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Supplementary Information for
Human Intermediate Progenitor Diversity during Cortical Development

Mark-Phillip Pebworth (0000-0001-8224-9480)[1 2], Jayden Ross (0000-0002-55698624)[1 2], Madeline Andrews (0000-00020-51540-5081)[1 2], Aparna Bhaduri (0000-00034625-6899)[1 2], Arnold R. Kriegstein (0000-0001-5742-2990)[1 2 *]

Arnold R. Kriegstein
Email: Arnold.Kriegstein@ucsf.edu

This PDF file includes:

Supplementary text
Figures S1 to S7

Other supplementary materials for this manuscript include the following:

Datasets S1-S5

Figure Legends:

Fig. S1: IPCs in the oSVZ appear more proliferative than those in the iSVZ during early neurogenesis, since EOMES nuclei co-stain with KI67 far more often in the oSVZ than the iSVZ. Images are from GW14, GW16, and GW18 and are the images quantified in Fig 1 C of the main text.

Fig. S2: EOMES & TBR 1 immunohistochemistry reveals a laminar pattern of transcription factor expression in the iSVZ during early neurogenesis. This evidence suggests that most iSVZ IPCs differentiate without migrating into the oSVZ.

Fig. S3: Further verification of EOMES staining pattern during late neurogenesis. A) Second EOMES antibody. Further verification of EOMES staining pattern during late neurogenesis. A) Second EOMES antibody validates that most IPCs disappear from oSVZ. Staining shows GW14, GW16, GW20 and GW22 staining with a second EOMES antibody. B) Markers for RG & neuronal tracts (VIM, HOPX, & L1CAM) stain oSVZ as expected. Cytoarchectiture of oSVZ confirmed to be normal for samples.

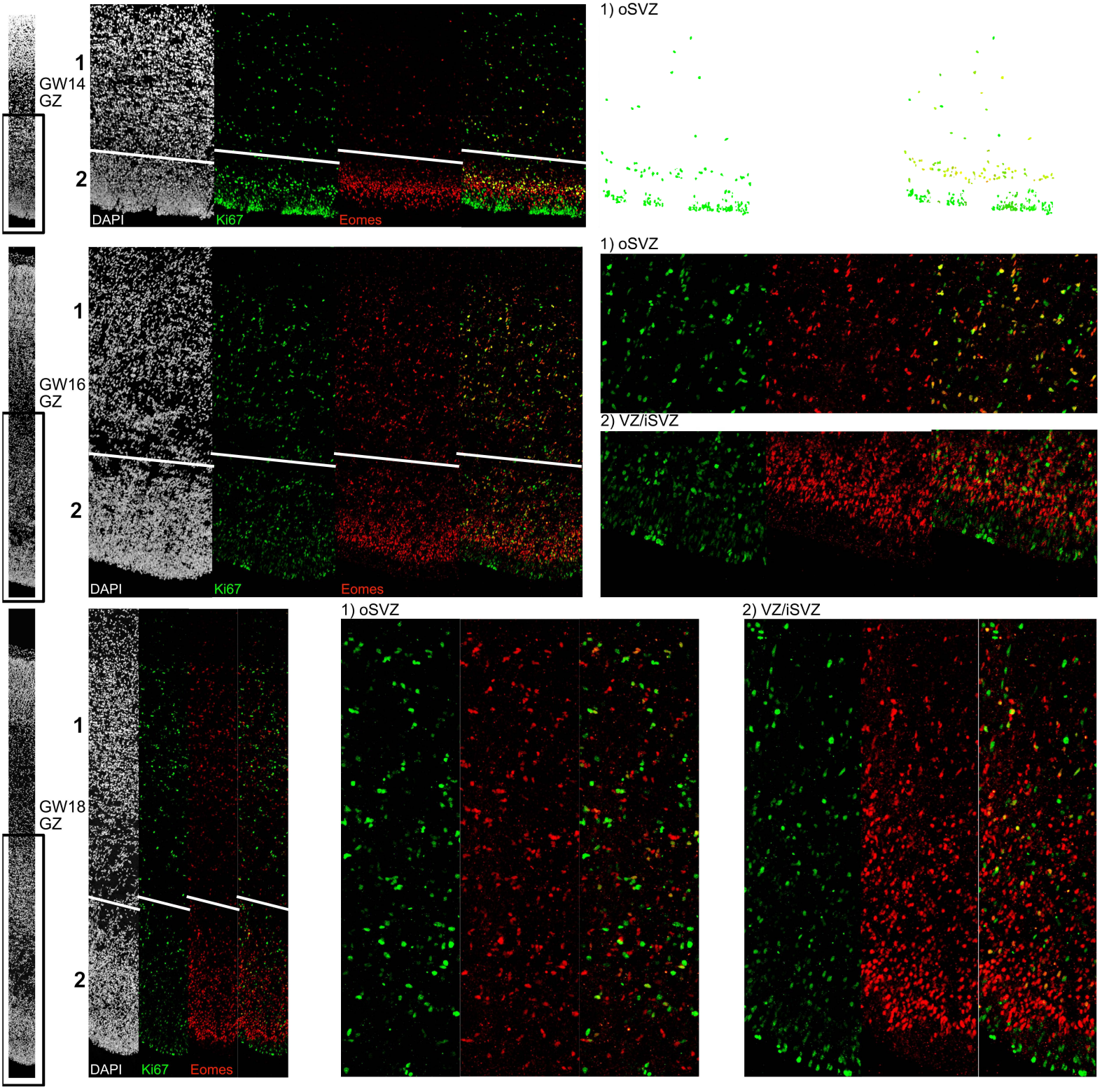
Fig. S4: Staining for neuronal markers support disappearance of neurogenesis from the oSVZ during late neurogenesis. A) Markers expressed in the neuronal lineages (NeuroD1, NHLH2, TBR1), and which begin in IPCs, are expressed throughout the GZ at GW18. B) By GW20, the same neuronal markers are no longer expressed throughout the germinal zones, and tend to be found in the cortical plate, or in the iSVZ.

Fig. S5: The original dataset and its comparison with the clusters and markers from 1000 scrambled iterations. A) Full clustering and annotations from the original dataset. Several clusters were marked by mitochondria genes, and were annotated as low quality. One cluster was dominated by ribosomal genes. One other cluster was Archetypic, and did not have a significant difference from our scrambled dataset. B) Cell Cycle annotations, conducted through Seurat, identified the three dividing clusters. C) Significant Cluster markers against the number of cells found in the cluster for our original dataset and our scrambled datasets. Significant markers had a p-value of less than 0.05 after Bonferroni correction. The vast majority of clusters had a significant number of markers far outside the range of p-values attained through scrambled data. However, Archetypic, and other low quality clusters did not. D). Cluster size vs Number of Markers for clusters from the original and scrambled data. As the number of cells decreases, the number of significant markers increases. Most, but not all, clusters have a high number of marker genes compared to the number of cells in the clusters, as compared to the scrambled data.

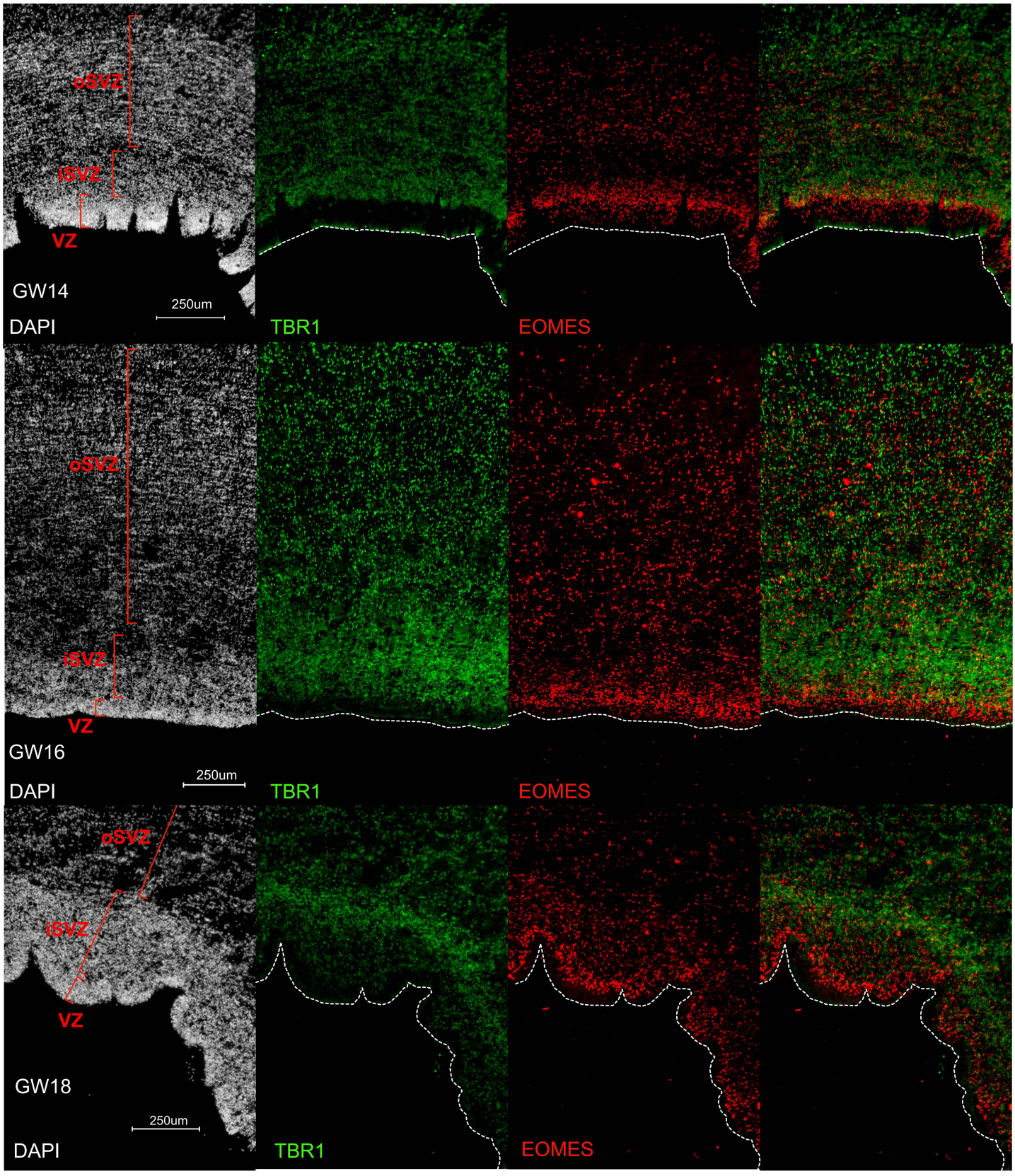
Fig. S6: Subclusters of RG-Like IPCs do not appear to correlated well with RG-subtypes. A) RG-Like IPCs can be clustered further bioinformatically. B) RG-subtypes genes do not appear to uniquely distinguish one subclusters from another. *PAX6* and *SOX2* included to show high levels of RG-like progenitor genes. C). Gene sets for each RG subtype also do not appear to distinguish subclusters. Gene set expression generated by projecting the marker gene set for each RG subtype into PCA space.

Fig. S7: A) PPP1R17 is restricted to the GZ. Image taken at GW18. B) Most PPP1R17+ cells do not co-express EOMES. Graph represents the fraction of PPP1R17 cells that co-express EOMES. Values represent the average from an image at GW14, GW16, & GW18. Images were quantified to generated values for the germinal zone, before the same image was divided up into iSVZ & oSVZ. Errors bars represent standard deviation. C) DNM3 is expressed in the cortical plate at GW14. D) PPP1R17+/EOMES- Cells can also express TBR1, marking them as potentially newborn neurons.

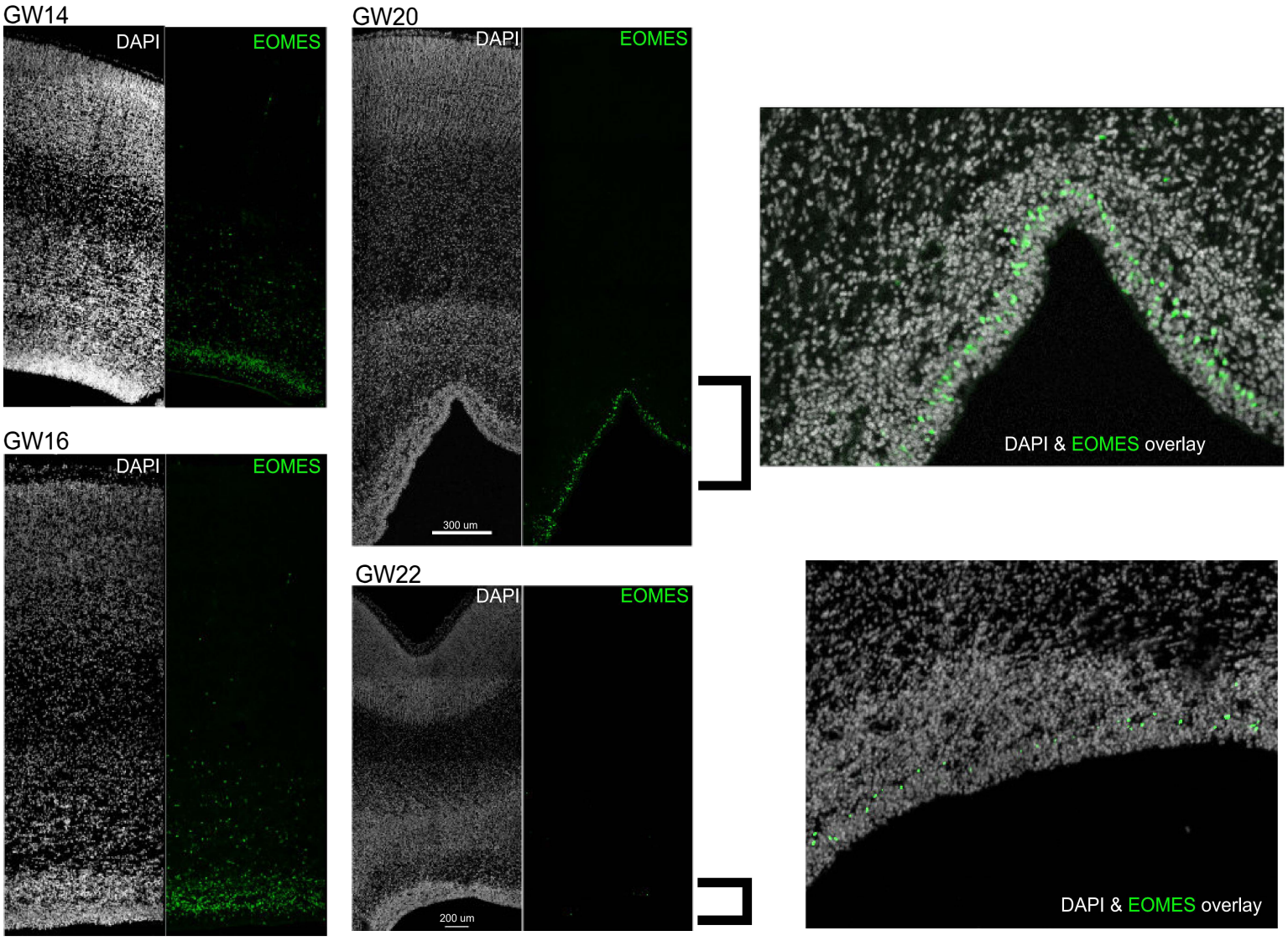
A) IPCs in the oSVZ are more proliferative than in the iSVZ



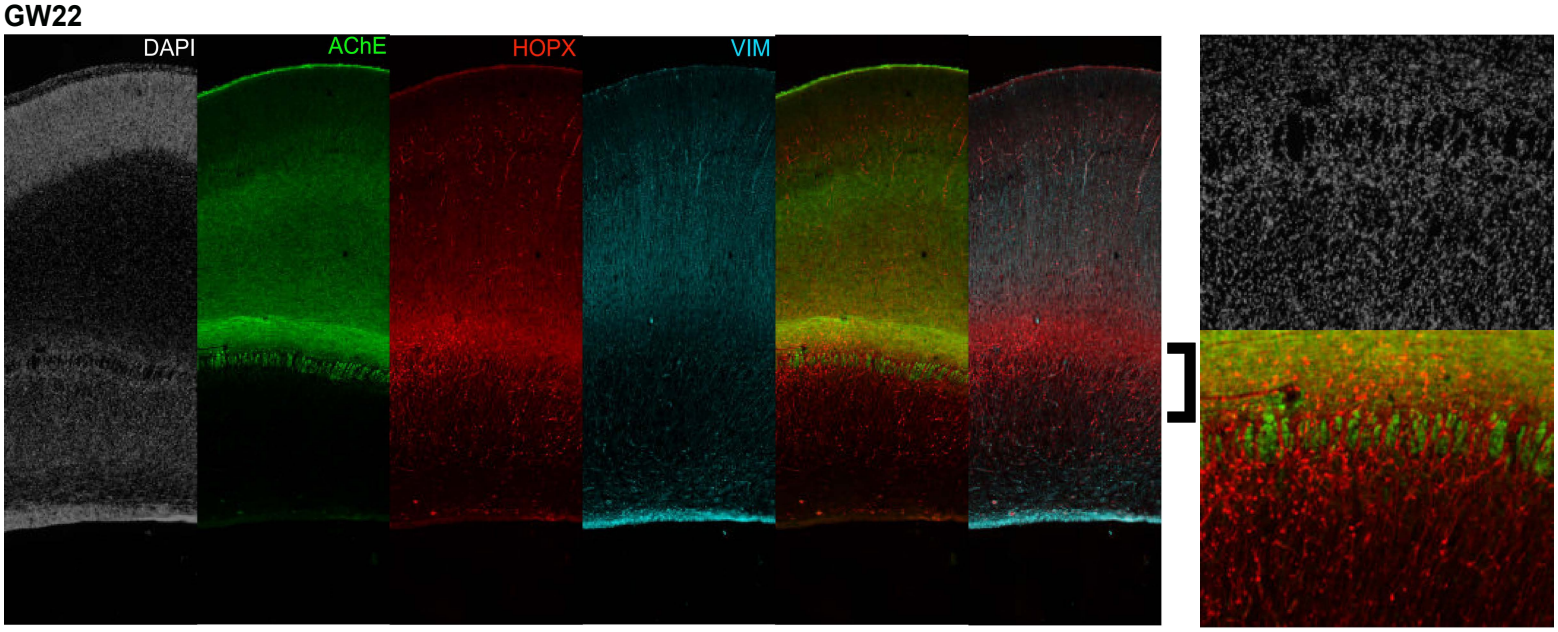
A) EOMES and TBR1 form a laminar pattern in in the iSVZ during early neurogenesis



A) Second EOMES antibody validates that most IPCs disappear from oSVZ

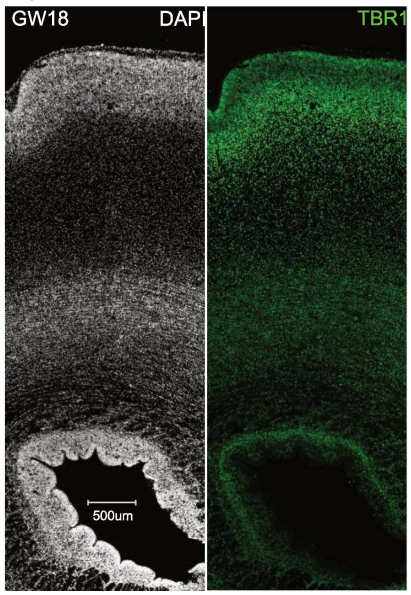


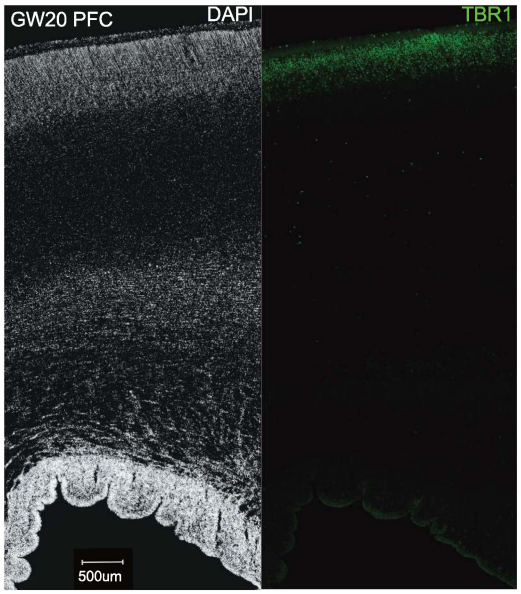
B) Markers for RG & neuronal tracts (VIM, HOPX, & L1CAM) stain oSVZ

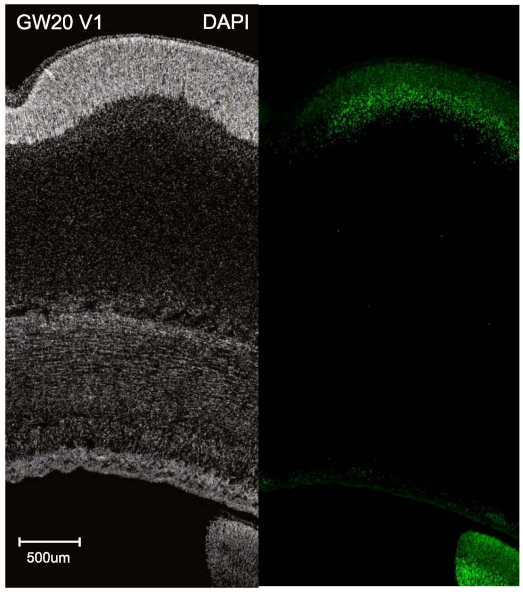


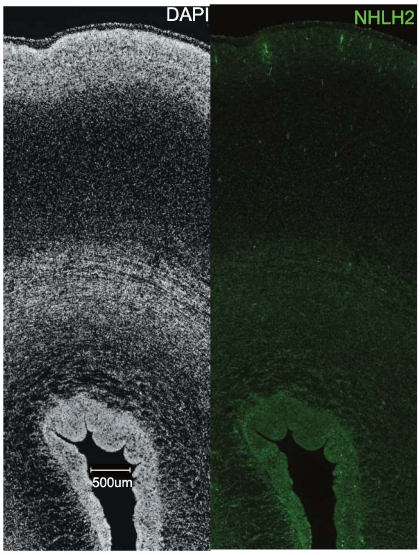
A) Neuronal lineage markers present in the GZ at GW18

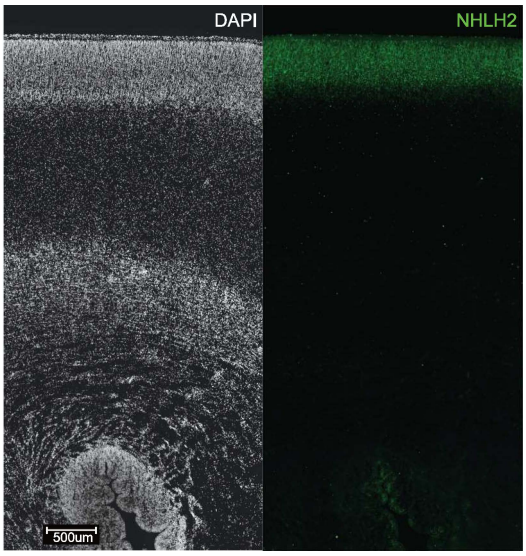
B) Neuronal lineage markers largely absent in the GZ at GW20

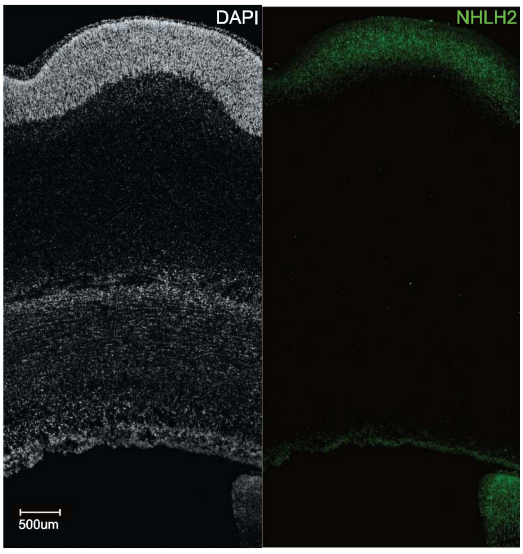


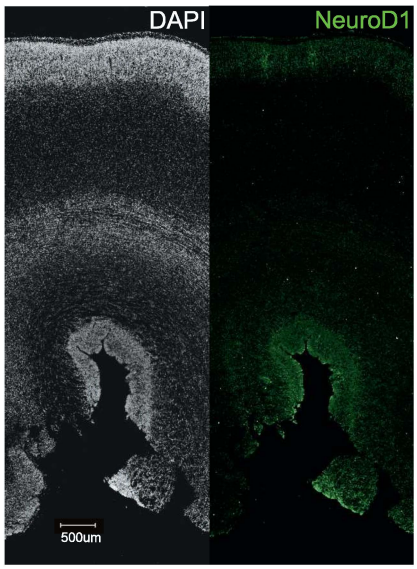


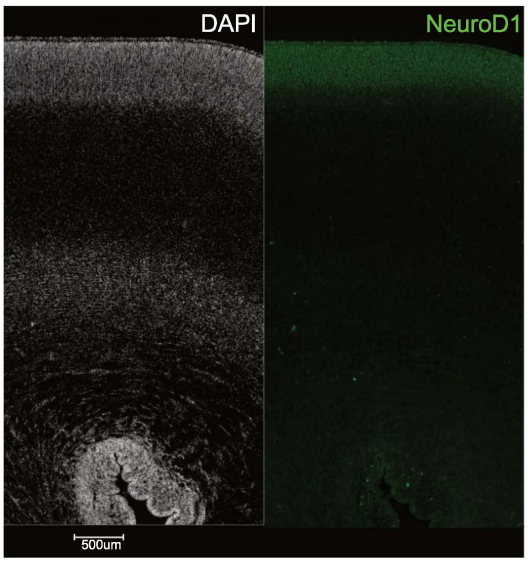


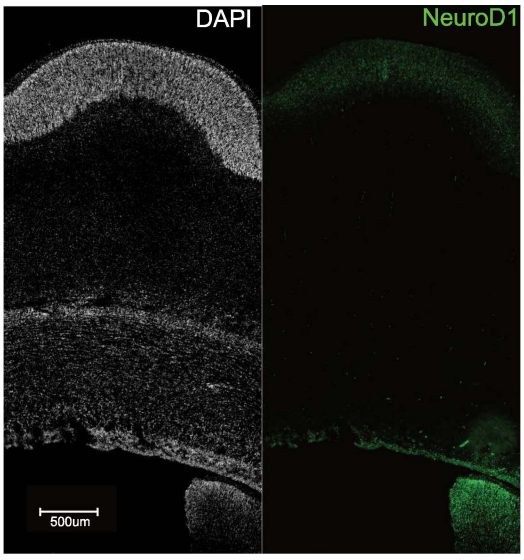




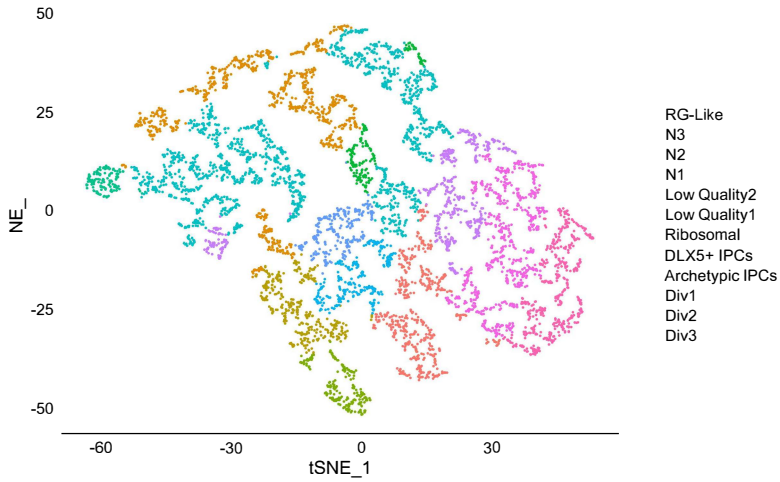




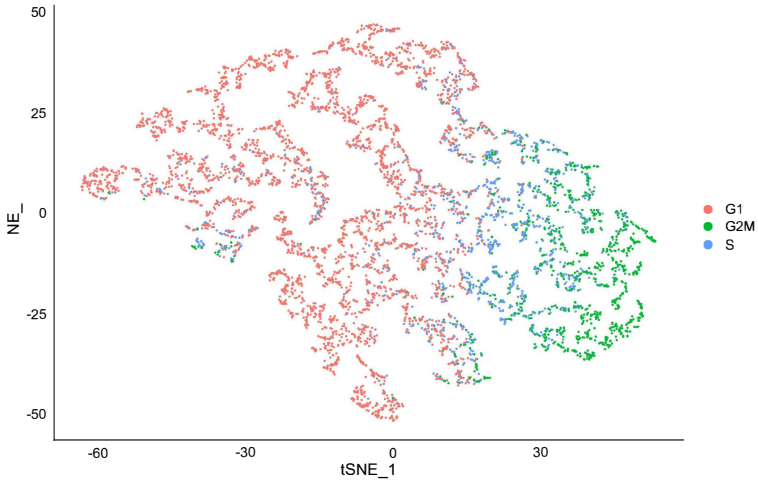




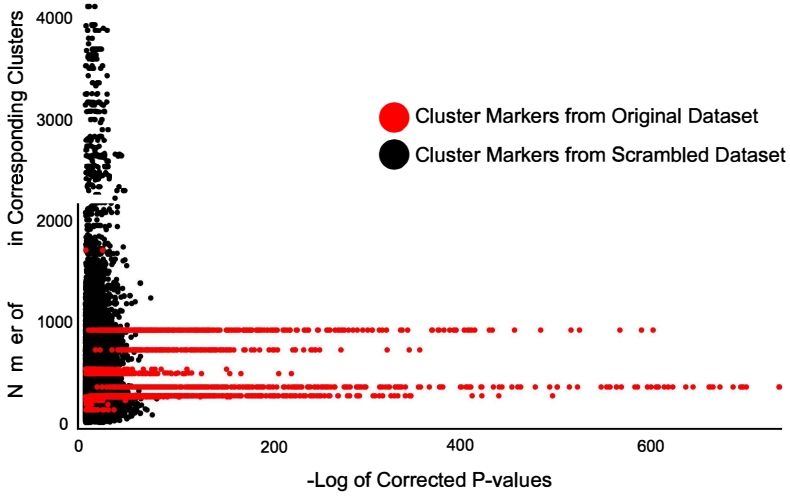
A) tSNE Projection including Ribosomal & Mitochondrial Clusters



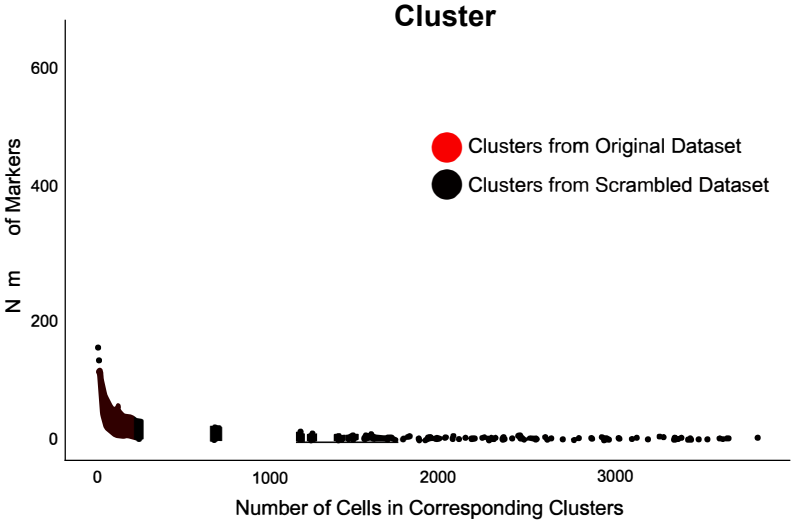
B) Cell Cycle Annotations

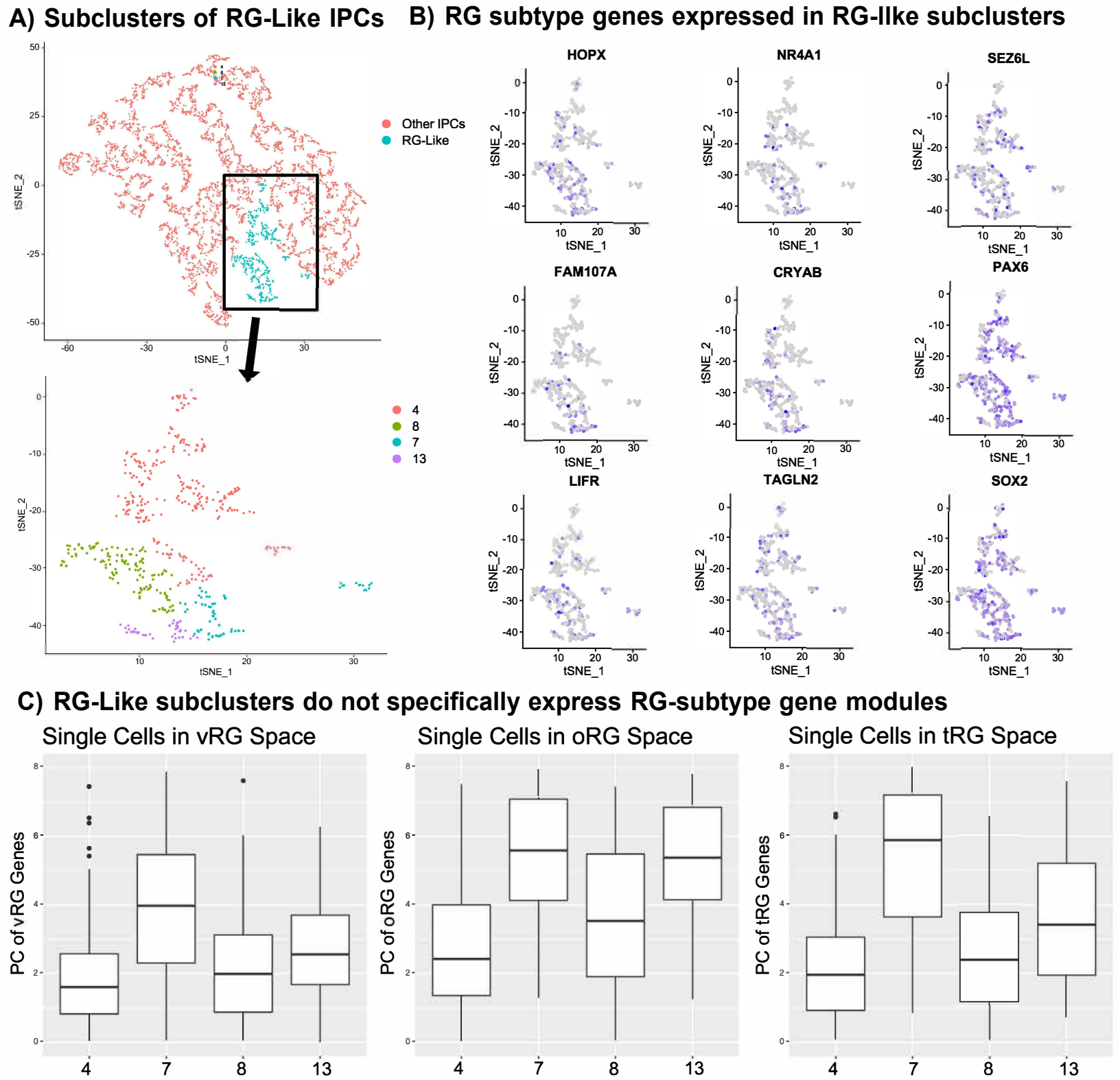


C) Significant Markers for Original and Scrambled Clusters

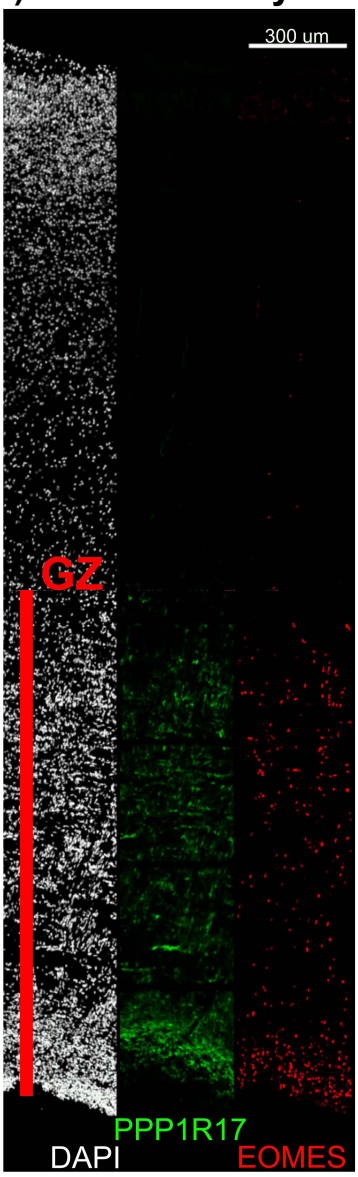


D) Distribution of Size and Number of Markers/ Cluster

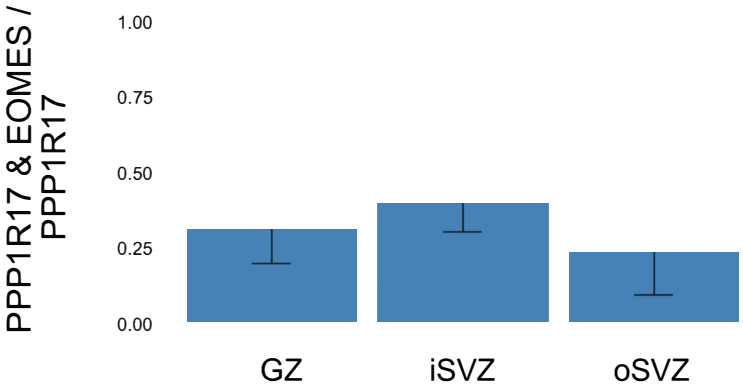



A) Subclusters of RG-Like IPCs
B) RG subtype genes expressed in RG-llke subclusters
Other IPCs
RG-Like
4
8
7
13
tSNE_1
tSNE_2
HOPX
NR4A1
SEZ6L
FAM107A
CRYAB
PAX6
LIFR
TAGLN2
SOX2
C) RG-Like subclusters do not specifically express RG-subtype gene modules
Single Cells in vRG Space
Single Cells in oRG Space
Single Cells in tRG Space
PC of vRG Genes
PC of oRG Genes
PC of tRG Genes
RG-like IPC Subclusters

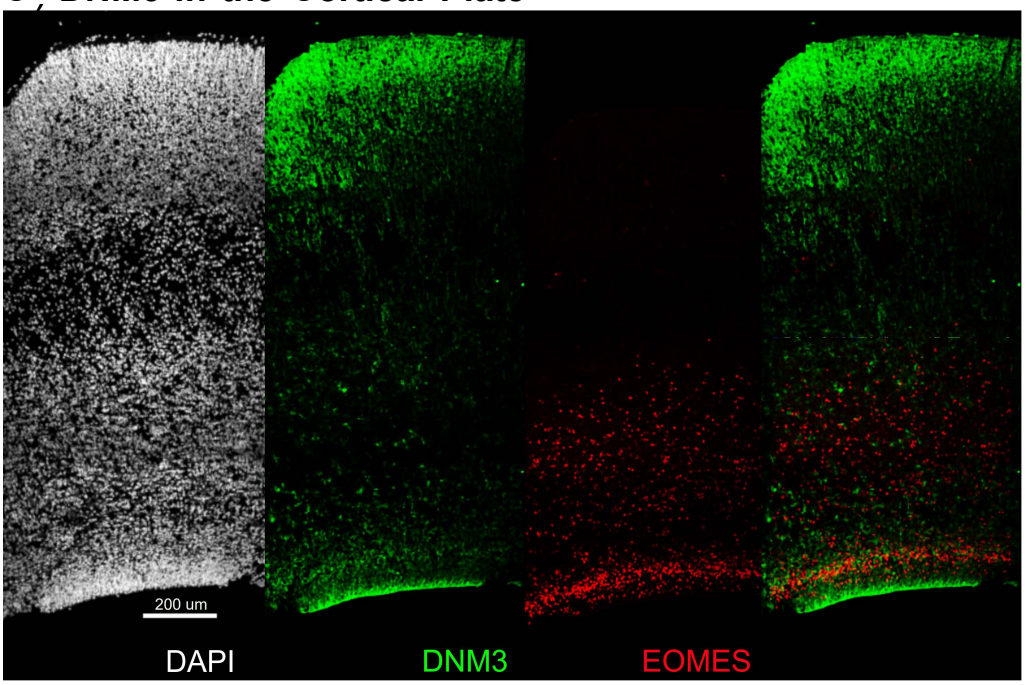
A) PPP1R17 only in GZ



B) Most PPP1R17+ cells are not EOMES+



C, DNM3 in the Cortical Plate



D) PPP1R17+/EOMES- Cells Express TBR1