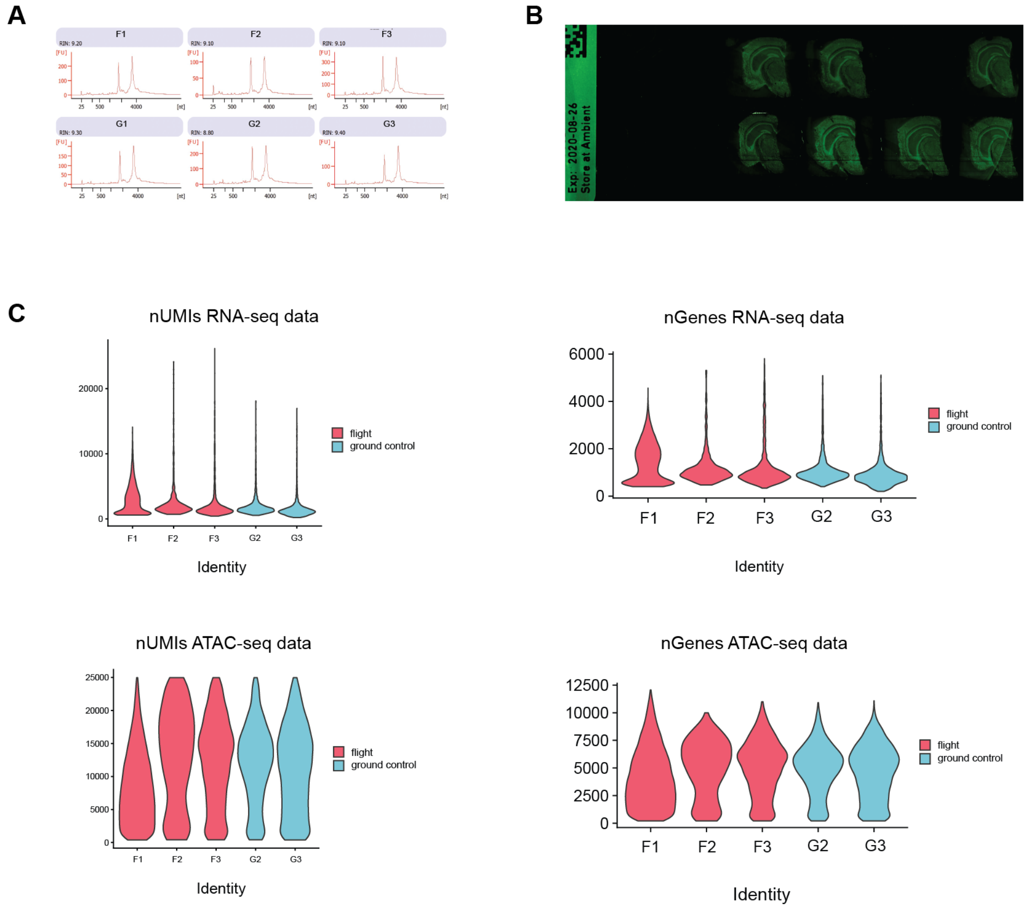
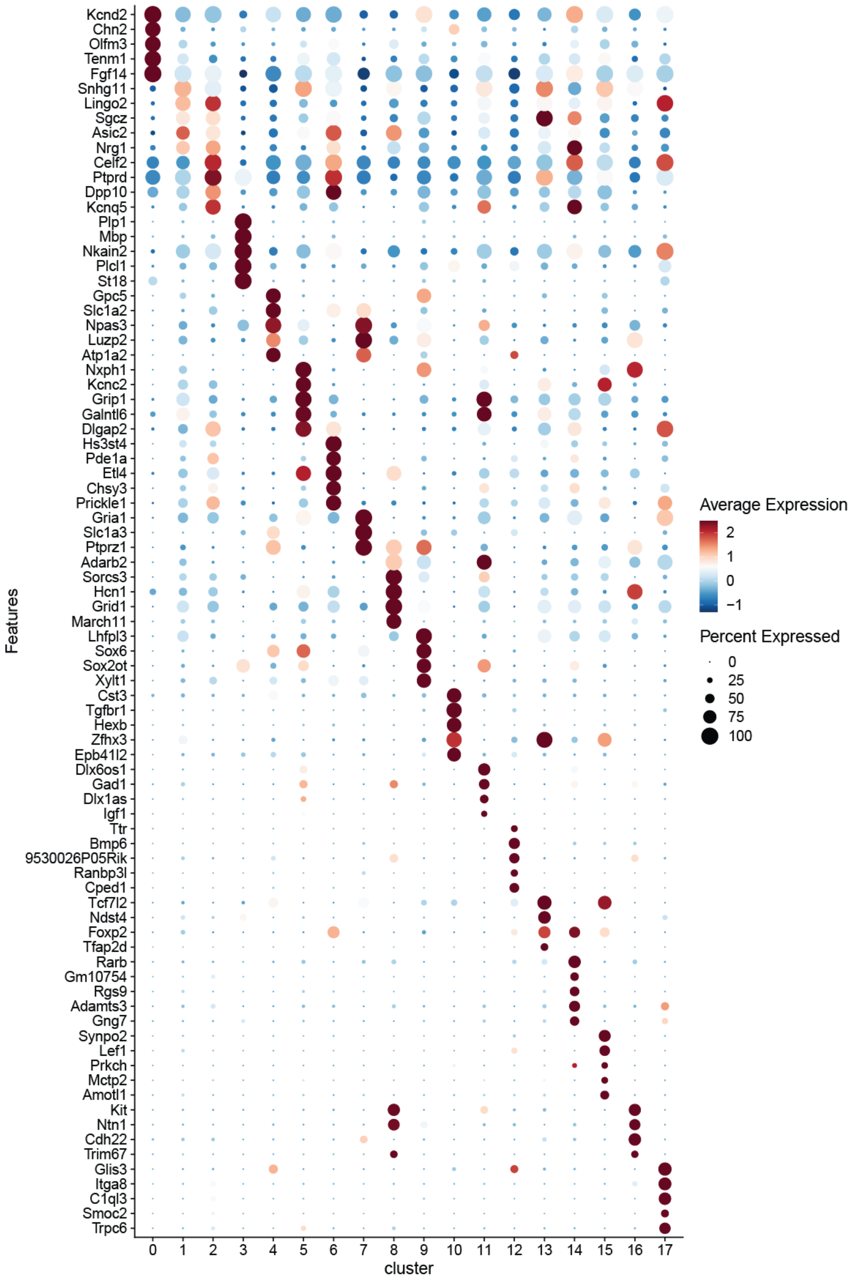
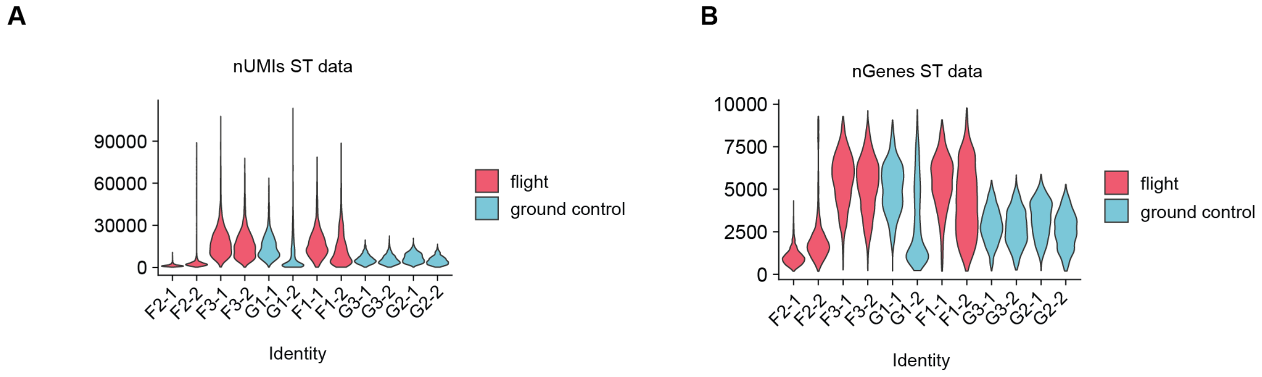
Supplementary Information



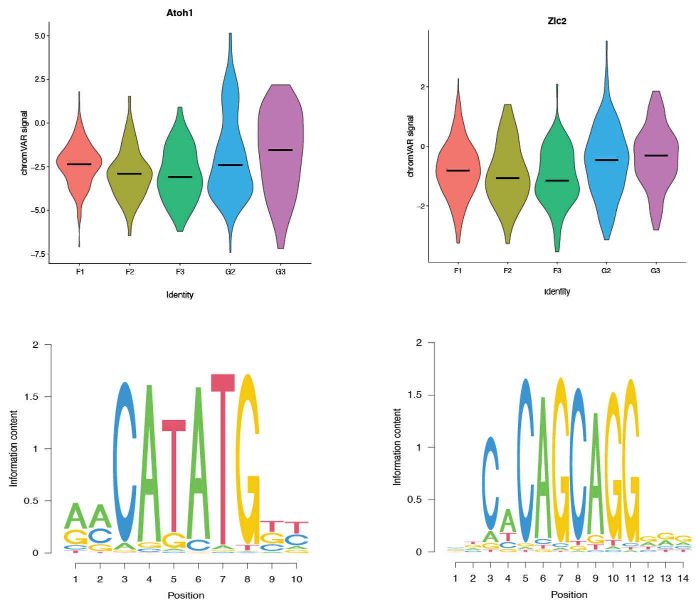
Supplementary Figure 1: A, Bioanalyzer traces showing RIN values for all brain samples. **B**, Fluorescent footprints of brain tissue section obtained from Visium Tissue Optimization protocol by 10x Genomics (for detailed protocol see: UserGuide_RevB, CG000238), each section footprint represents different times tested during permeabilization step, empty position represents negative control, section untreated with permeabilization mix. **C**, Distributions of unique molecules/genes per nucleus per sample in single nuclei multiomics dataset (RNA-seq, ATAC-seq) visualized as violin plots colored by sample condition.



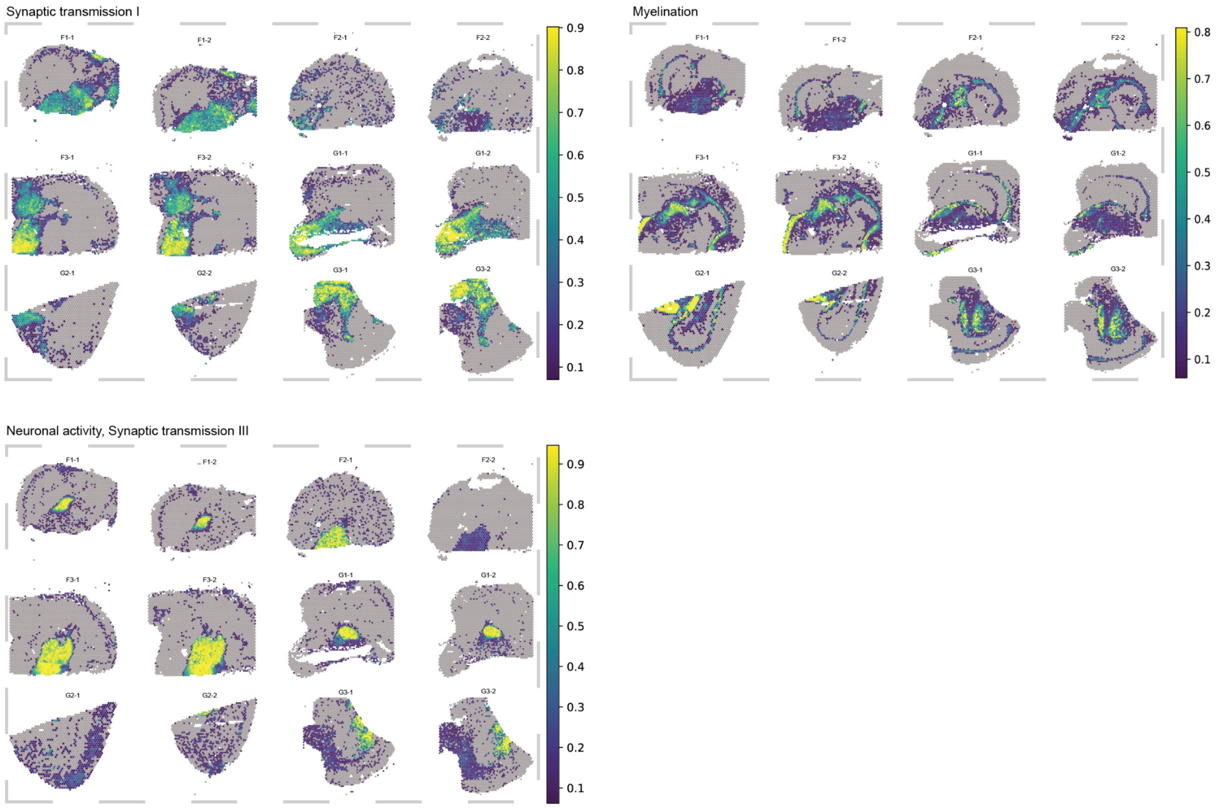
Supplementary Figure 2: Marker genes for each multiomics cluster visualized as Dot Plot.



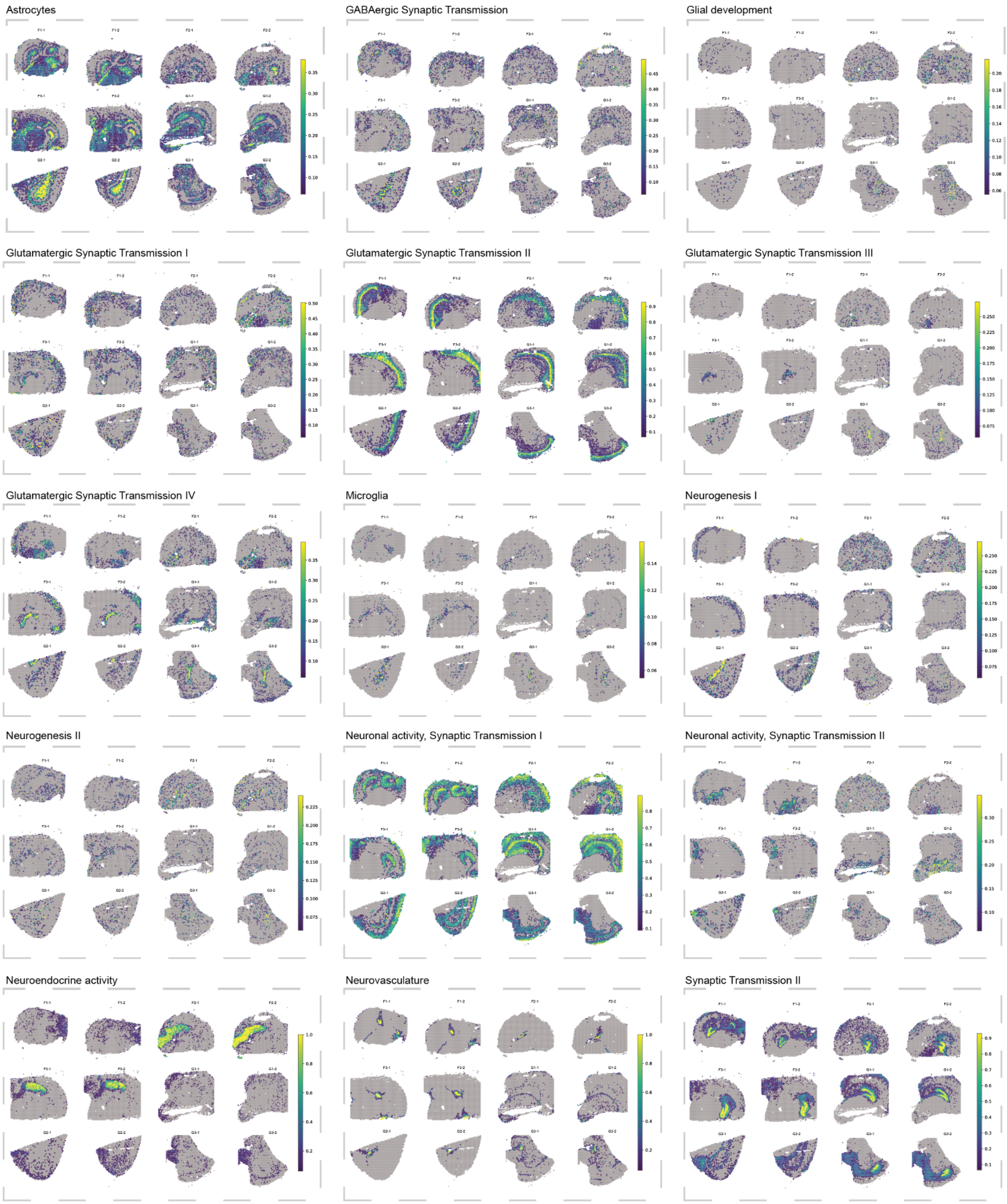
Supplementary Figure 3: A, Distributions of unique molecules per spot per tissue section in ST datasets visualized as violin plots colored by sample condition. **B,** Distributions of genes per spot per tissue section in ST datasets visualized as violin plots colored by sample condition.



Supplementary Figure 4: Motif analysis (Atoh1 and Zic2 in cluster 4).



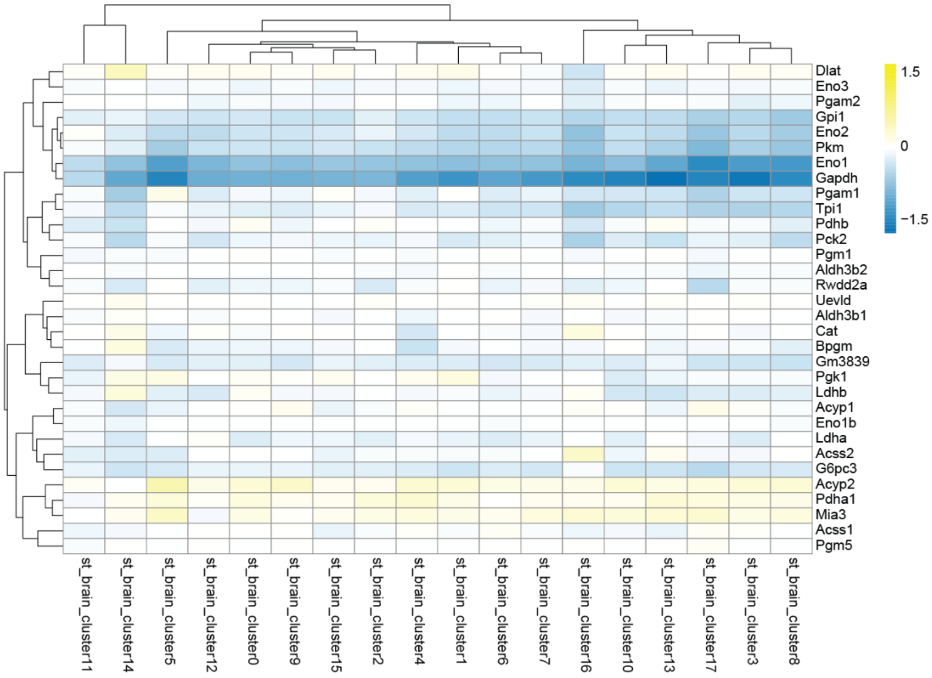
Supplementary Figure 5: Spatial localization of multiomics clusters 1, 3 and 15 (Synaptic Transmission I, Myelination and Neuronal activity, Synaptic Transmission III) visualized on all brain sections.



Supplementary Figure 6: Spatial localization of the other multiomics clusters visualized on all brain sections.

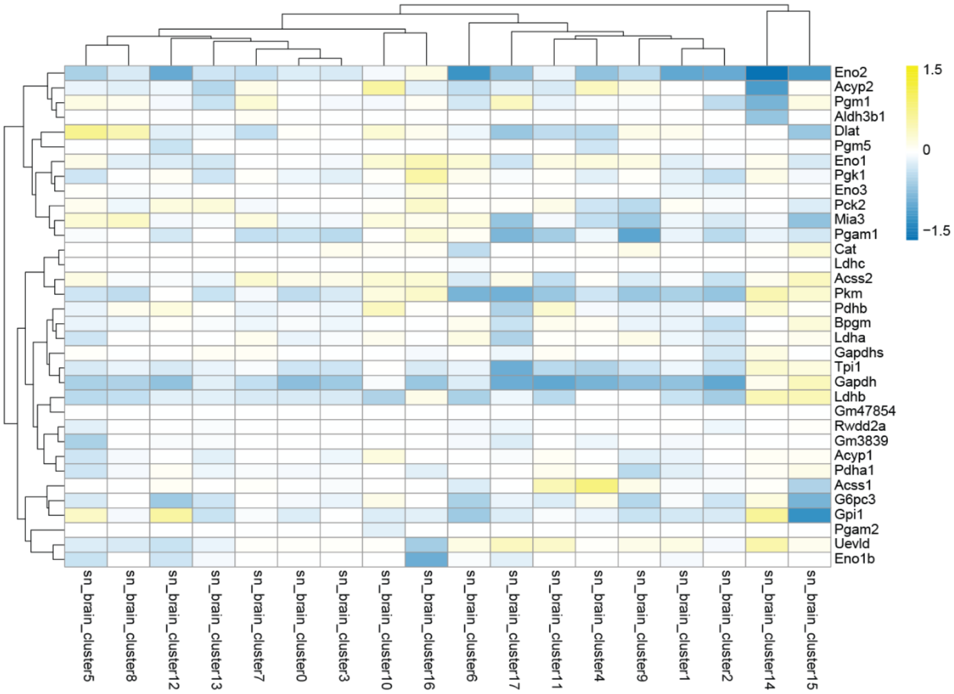
Glycolysis_gluconeogenesis - ST

Heatmap of log2 FC flight vs ground



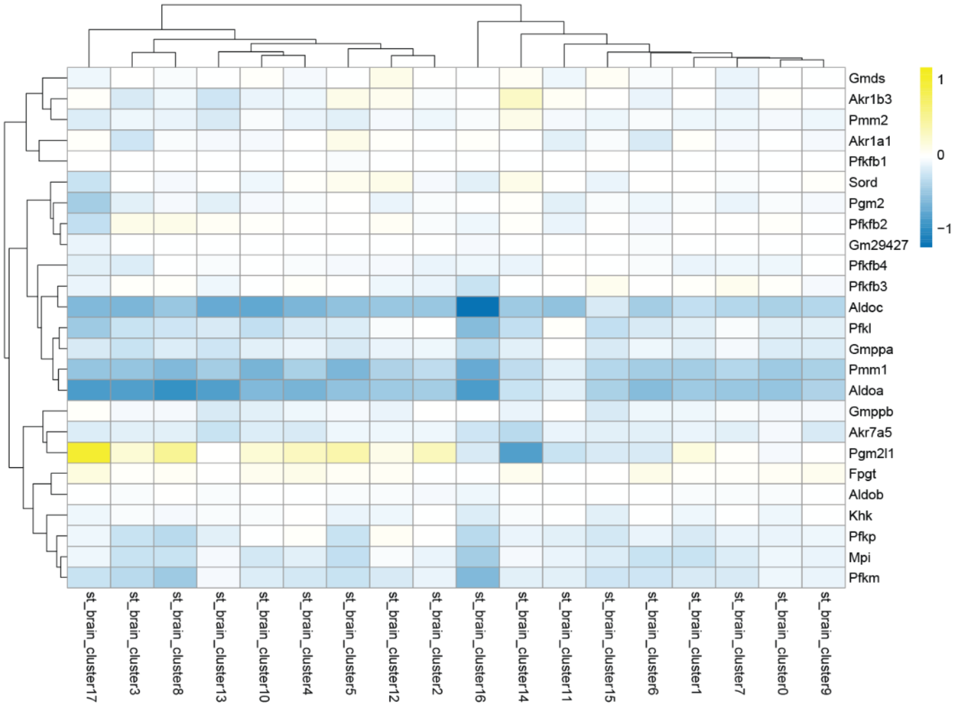
Glycolysis_gluconeogenesis - multiomics

Heatmap of log2 FC flight vs ground

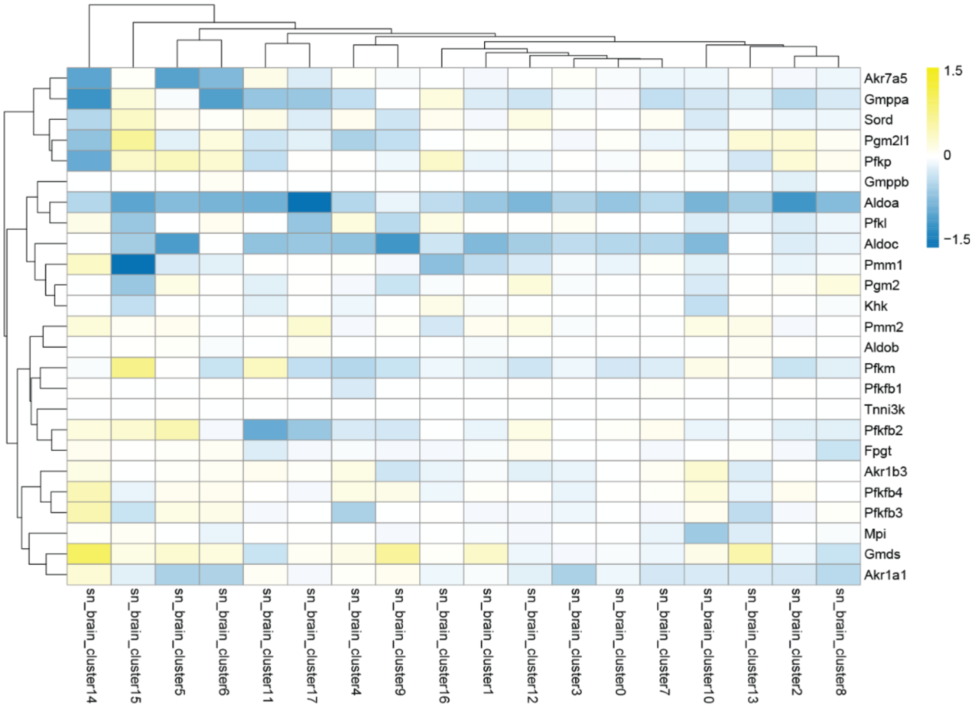


Supplementary Figure 7: Heatmap showing log2 FC between flight and ground control samples in glycolysis and gluconeogenesis pathways in both ST and multiomics datasets.

Fructose and mannose metabolism - ST
Heatmap of log2 FC flight vs ground



Fructose and mannose metabolism - multiomics
Heatmap of log2 FC flight vs ground



Supplementary Figure 8: Heatmap showing log2 FC between flight and ground control samples in fructose and mannose metabolism pathway in both ST and multiomics datasets.

Supplementary Table 1: Multiomics cluster marker genes. Marker genes identified for each of the 18 clusters identified in the multiomics clusters of RR3 brain data.

Supplementary Table 2: Multiomics clusters annotations. Functional annotations of the 18 (0-17) multiomics clusters identified in the multiomics brain dataset according to functions. Cluster Annotation column denotes the shorter names for these cluster annotations.

Supplementary Table 3: Differentially expressed genes (DEGs) for multiomics clusters. DEGs identified for the 18 multiomics clusters in the spaceflight samples of RR3 brain data.

Supplementary Table 4: ST clusters annotation. Categorization of the 18 (0-17) ST clusters identified in the spatial dataset according to their spatial location and function.

Supplementary Table 5: ST cluster marker genes. Marker genes identified for the 18 spatial (ST) clusters in RR3 brain data.

Supplementary Table 6: DEGs for ST clusters. Differentially expressed genes for spaceflight samples found across the 18 ST RR3 brain clusters.

Supplementary Table 7: Deconvolution results using Stereoscope. ST clusters that matched with celltype proportions (multiomics clusters) found by deconvolution using Stereoscope.

Supplementary Table 8: Motif analysis. Top 10 motifs analysed for multiomics clusters using ATACseq peaks.

Supplementary Table 9: Metabolic analysis results for ST dataset. FGSEA results with adjusted p-values for spatial (ST) RR3 brain clusters.

Supplementary Table 10: Metabolic analysis results for multiomics dataset. FGSEA results with adjusted p-values for multiomics (snRNA-seq and snATAC-seq combined) RR3 brain clusters.