

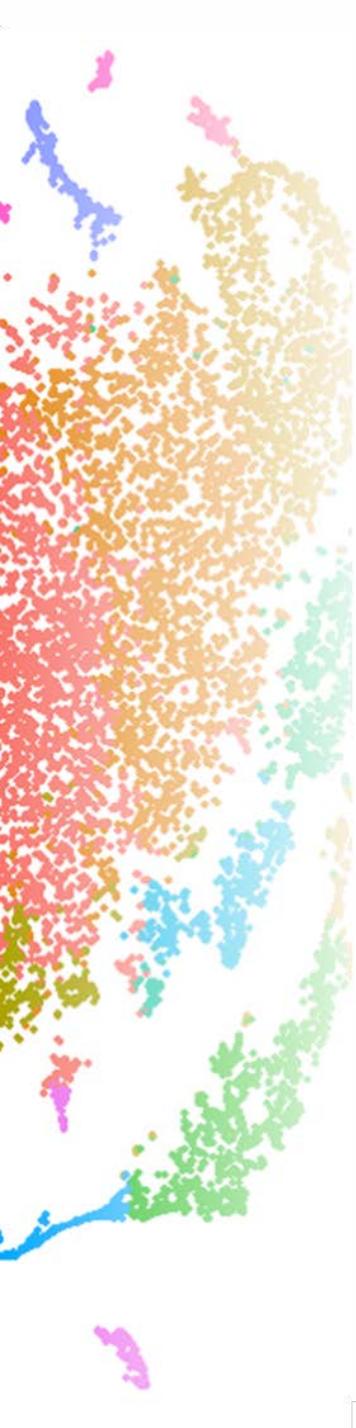
Understanding the development of **Left/Right** asymmetry in
the zebrafish brain by single cell transcriptomic approach.

Aurelie Quillien
Marion Aguirrebengoa
Patrick Blader's Lab

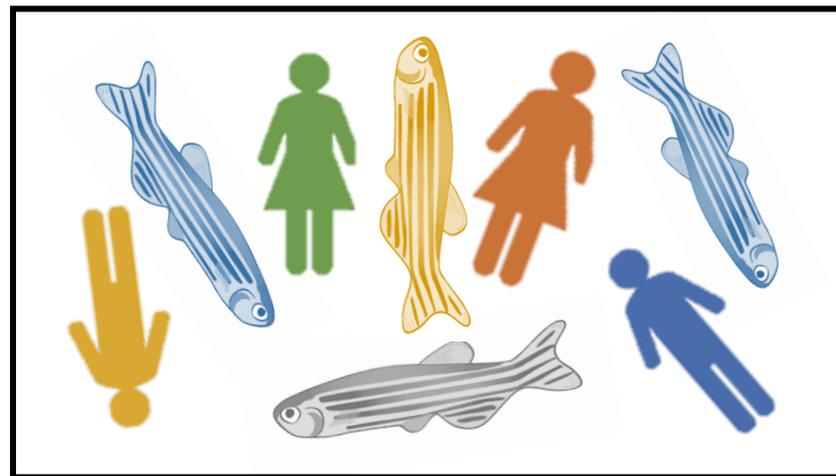
EMEA 10x Genomics User Day |
10/03/2020



C B I
Centre de Biologie
Intégrative - Toulouse

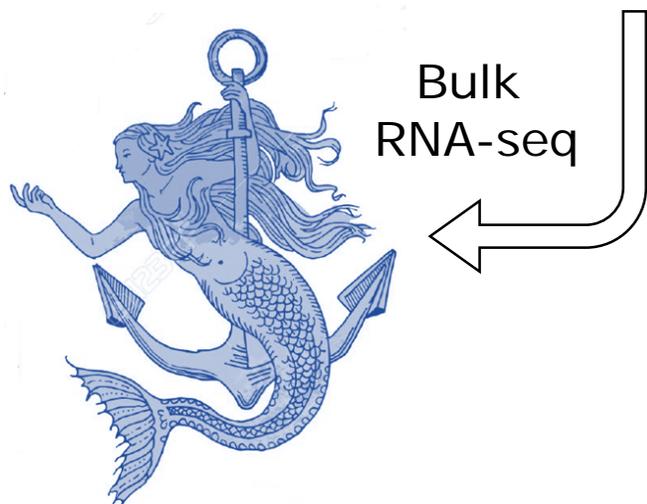
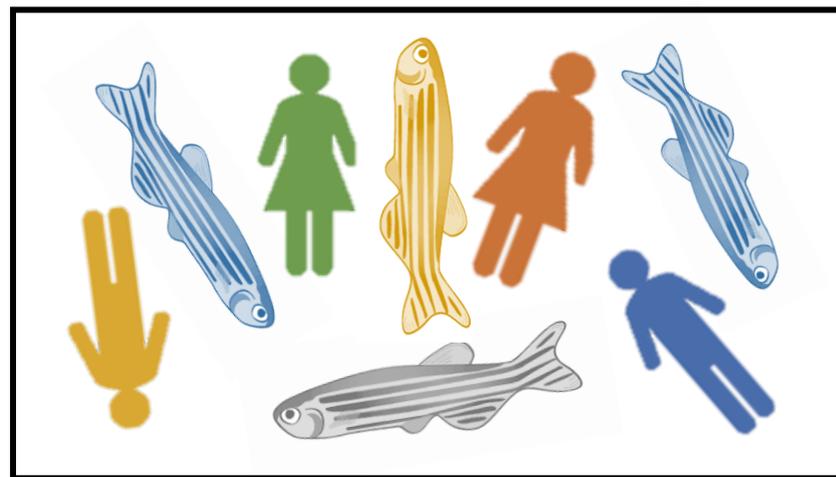


Sample



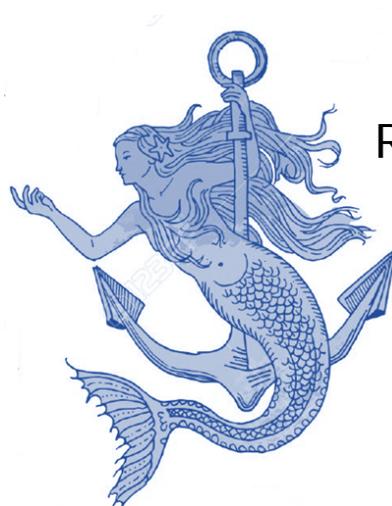
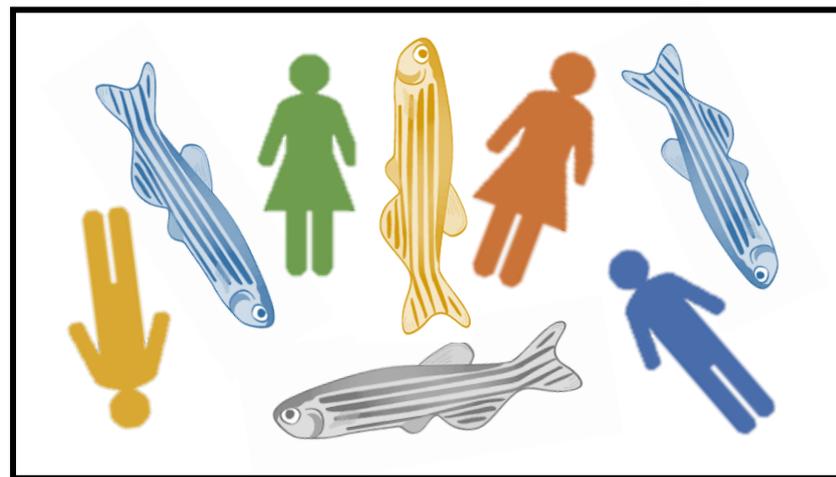


Sample

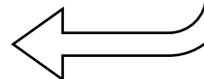




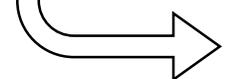
Sample



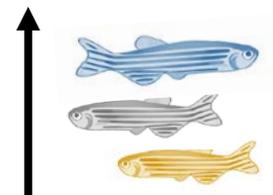
Bulk
RNA-seq



scRNA-seq



Fin



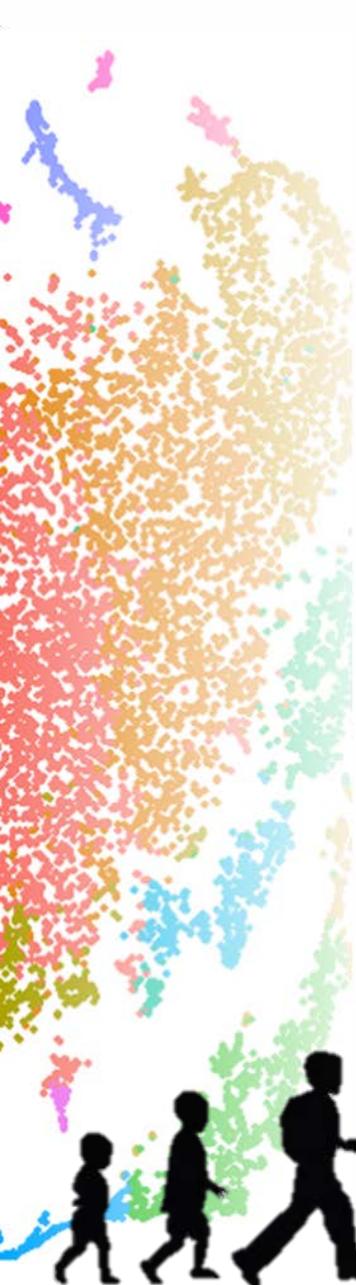
Fur



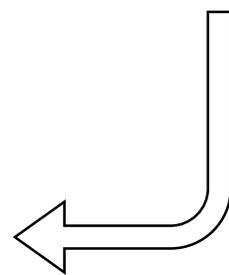


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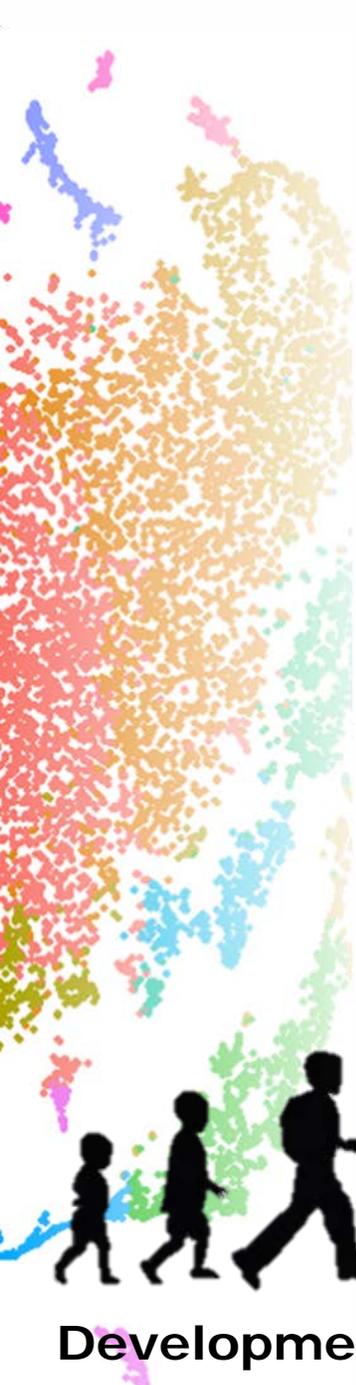




Sample



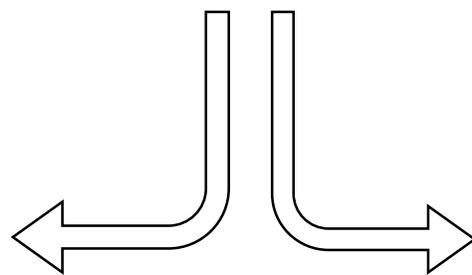
Developmental stage heterogeneity



Sample



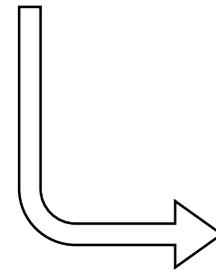
Developmental stage heterogeneity



Spatial heterogeneity



Sample



Left



Right



The vertebrate brain is lateralized : Left/Right asymmetry of cognitive functions and behavior

Handedness



Right 90%



Left 10%

Eye Preference in visual tasks



© Aaron Wong (Singapore)

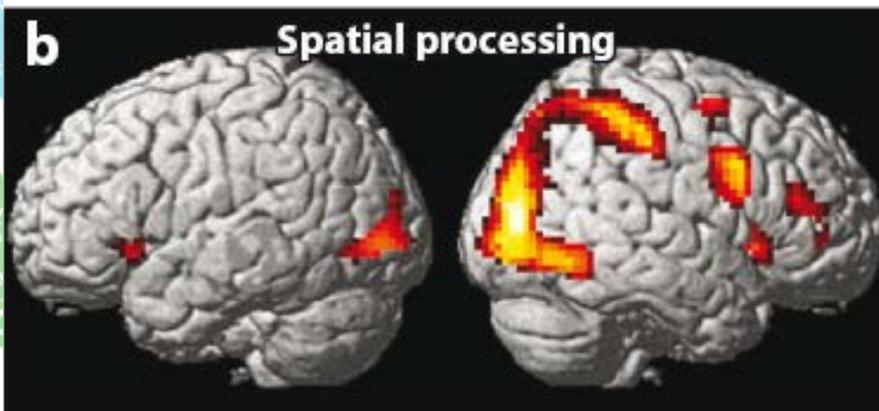
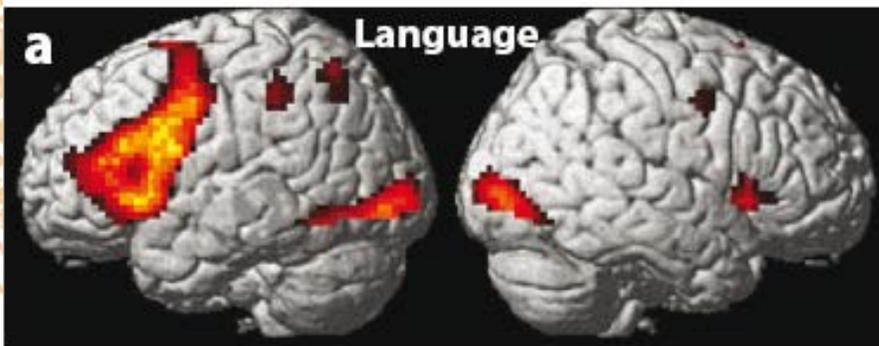


© Franklin Ramirez (Honduras)



The vertebrate brain is lateralized :
Left/Right asymmetry of cognitive functions and behavior

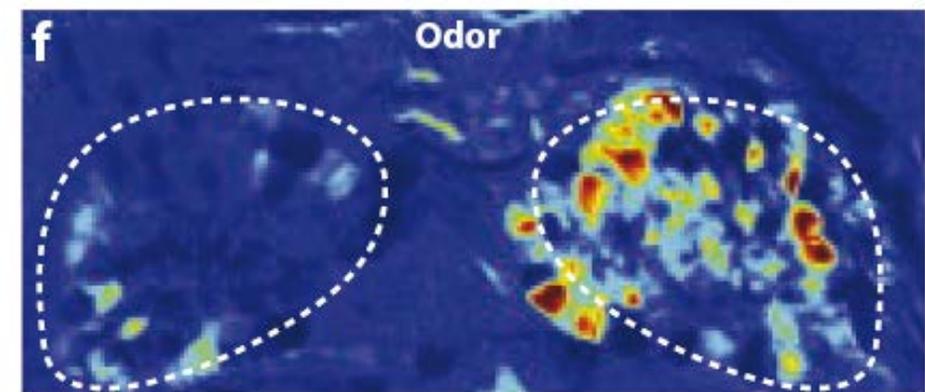
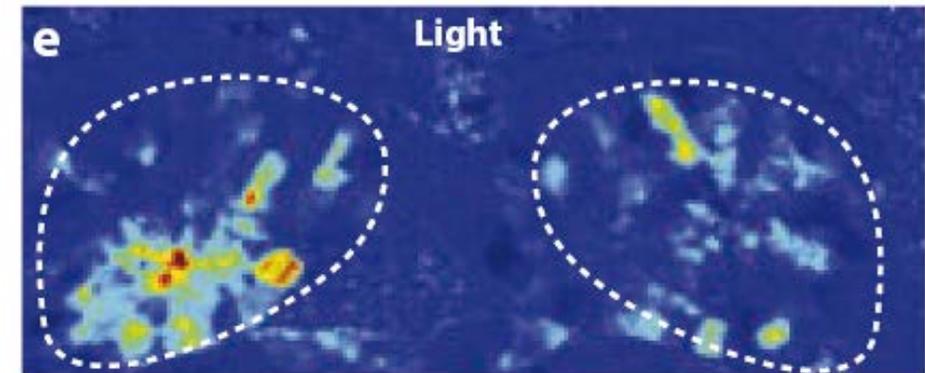
Brain activation (MRI)



Left

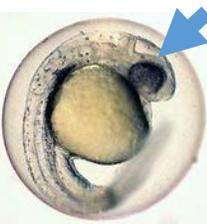
Right

Neuronal activity in the zebrafish epithalamus



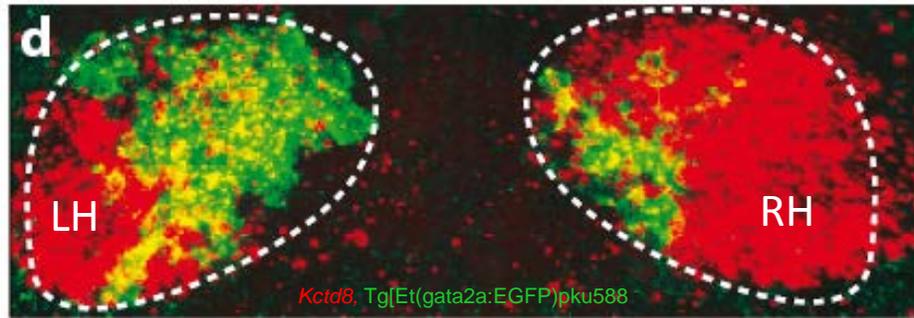
Left

Right

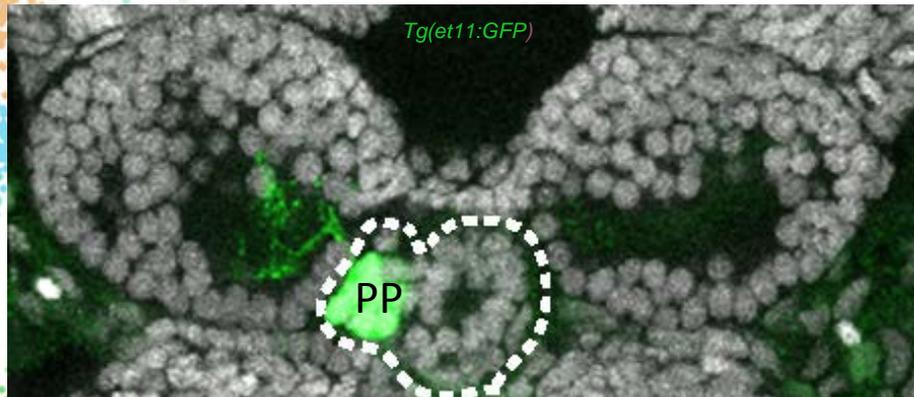


The vertebrate brain is lateralized :
Left/Right asymmetry at the Neuroanatomical Level

Molecular asymmetry



Neuroanatomical asymmetry

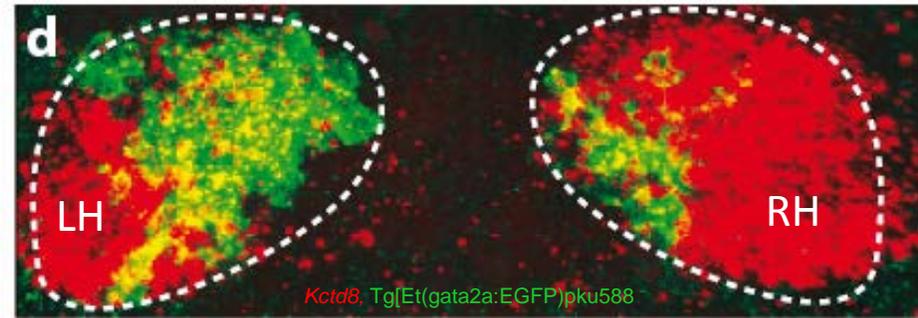


Left

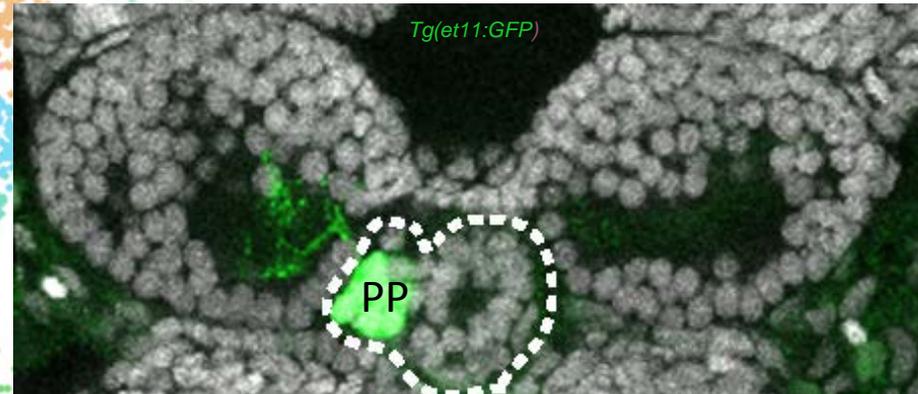
Right

How the **Left/Right** asymmetry is established in the zebrafish epithalamus?

Molecular asymmetry

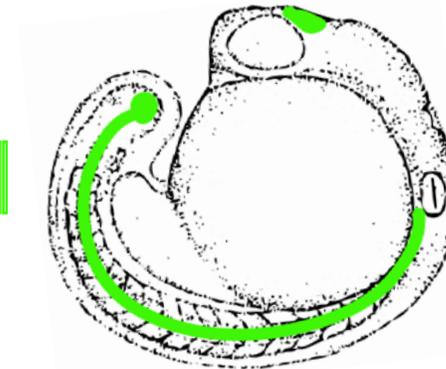
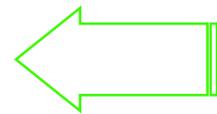


Neuroanatomical asymmetry



Left

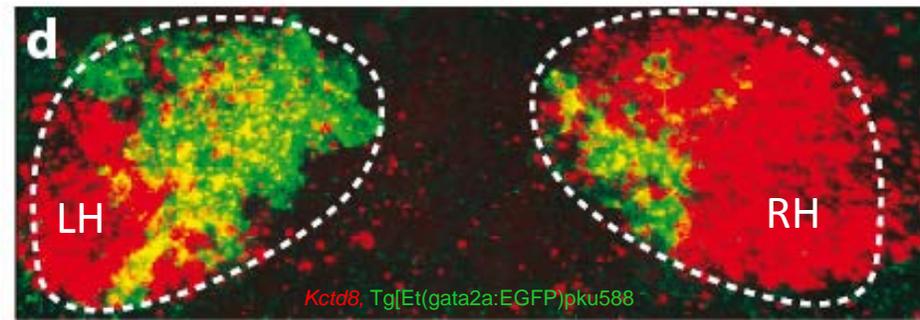
Right



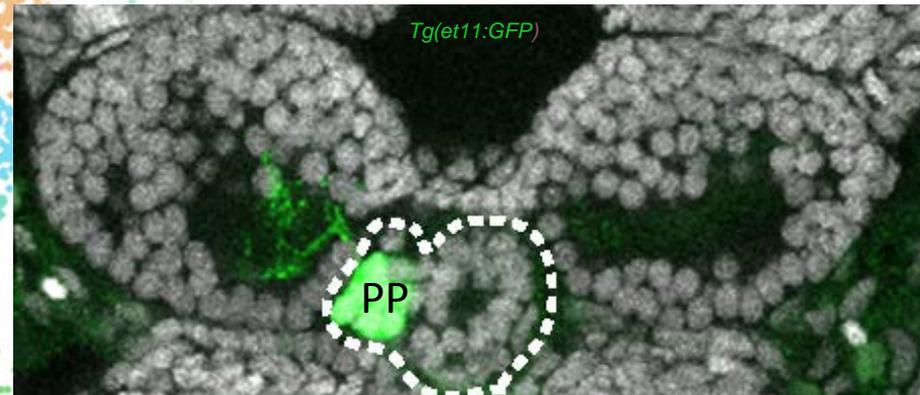
18-22h

How the **Left/Right** asymmetry is established in the zebrafish epithalamus? The NODAL signaling

Molecular asymmetry

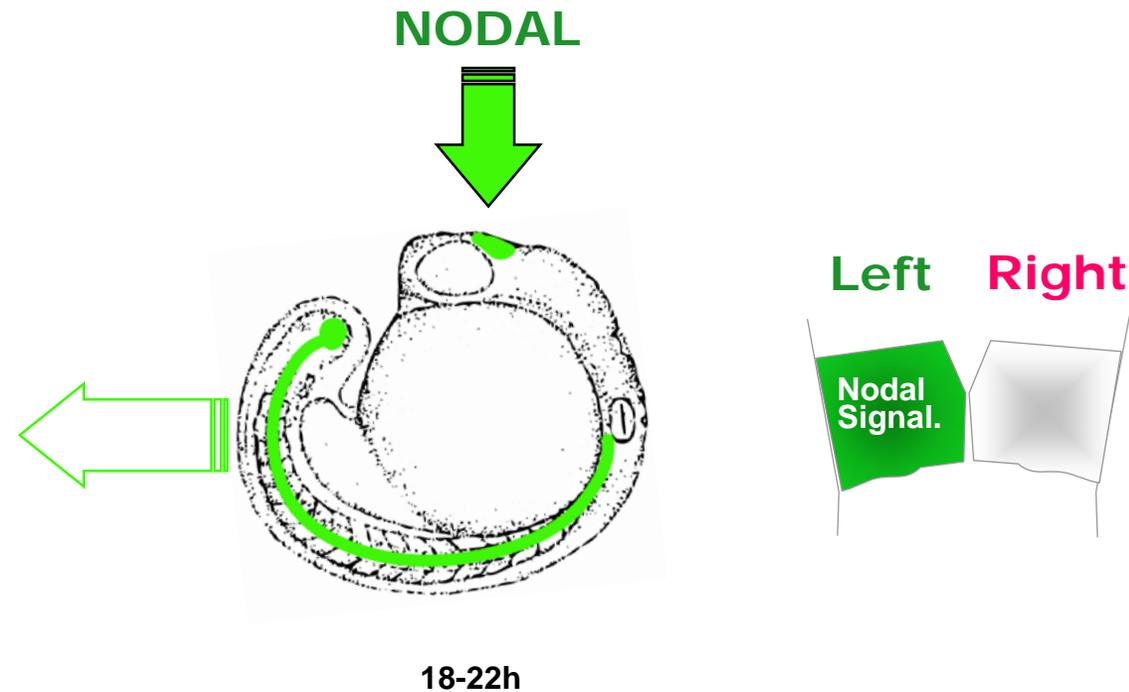


Neuroanatomical asymmetry

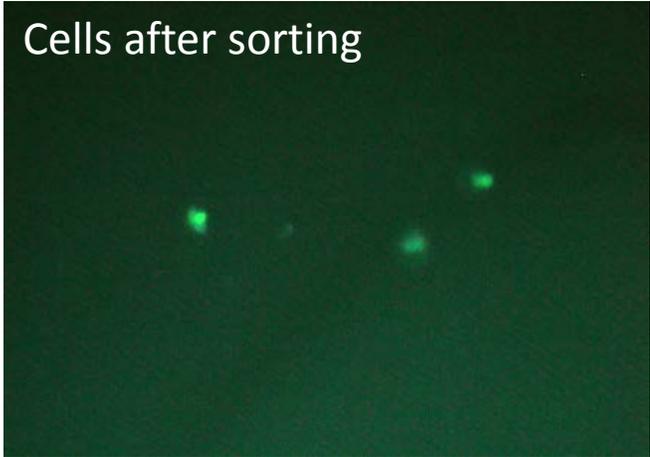
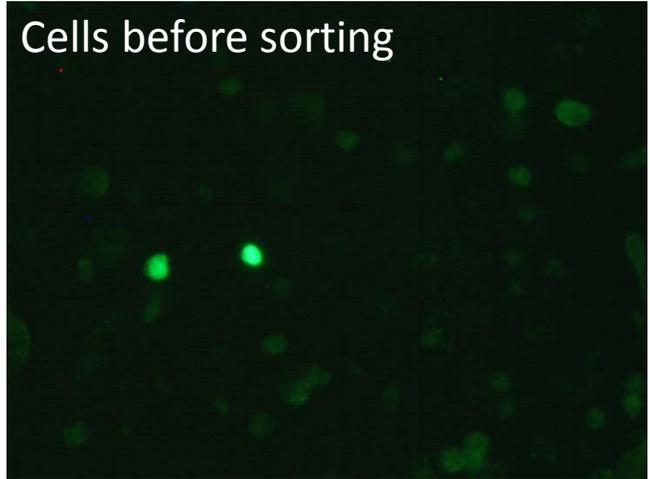
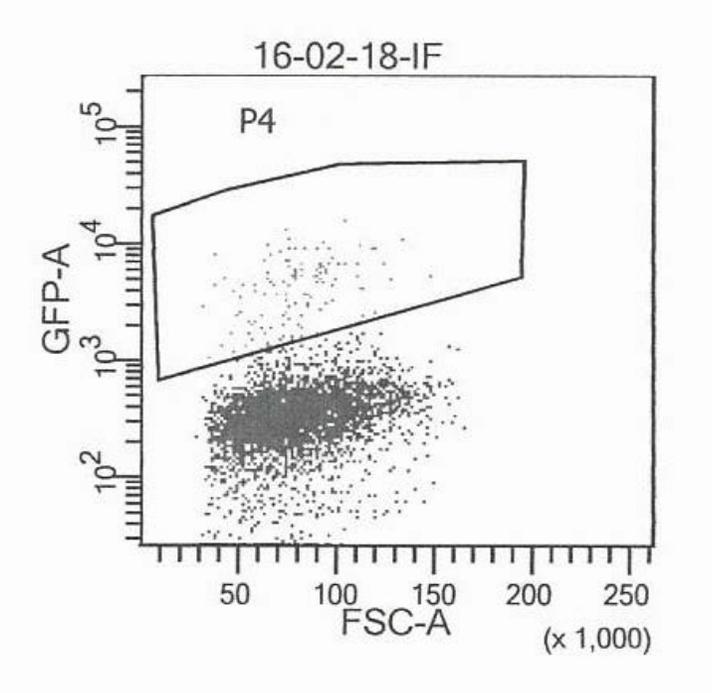
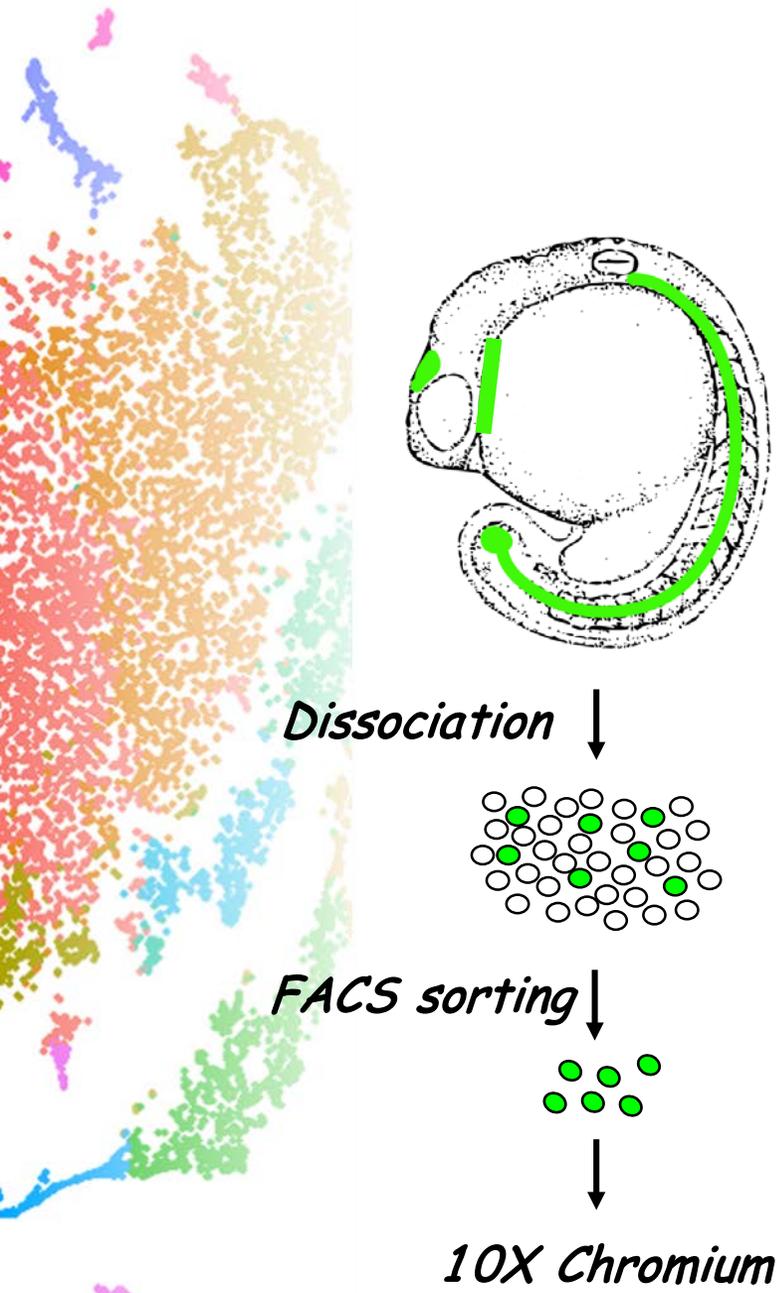


Left

Right



Cells preparation for scRNA-seq





Estimated Number of Cells

9,422

Mean Reads per Cell

32,663

Median Genes per Cell

1,983

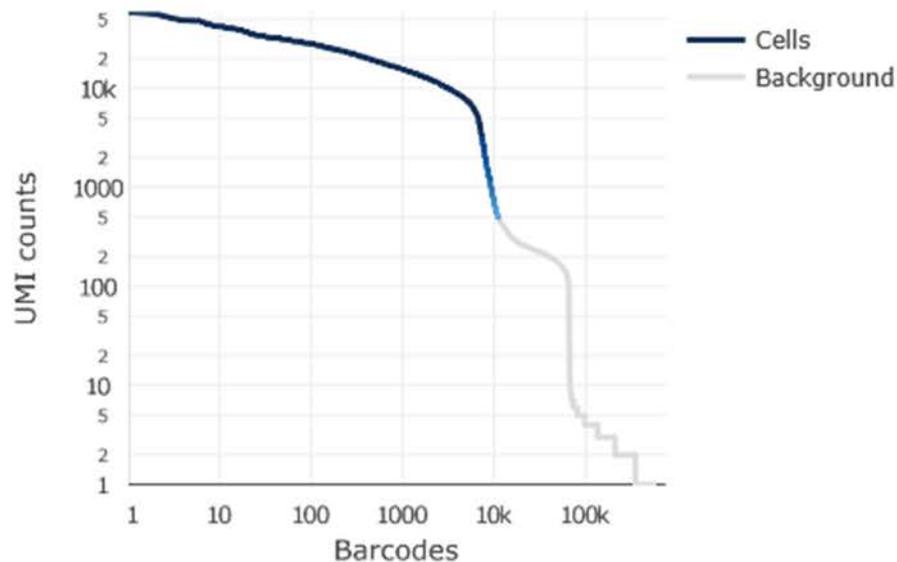
Sequencing

| | |
|---------------------------|-------------|
| Number of Reads | 307,754,089 |
| Valid Barcodes | 98.2% |
| Sequencing Saturation | 56.6% |
| Q30 Bases in Barcode | 97.3% |
| Q30 Bases in RNA Read | 64.1% |
| Q30 Bases in Sample Index | 91.6% |
| Q30 Bases in UMI | 97.6% |

Mapping

| | |
|--|-------|
| Reads Mapped to Genome | 87.4% |
| Reads Mapped Confidently to Genome | 85.1% |
| Reads Mapped Confidently to Intergenic Regions | 4.3% |
| Reads Mapped Confidently to Intronic Regions | 3.8% |
| Reads Mapped Confidently to Exonic Regions | 76.9% |
| Reads Mapped Confidently to Transcriptome | 72.8% |
| Reads Mapped Antisense to Gene | 0.6% |

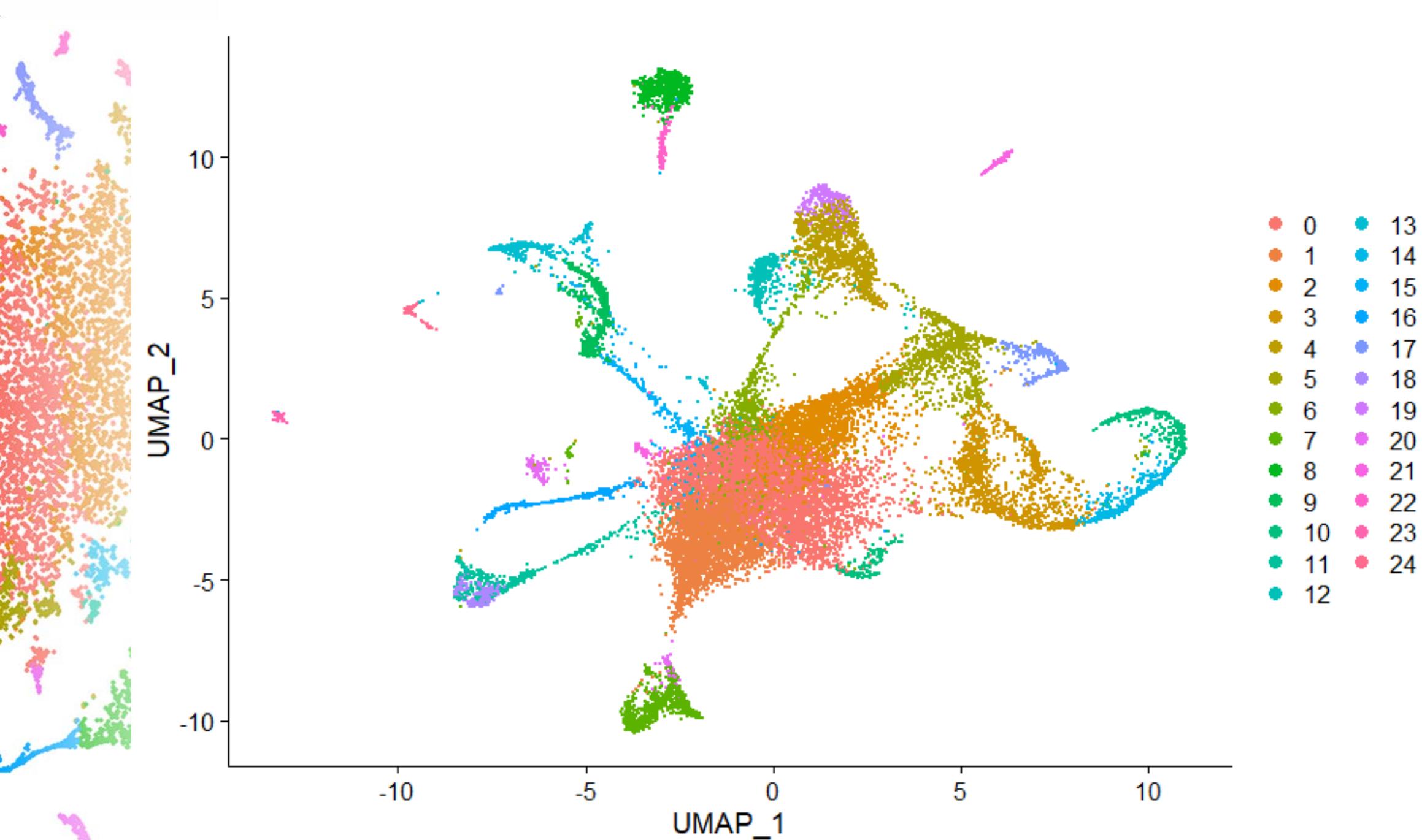
Cells

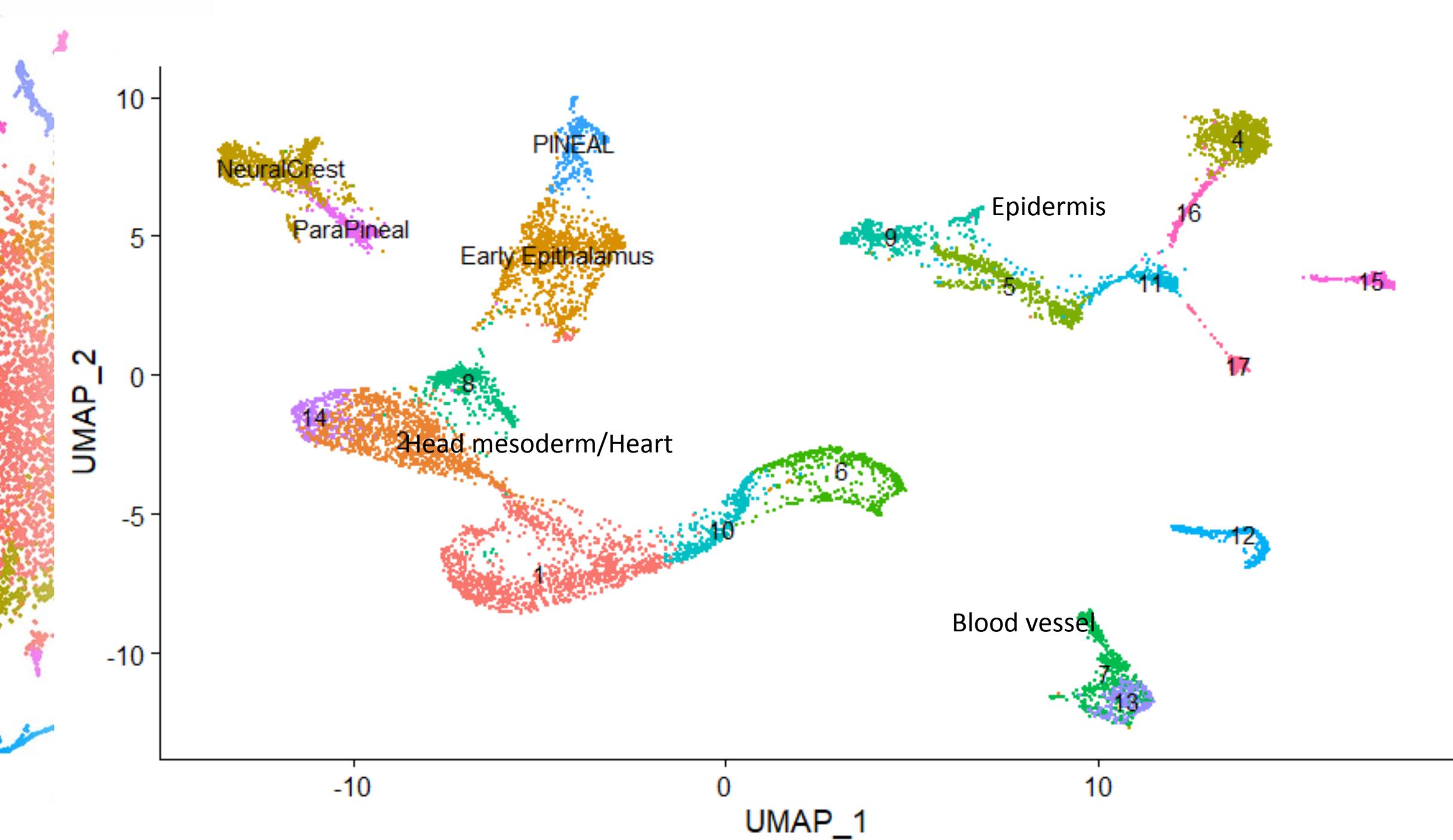


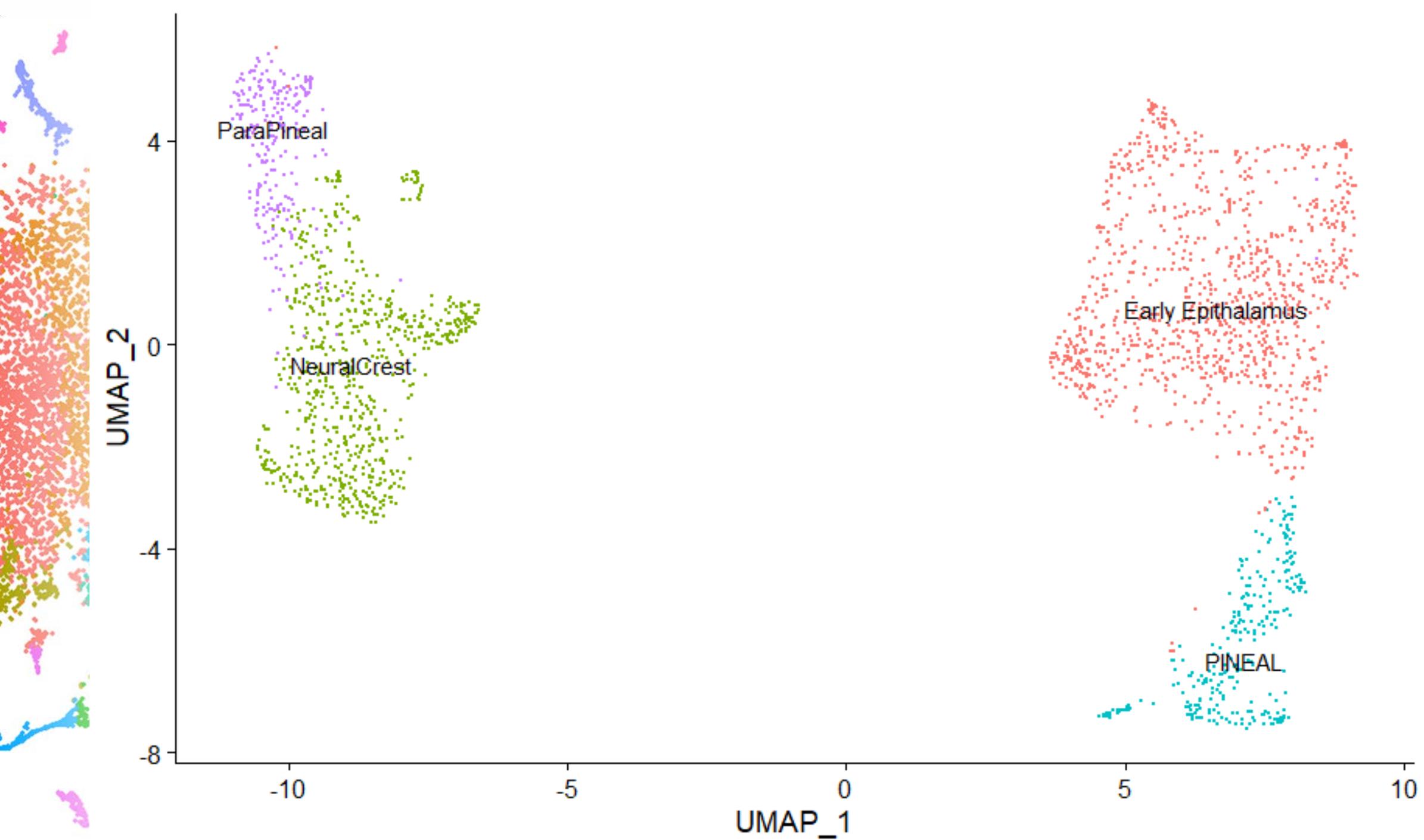
| | |
|----------------------------|--------|
| Estimated Number of Cells | 9,422 |
| Fraction Reads in Cells | 85.1% |
| Mean Reads per Cell | 32,663 |
| Median Genes per Cell | 1,983 |
| Total Genes Detected | 22,821 |
| Median UMI Counts per Cell | 7,911 |

Sample

| | |
|---------------------|------------------------|
| Name | Asym1-5000Cell |
| Description | |
| Transcriptome | zebrafish_GRCz11_exons |
| Chemistry | Single Cell 3' v2 |
| Cell Ranger Version | 3.0.2 |





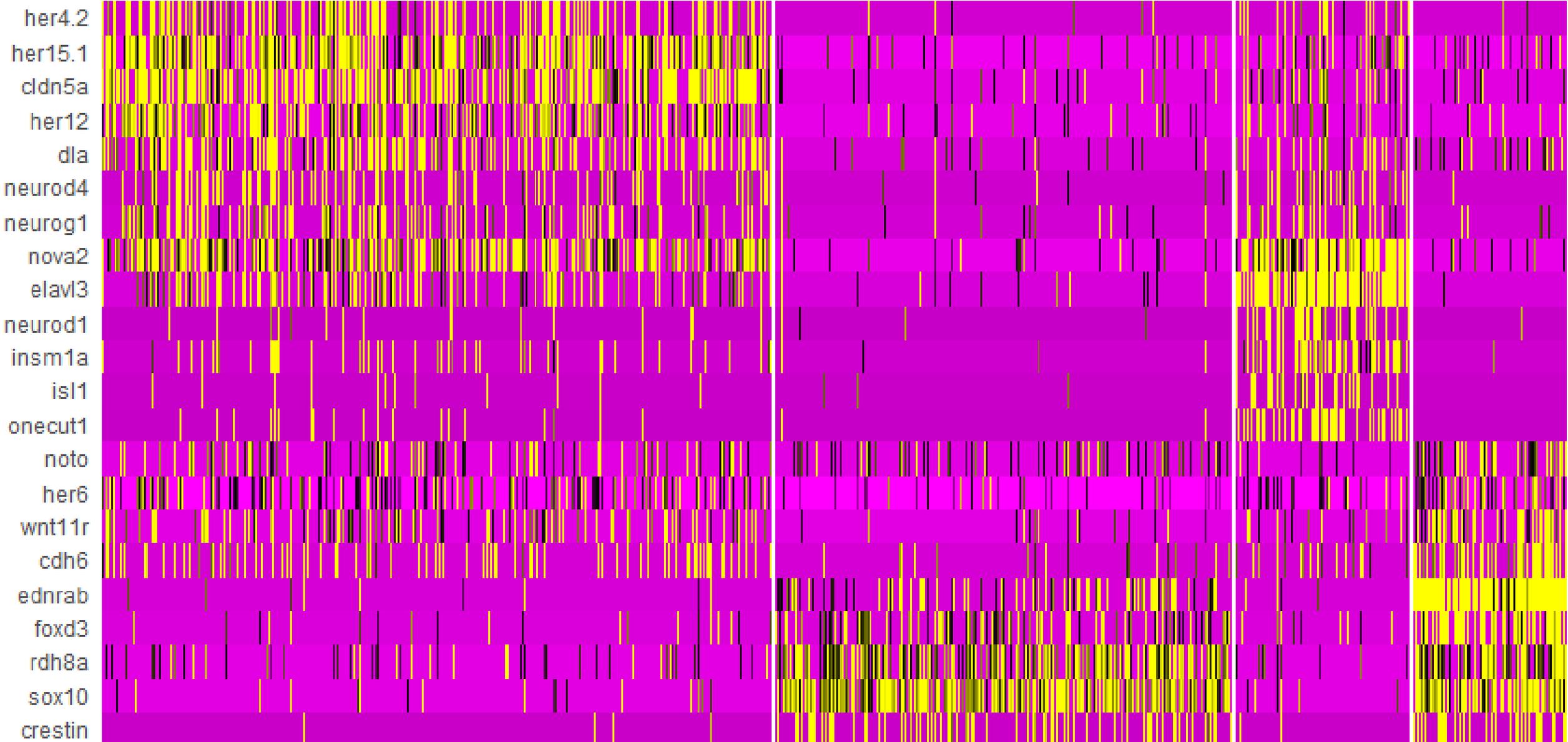


Early epithalamus

Neural Crest

Pineal

ParaPineal



Early epithalamus



neurog1



isl1

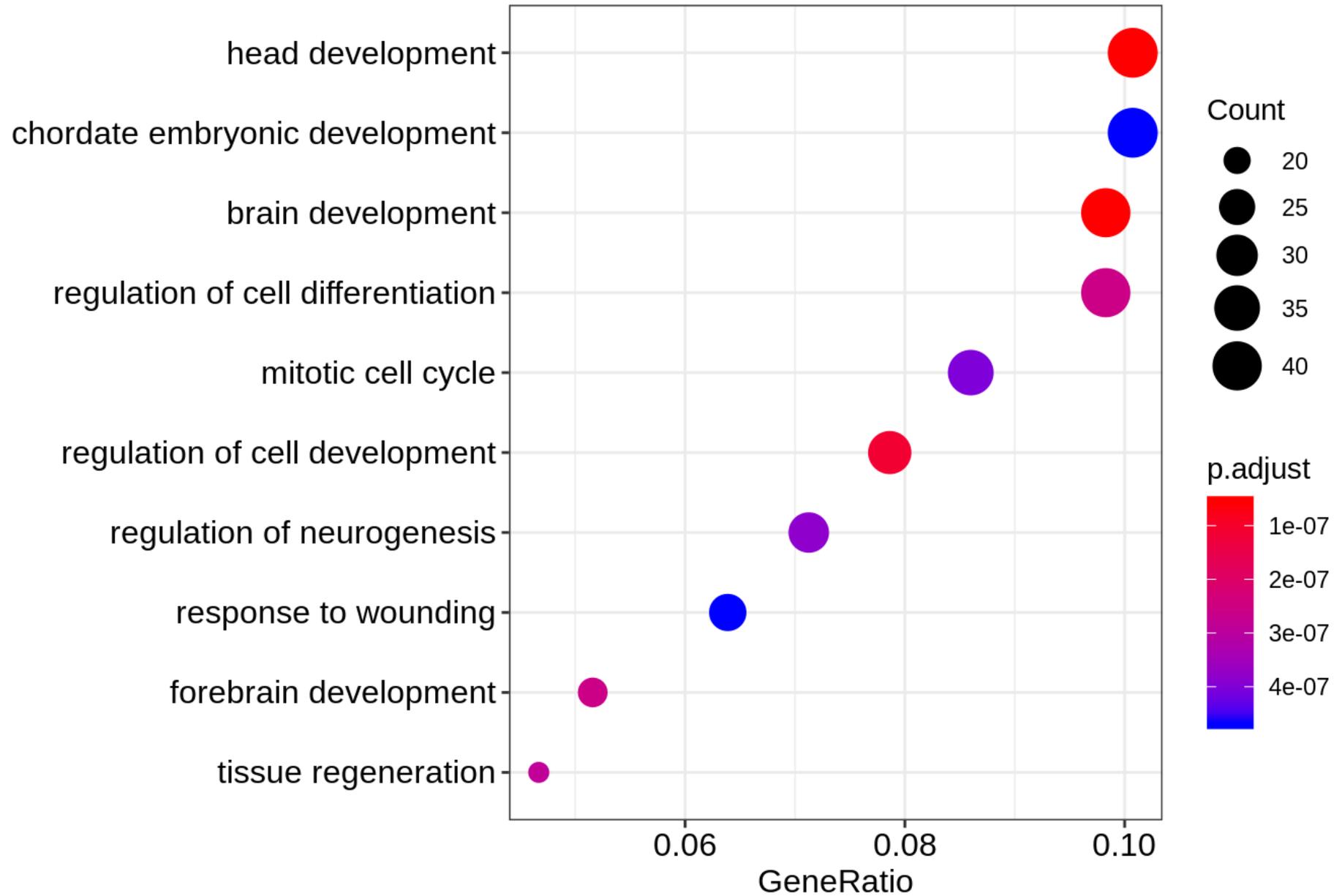


neurod4

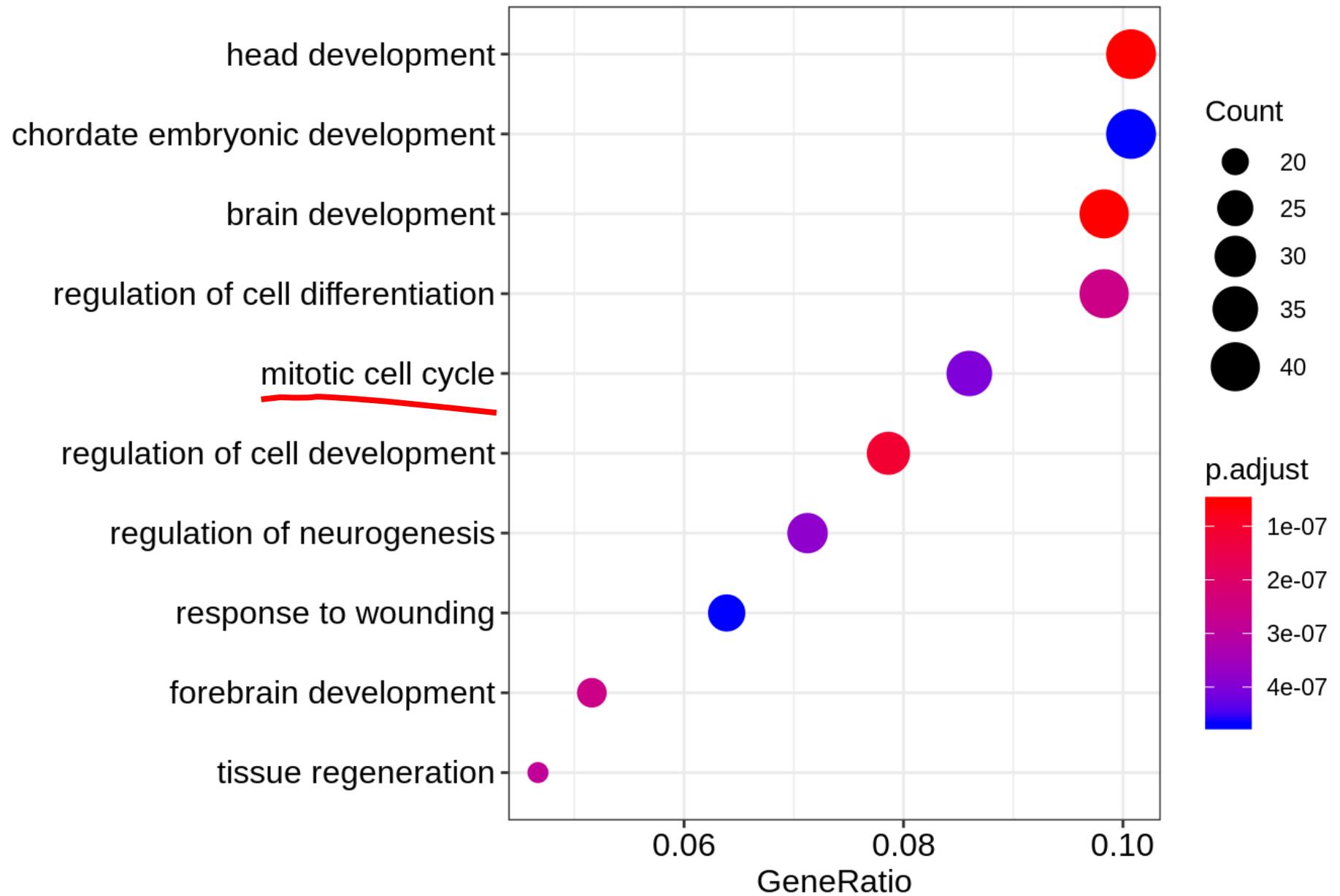


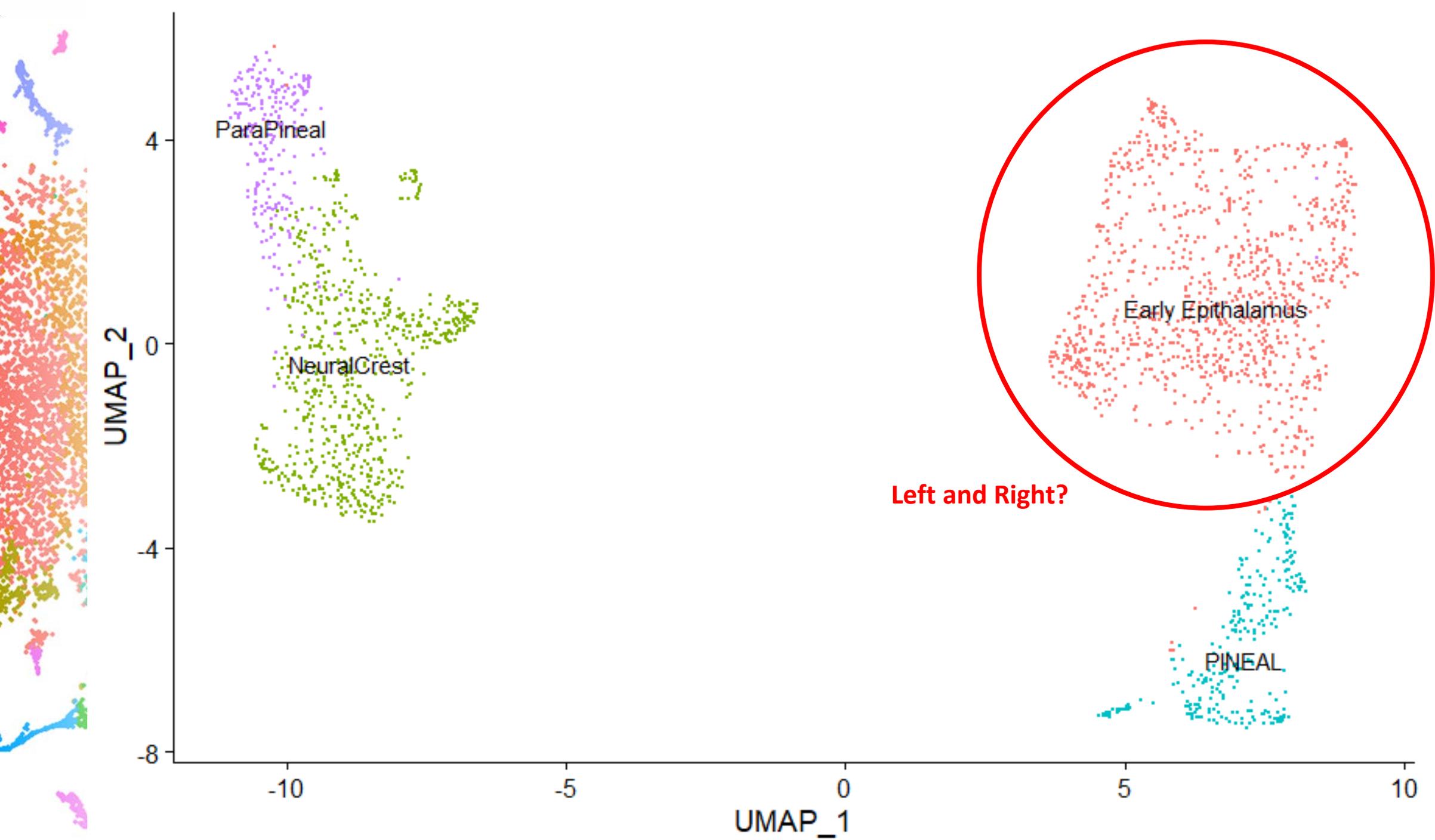
neurod1

GO term analysis

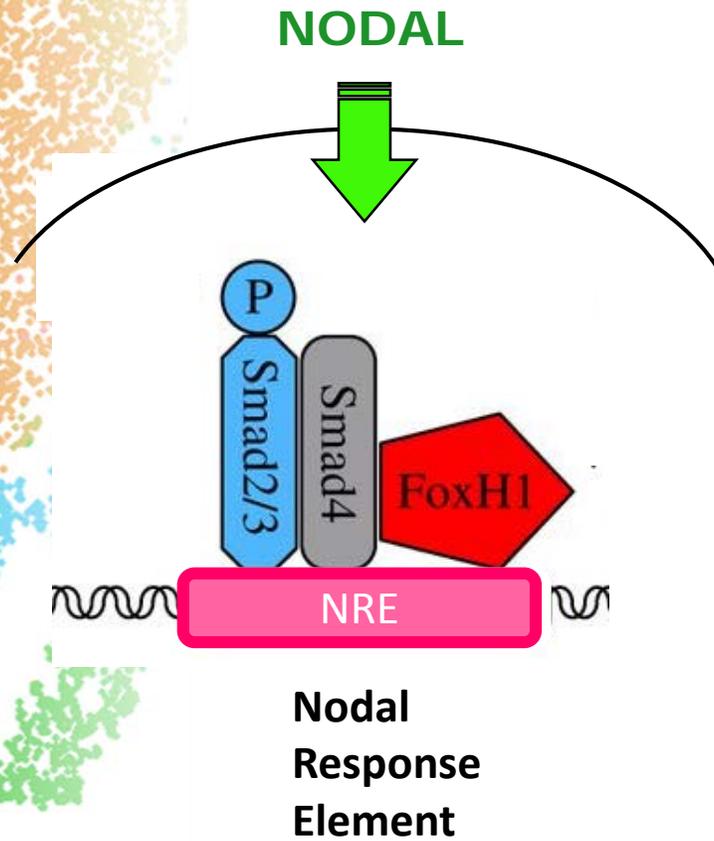


GO term analysis

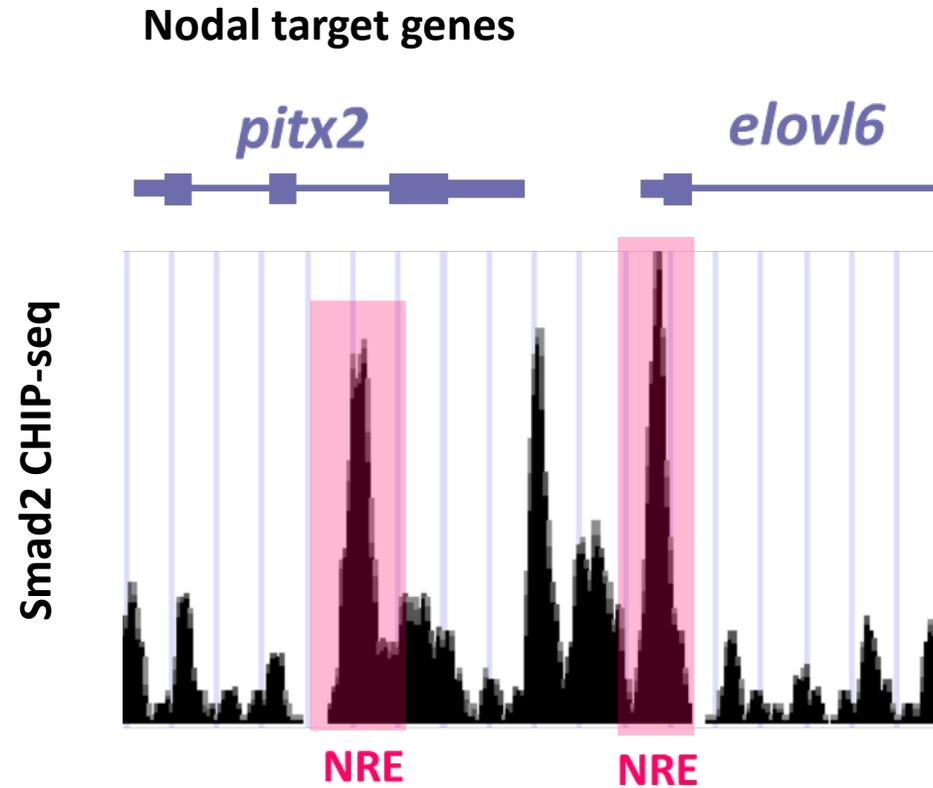
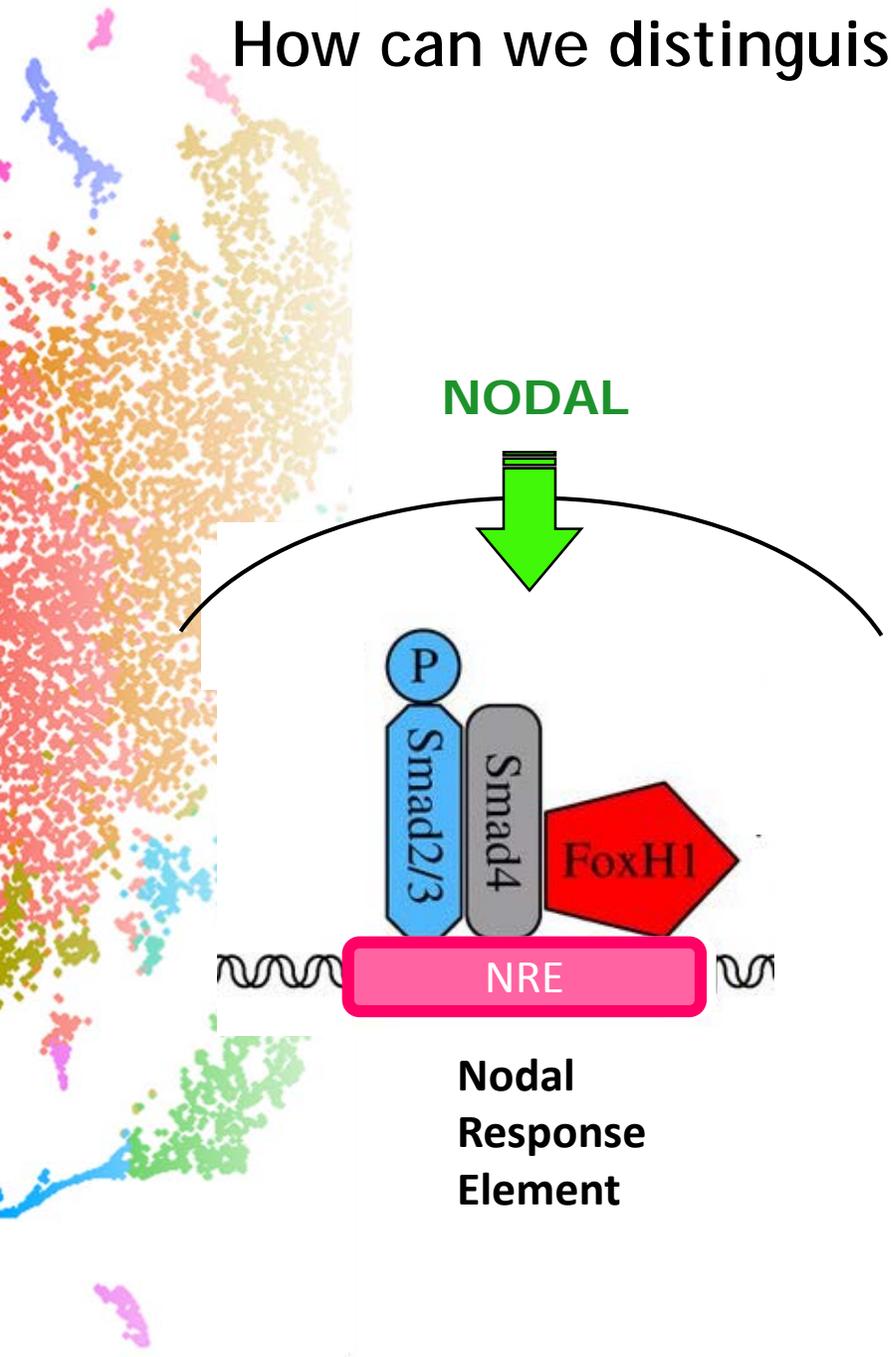




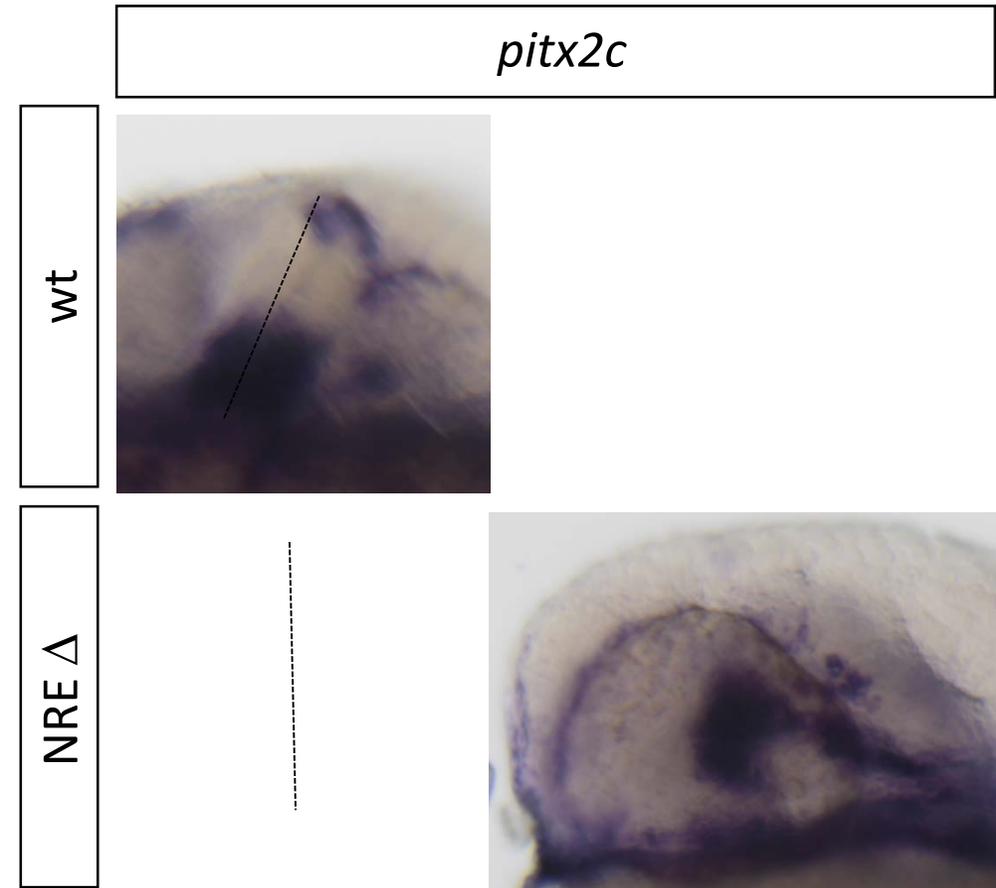
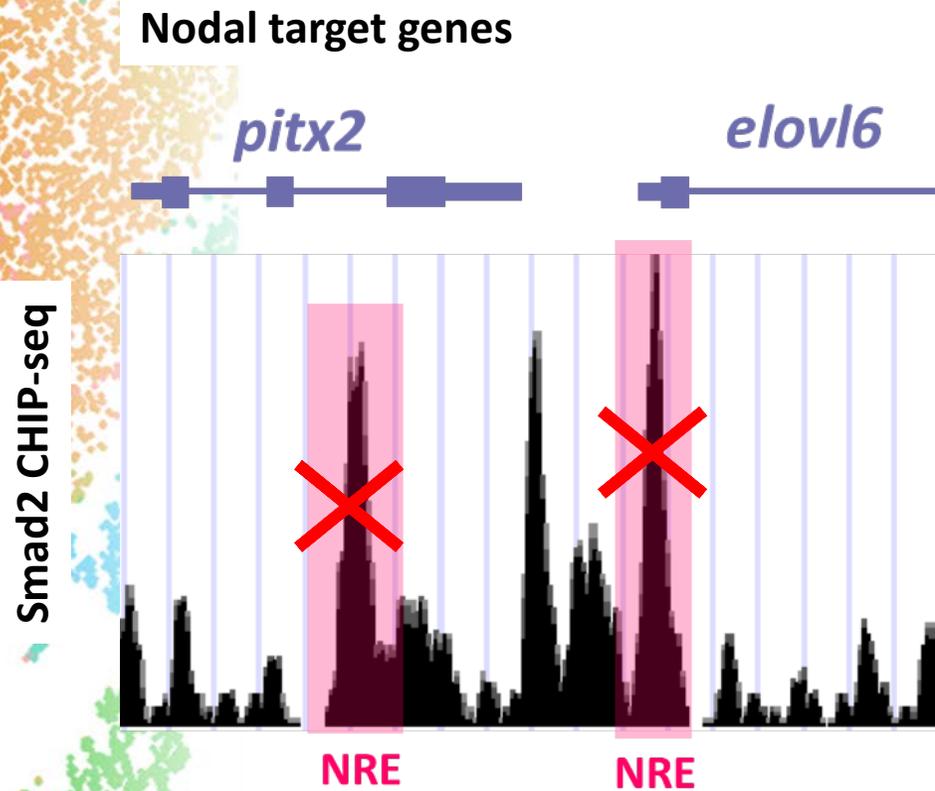
How can we distinguish the cell from the **left** side of the epithalamus?



How can we distinguish the cell from the **left** side of the epithalamus?



How can we distinguish the cell from the **left** side of the epithalamus?

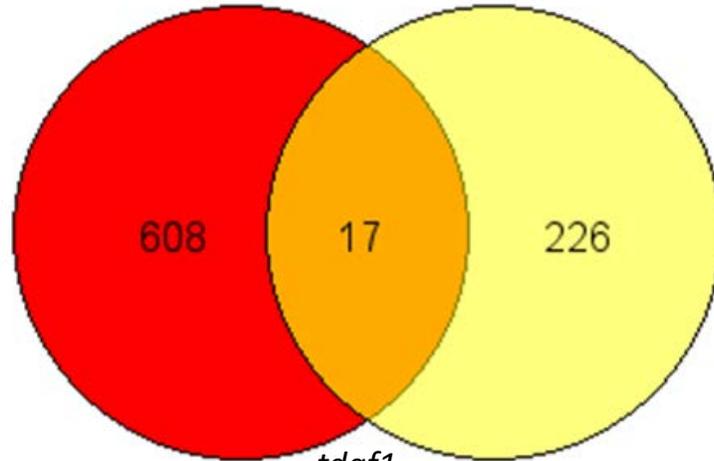


How can we distinguish the cell from the **left** side of the epithalamus?



Nodal target genes

Early Epithalamus genes



608

17

226

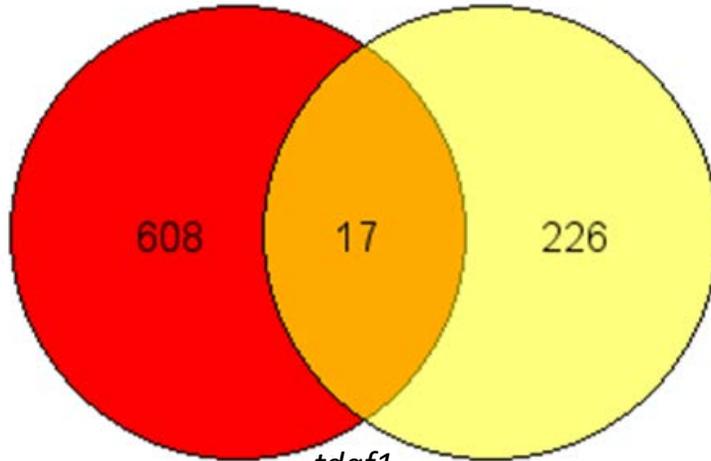
tdgf1
igf2b
irx7
bmper
lrrn1
rdh10a
cdc25b
lhx1a
efnb2a
smc1a1
pitx2
elovl6
lfng
noto
...

How can we distinguish the cell from the **left** side of the epithalamus?



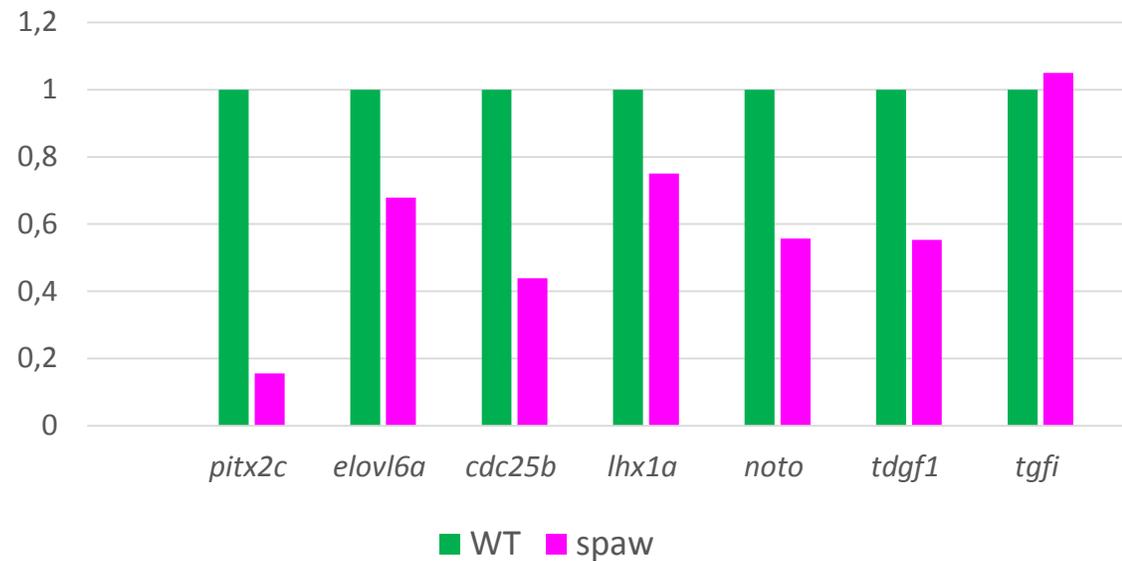
Nodal target genes

Early Epithalamus genes

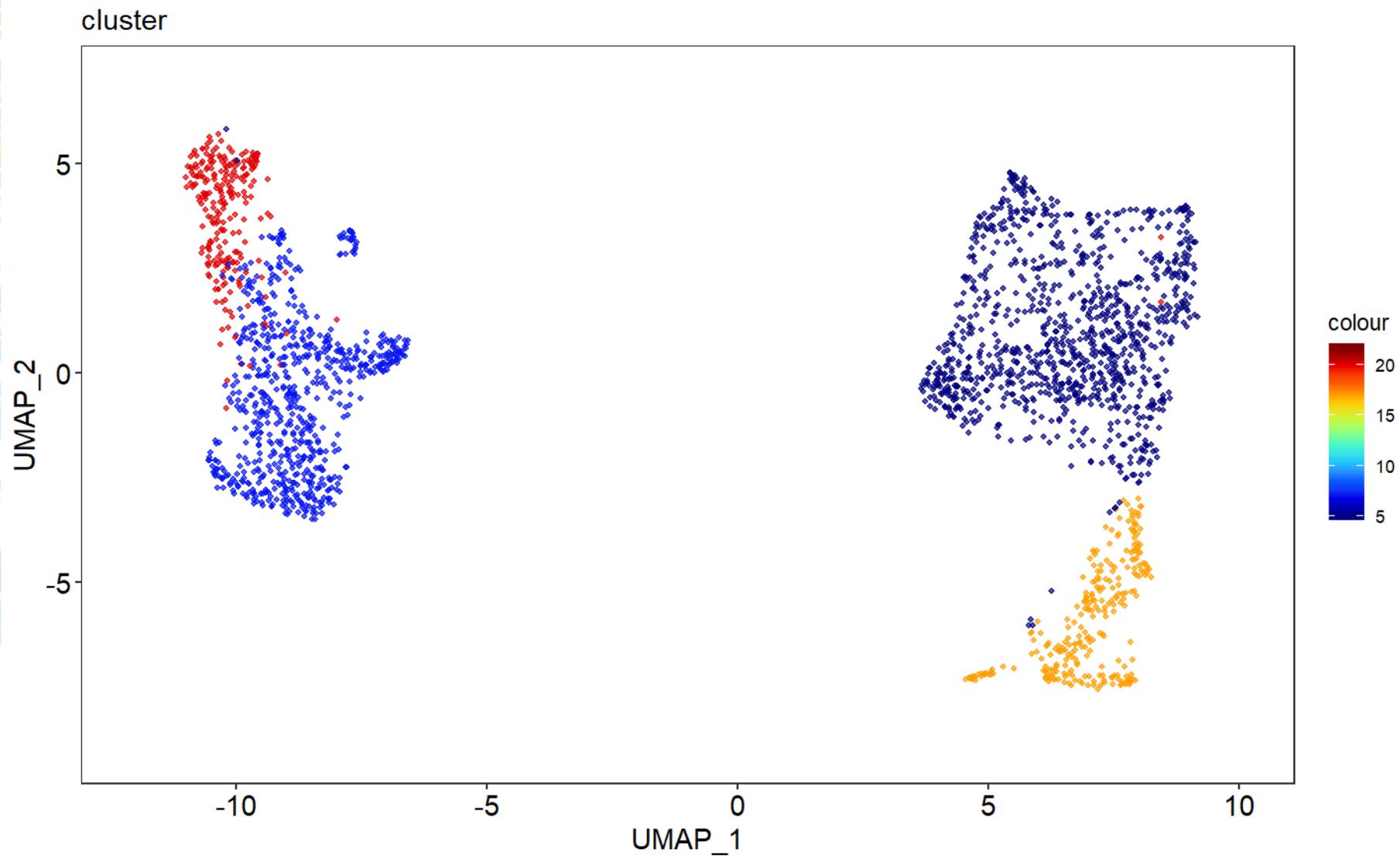
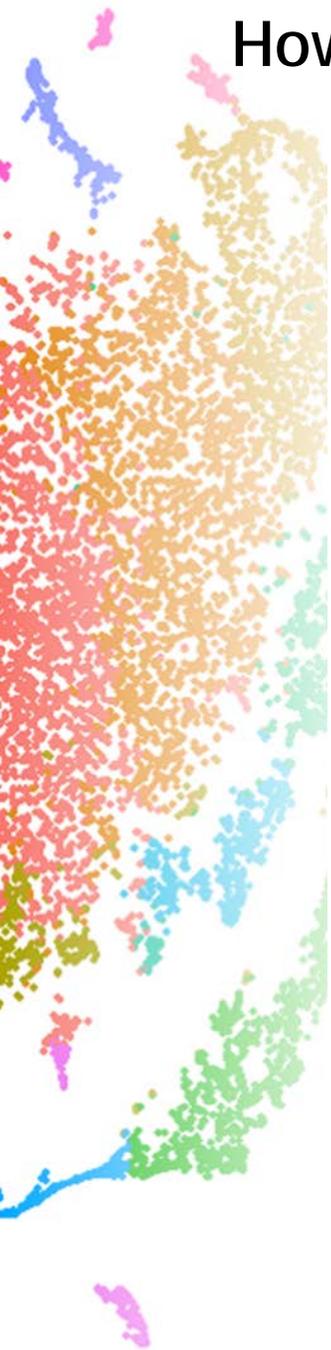


tdgf1
igf2b
irx7
bmper
lrrn1
rdh10a
cdc25b
lhx1a
efnb2a
smc1al
pitx2
elovl6
lfng
noto
...

Relative mRNA expression

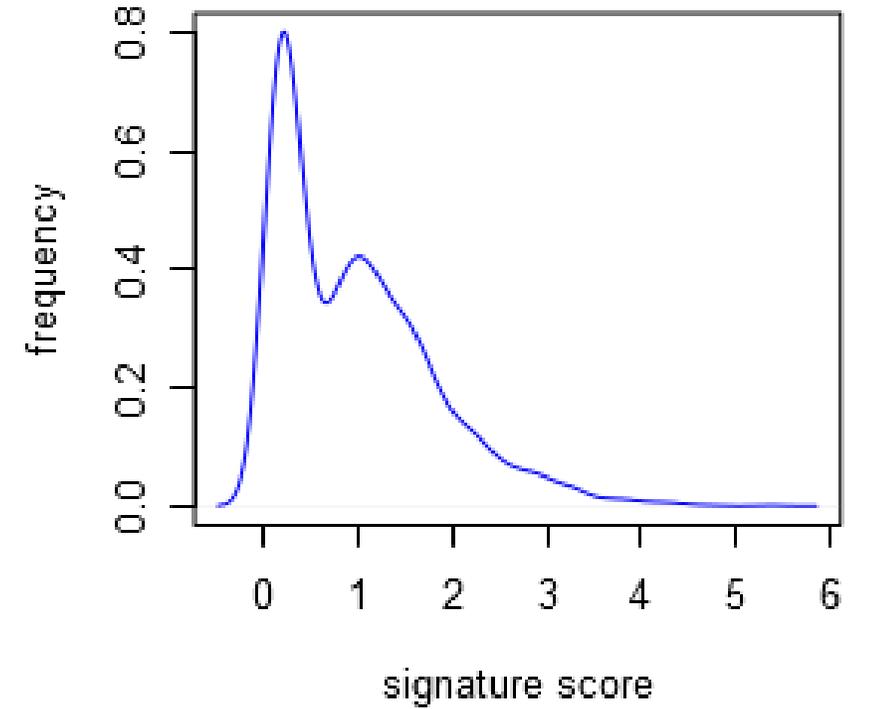
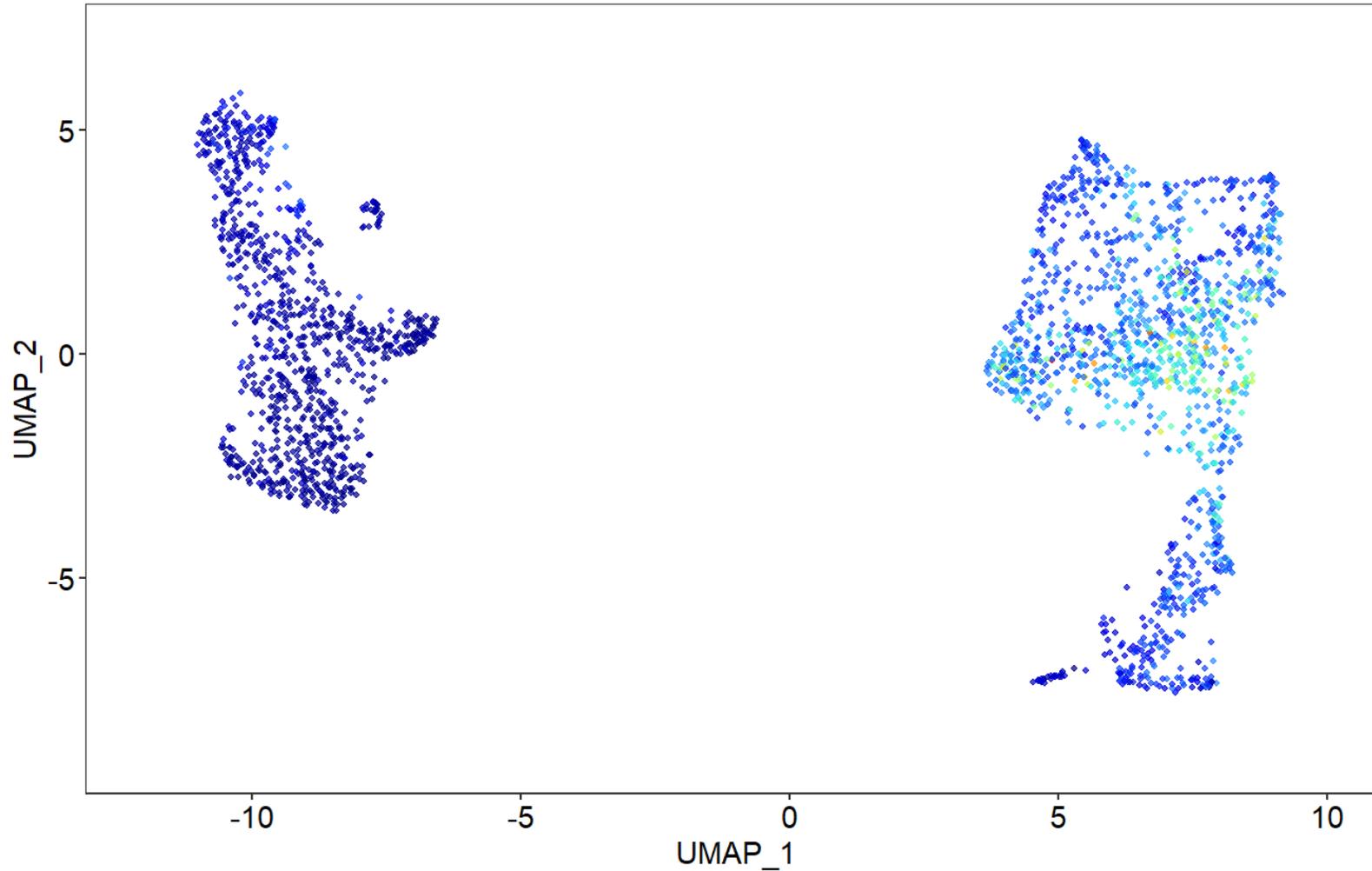


How can we distinguish the cell from the **left** side of the epithalamus?

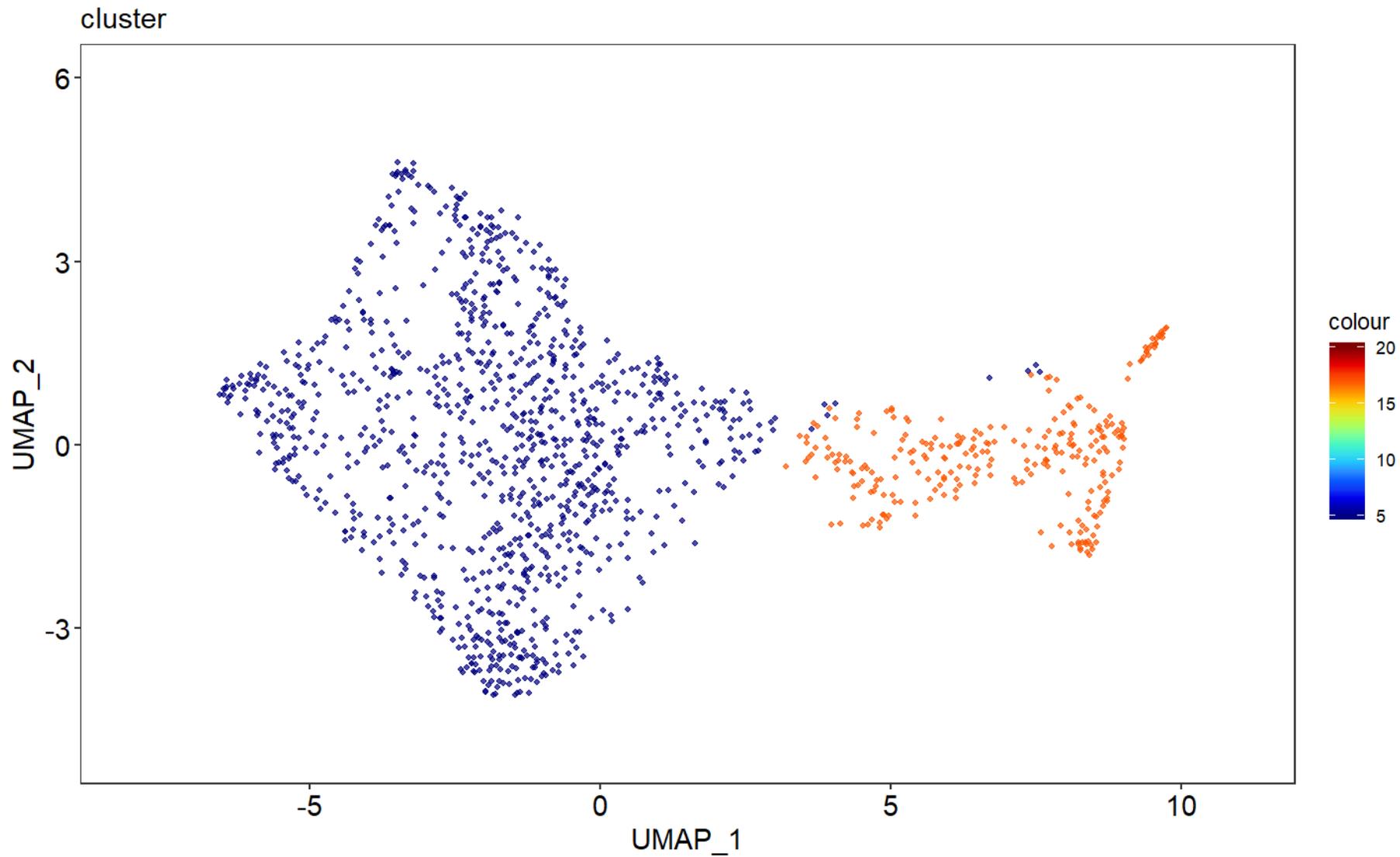


How can we distinguish the cell from the **left** side of the epithalamus?

Nodal target genes

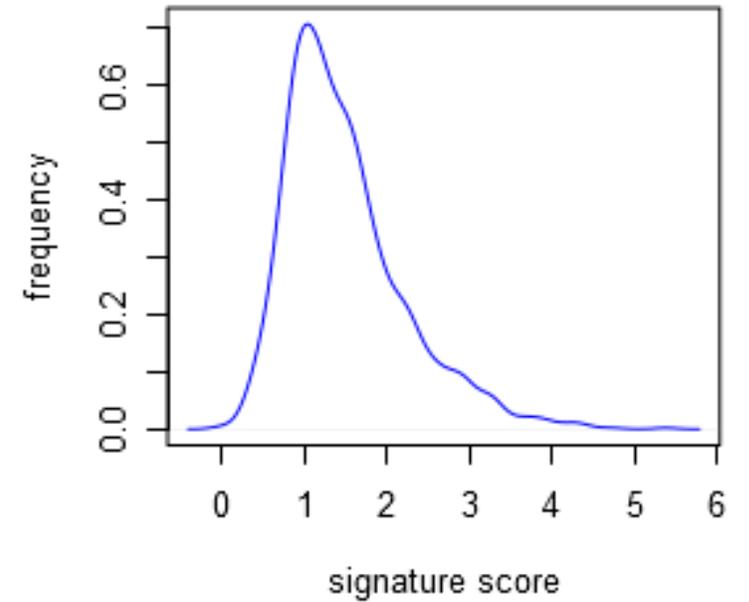
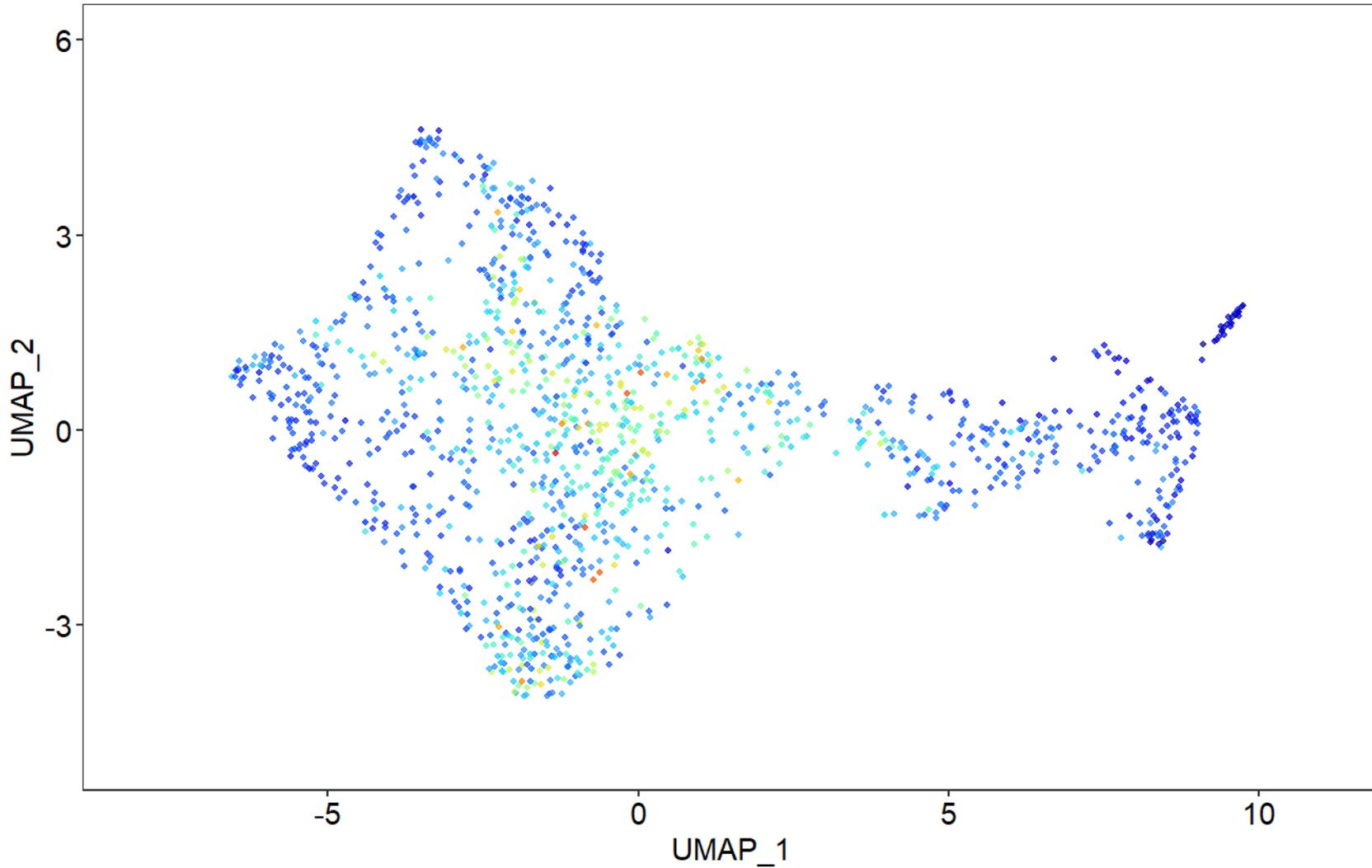


How can we distinguish the cell from the **left** side of the epithalamus?

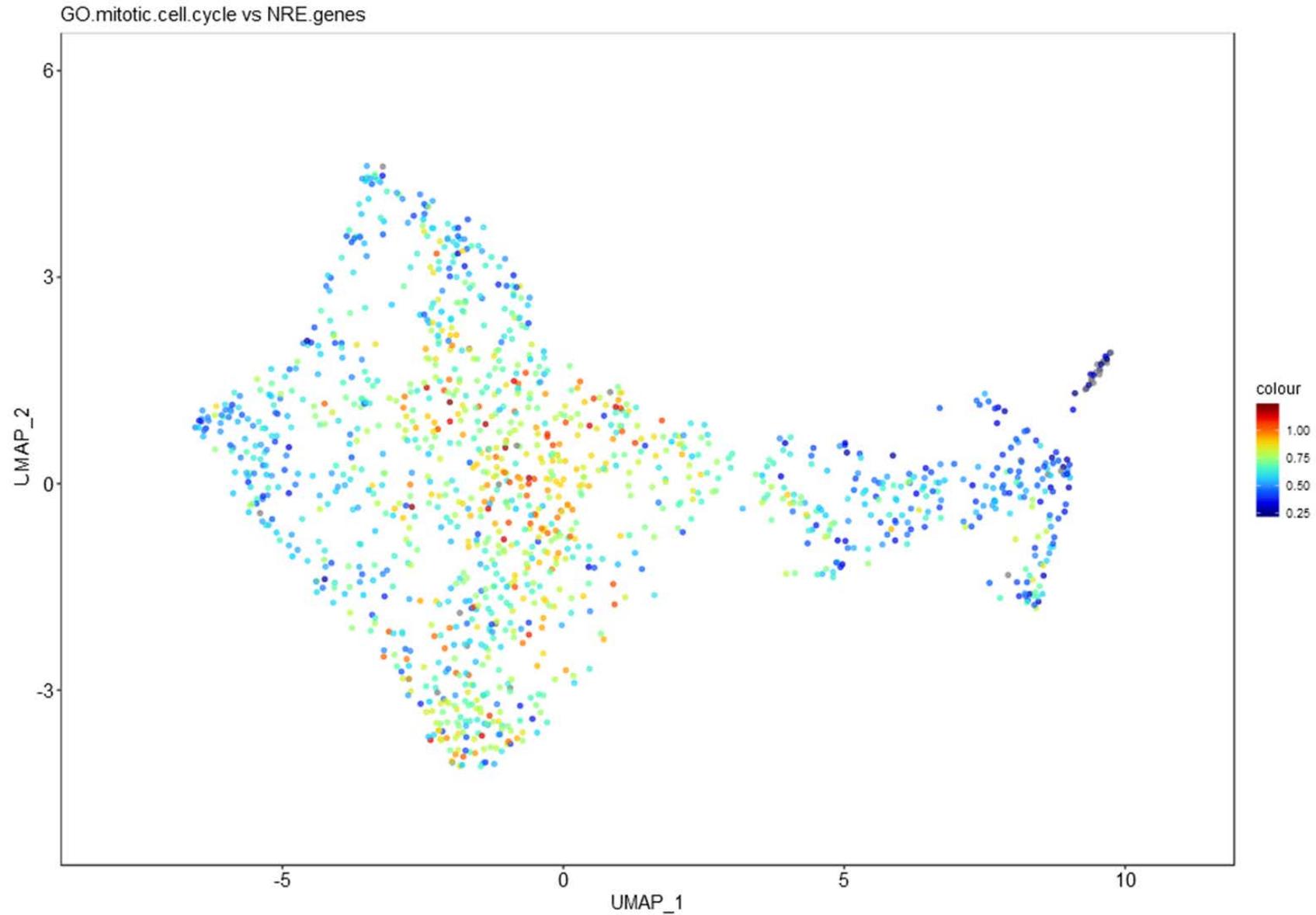


How can we distinguish the cell from the **left** side of the epithalamus?

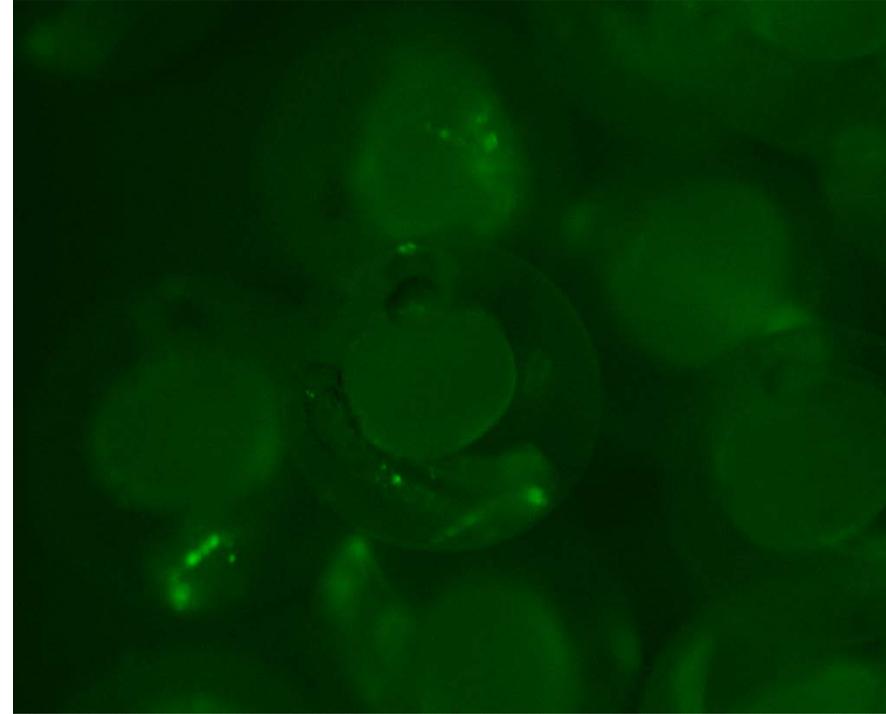
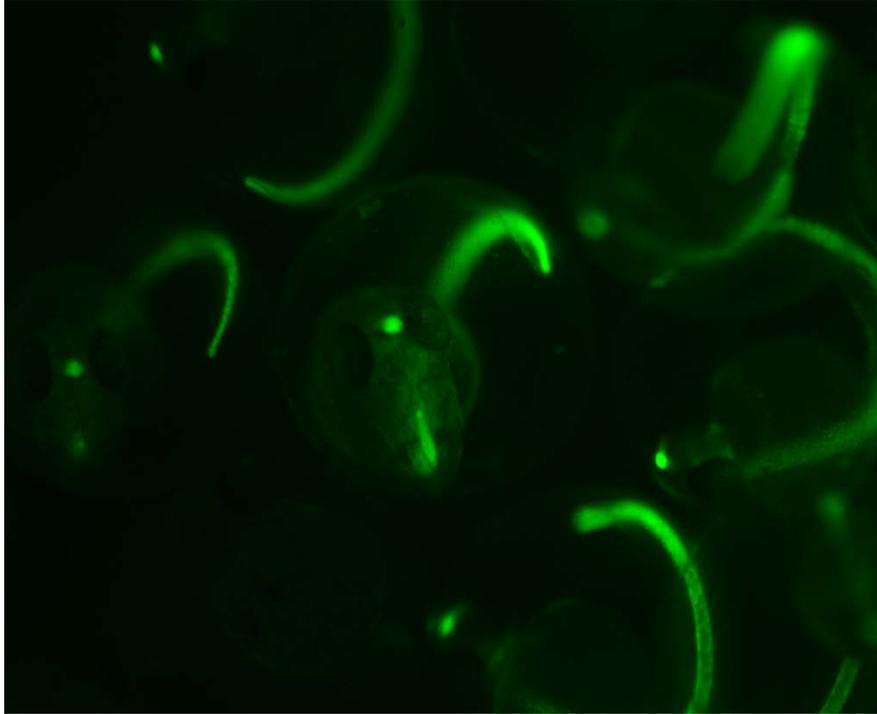
Nodal target genes



How can we distinguish the cell from the **left** side of the epithalamus?



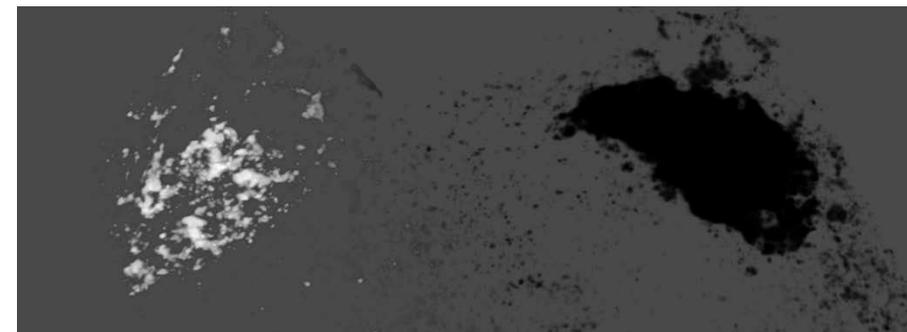
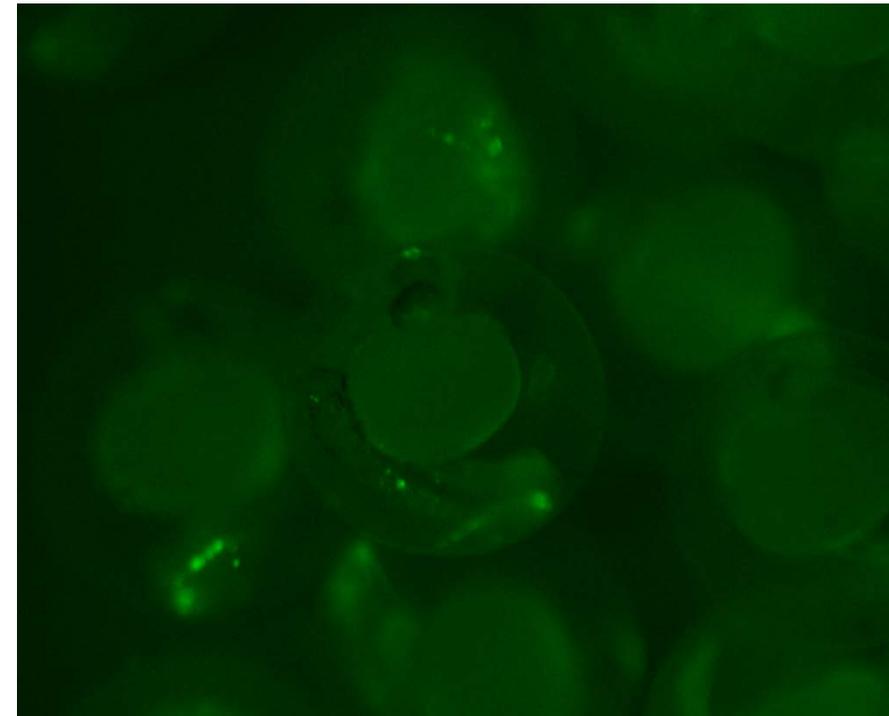
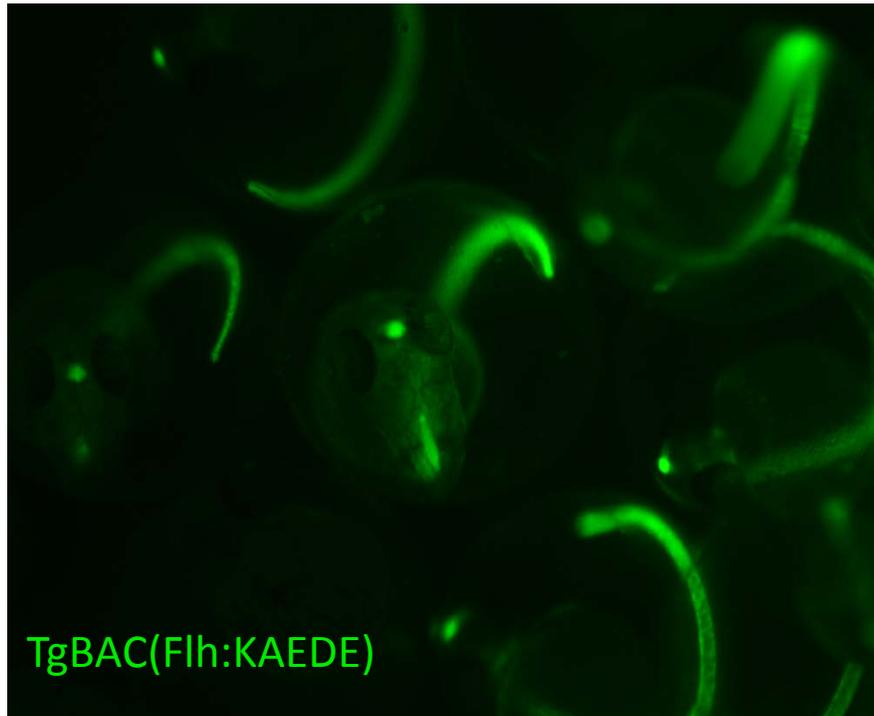
Testing new putative candidate: cdc25b



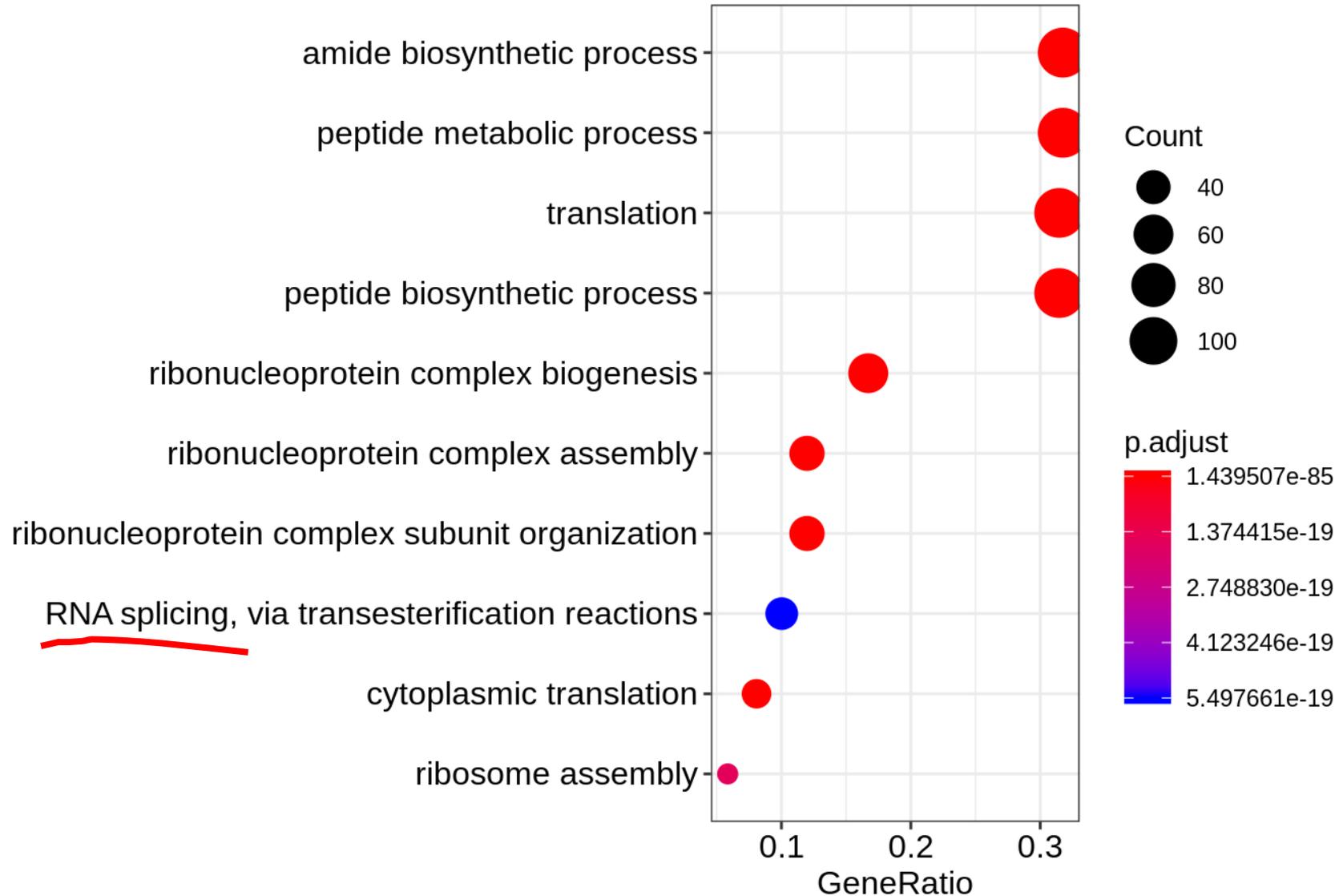
Testing new putative candidate: *cdc25b*

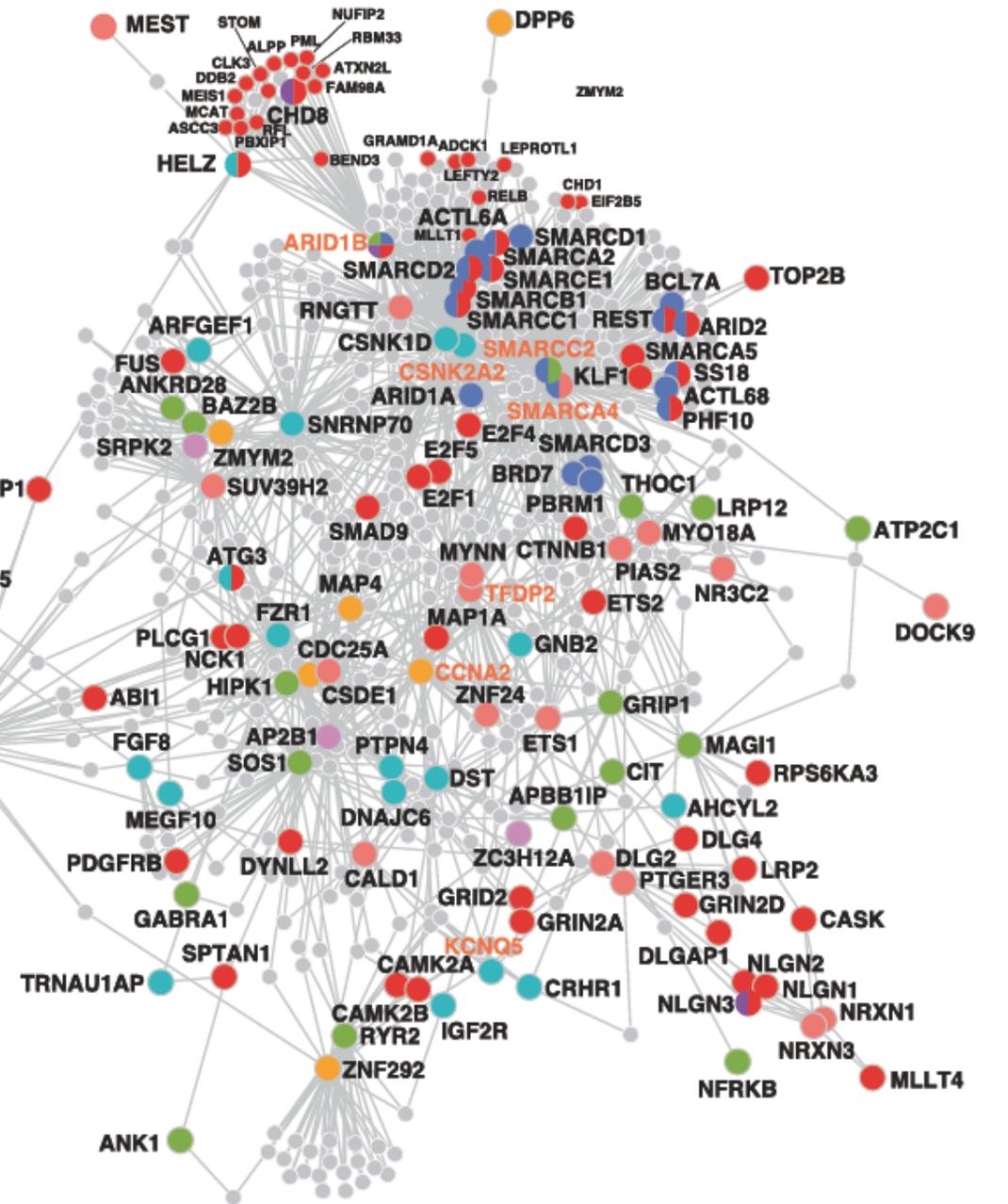
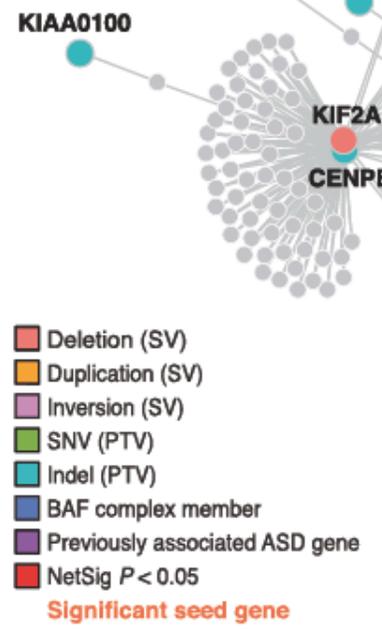
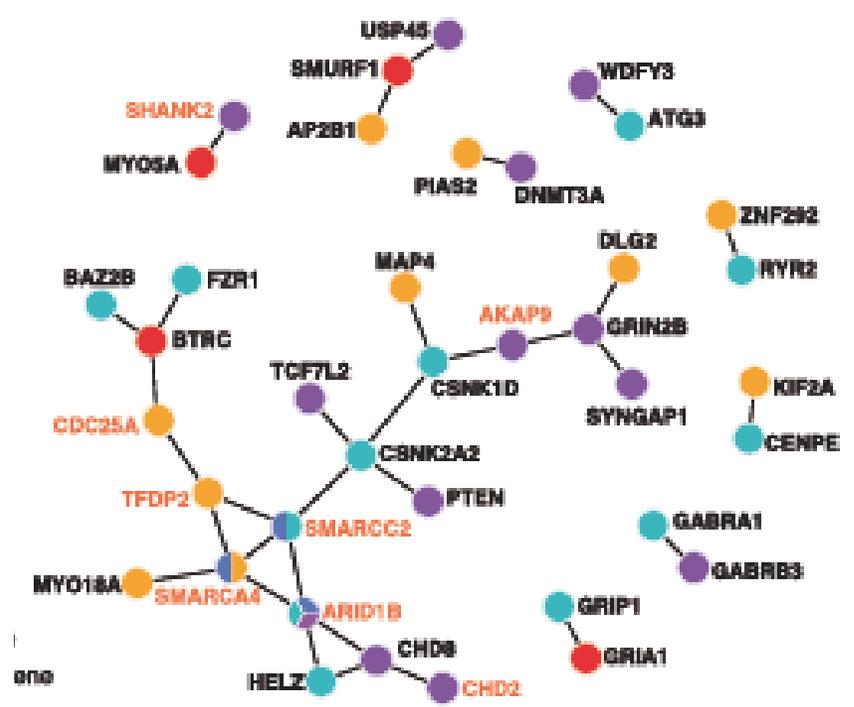
control

CRISPANT NRE *noto-cdc25b*



Why it is so hard to identify the cell from the **left** side of the epithalamus?





- Deletion (SV)
- Duplication (SV)
- Inversion (SV)
- SNV (PTV)
- Indel (PTV)
- BAF complex member
- Previously associated ASD gene
- NetSig $P < 0.05$
- Significant seed gene