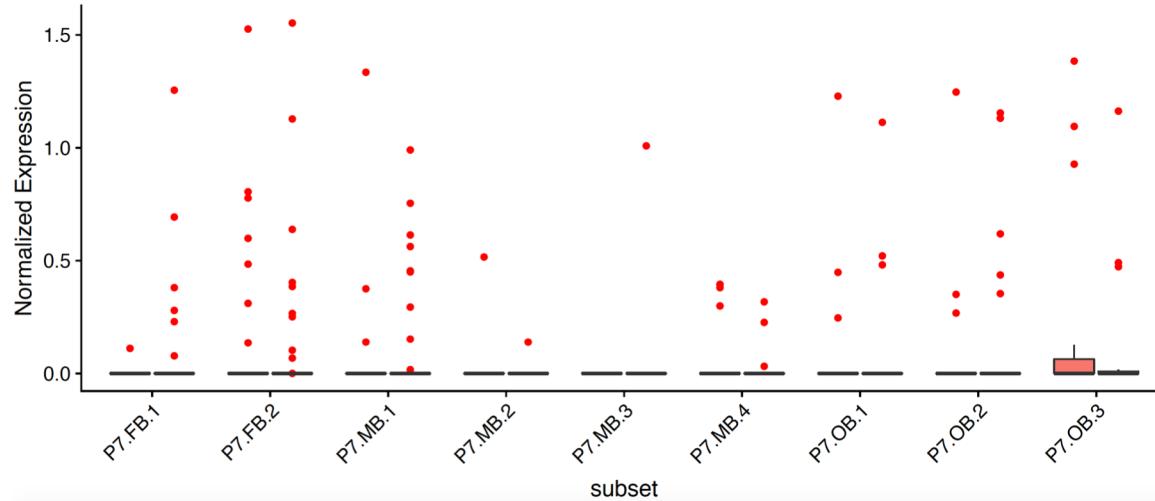
Gria3 Isoform level expression



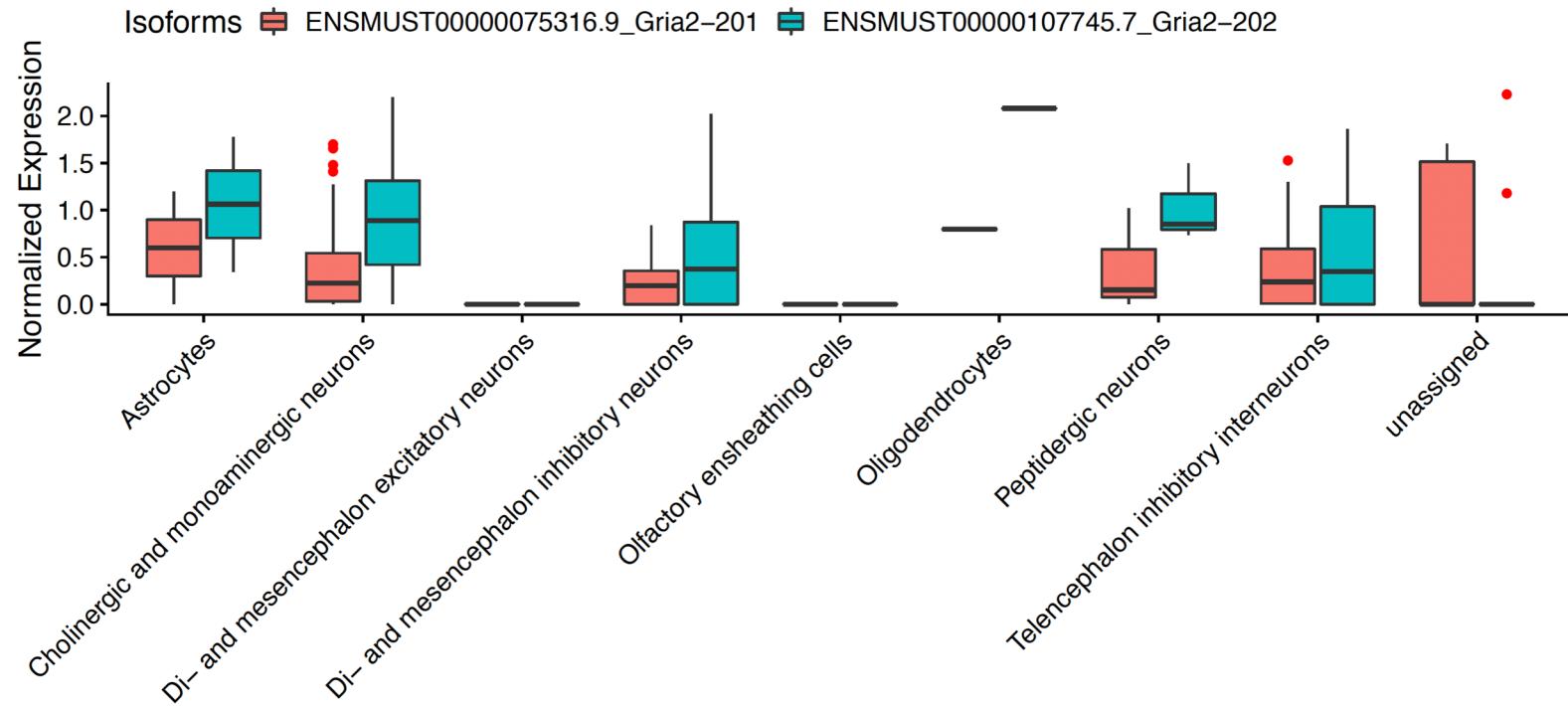
Prediction

- Astrocytes
- Cholinergic and monoaminergic neurons
- Di- and mesencephalon excitatory neurons
- Di- and mesencephalon inhibitory neurons
- Olfactory ensheathing cells
- Oligodendrocytes
- Peptidergic neurons
- Telencephalon inhibitory interneurons
- unassigned

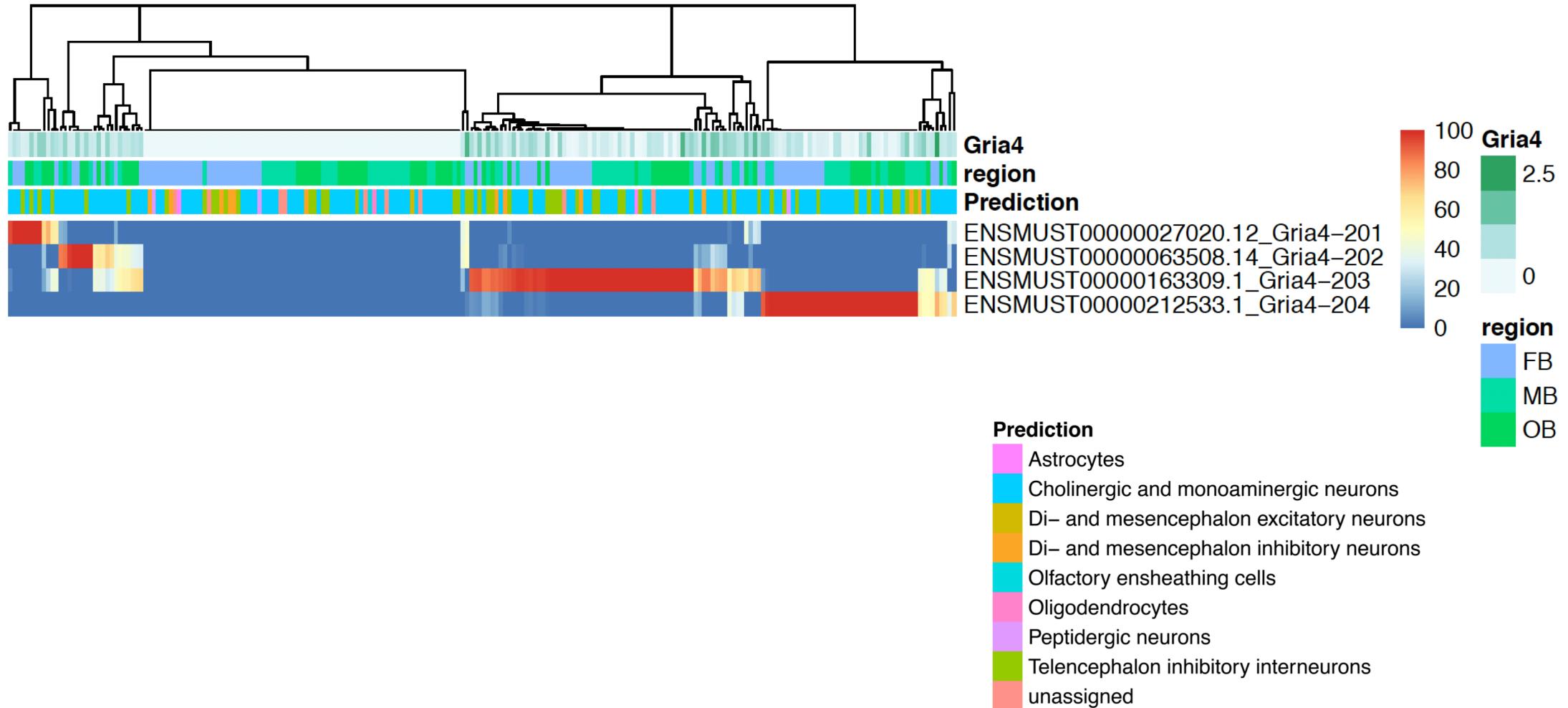
Isoforms ENSMUST00000027020.12_Gria4-201 ENSMUST00000063508.14_Gria4-202

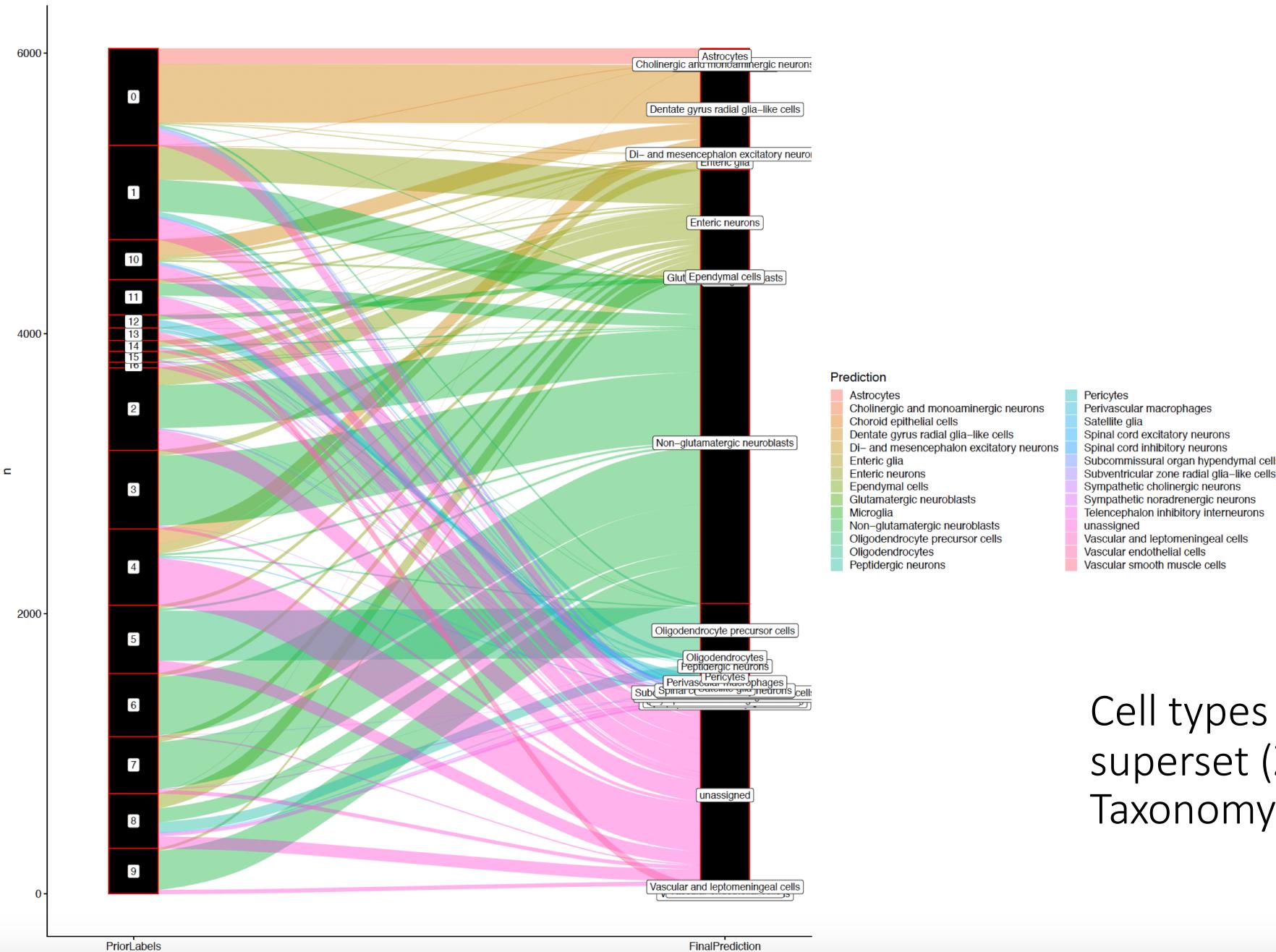


Gria4 Isoform level expression



Gria4 Isoform level expression





Cell types projection from KI-superset (Zeisel et al 2018, Taxonomy Rank 4)