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Supplemental information

Cell type characterization of spatiotemporal gene co-expression modules in Down syndrome brain

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Supplementary figures

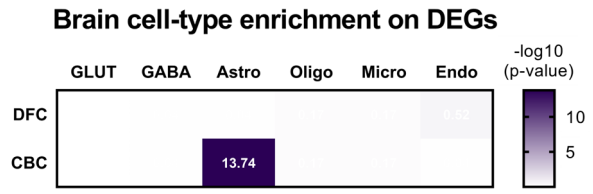

Figure S1. Cell-type enrichment analysis on DEGs, related to Figure 1 and Results
Cell-type enrichment analysis with cell type-enriched genes of brain cell types was conducted on the DEGs of the DFC and CBC. The -log10 (Benjamini–Hochberg-adjusted (BHA) *P*-value) values are shown in the enriched cell types (Fisher's exact test). DEGs in the CBC were significantly enriched with astrocyte-enriched genes. Conversely, DEGs in the DFC were not significantly enriched with any cell-type gene but were most enriched with endothelial cell-enriched genes.

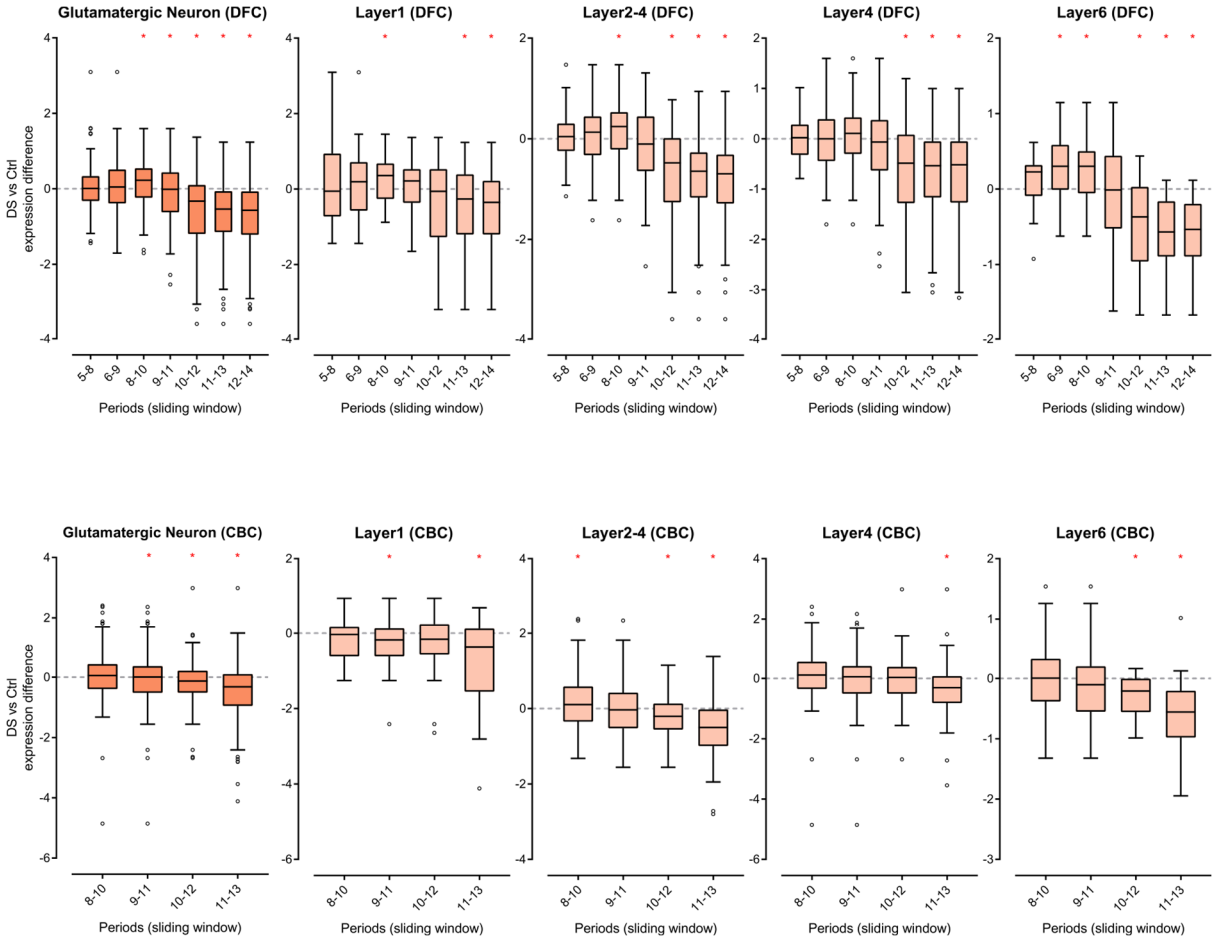

Figure S2. Differential expression patterns of genes related to glutamatergic neuron subtypes, related to Figure 1

Differential expression patterns of genes related to each glutamatergic neuron subtype between the DS and control are displayed on a log2 scale across brain regions and developmental stages. A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. Genes enriched in glutamatergic neuron subtypes showed similar differential expression patterns to whole glutamatergic neuronal genes. *, $P < 0.05$ (paired *t*-test).

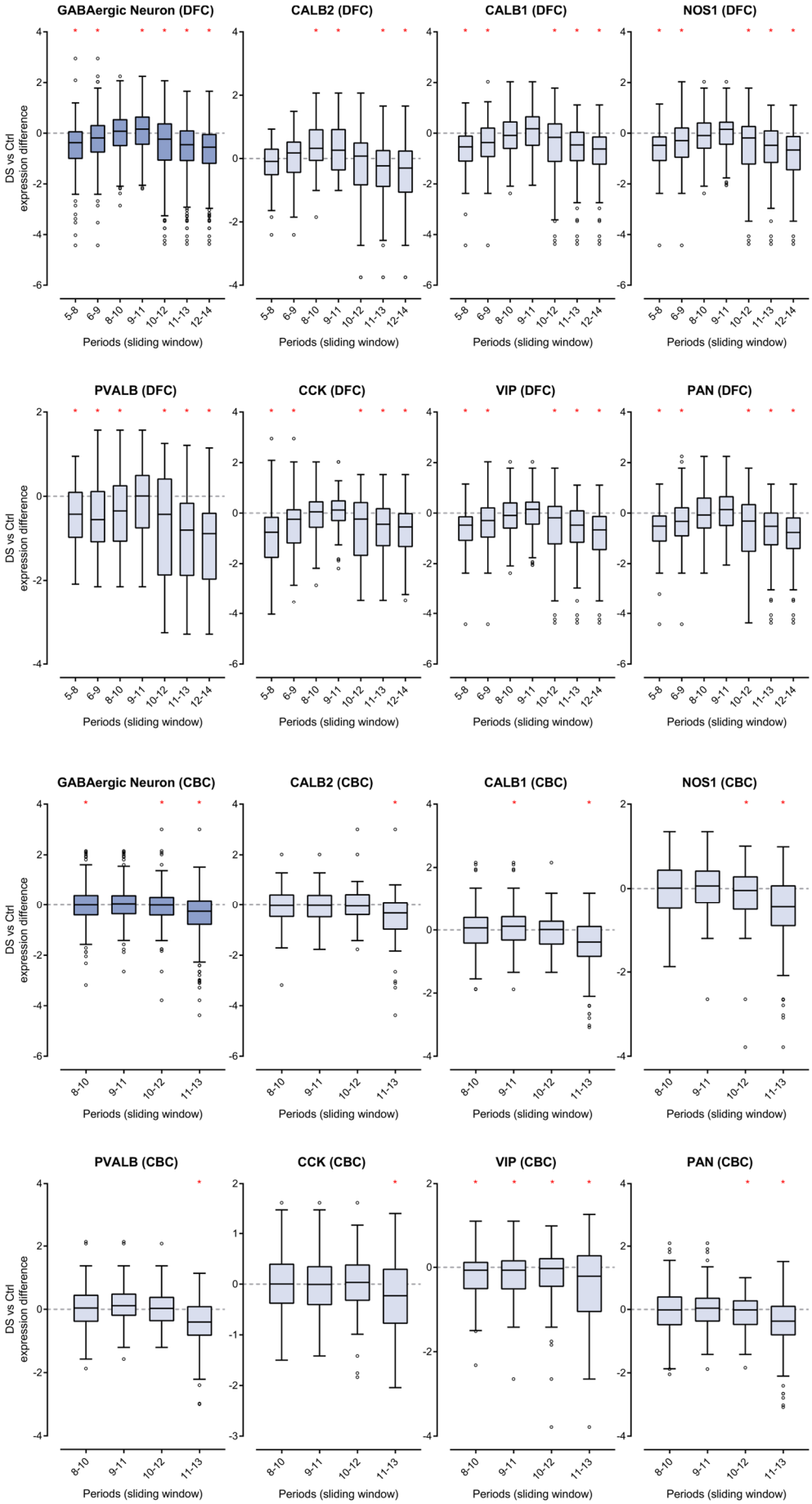
GABAergic Neuron (DFC)
CALB2 (DFC)
CALB1 (DFC)
NOS1 (DFC)
PVALB (DFC)
CCK (DFC)
VIP (DFC)
PAN (DFC)
GABAergic Neuron (CBC)
CALB2 (CBC)
CALB1 (CBC)
NOS1 (CBC)
PVALB (CBC)
CCK (CBC)
VIP (CBC)
PAN (CBC)
DS vs Ctrl expression difference
Periods (sliding window)
5-8
6-9
8-10
9-11
10-12
11-13
12-14

Figure S3. Differential expression patterns of genes related to GABAergic neuron subtypes, related to Figure 1

Differential expression patterns of genes related to each GABAergic neuron subtype between the DS and control are displayed on a log2 scale across brain regions and developmental stages. A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. Genes enriched in GABAergic neuron subtypes showed similar differential expression patterns to whole GABAergic neuronal genes. *, $P < 0.05$ (paired *t*-test).

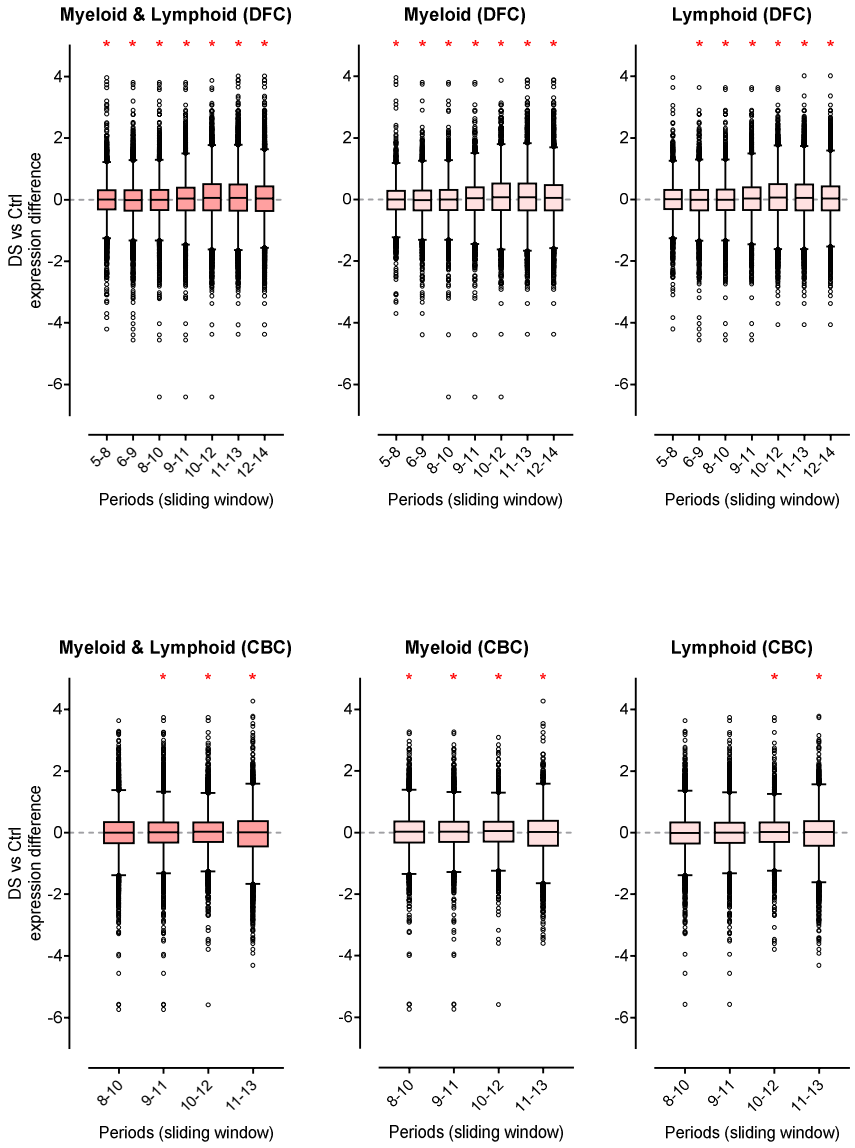

Figure S4. Differential expression patterns of immune cell type-related genes, related to Figure 1 and Discussion

Differential expression patterns of myeloid and lymphoid genes between DS and control are displayed on a log2 scale across brain regions and developmental stages. A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. Gene expression patterns were similar to those of microglia and endothelial cells; however, the expression differences between myeloid and lymphoid genes were smaller than those between microglia and endothelial cells. *, $P < 0.05$ (paired *t*-test).

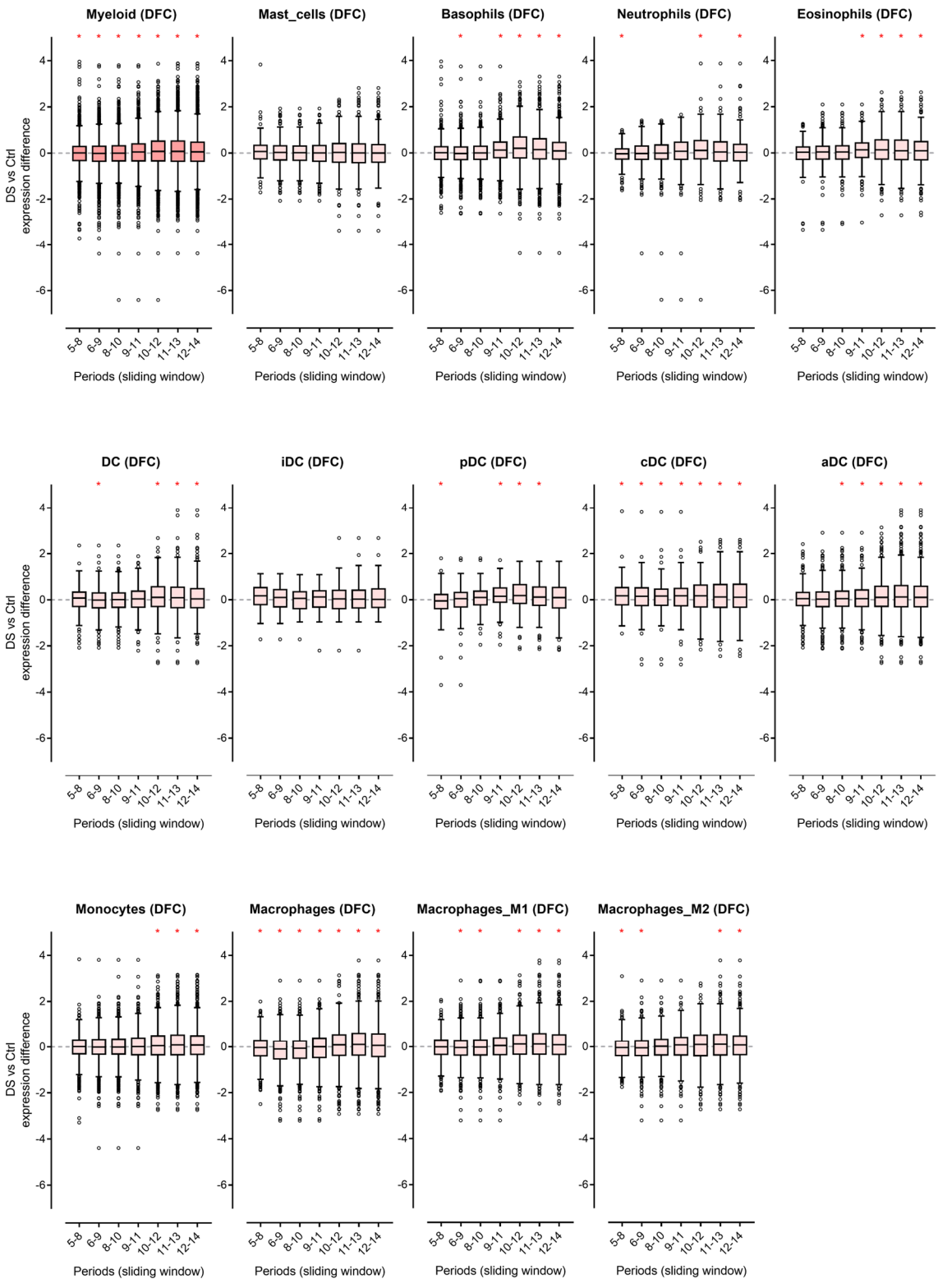
Myeloid (DFC)
Mast_cells (DFC)
Basophils (DFC)
Neutrophils (DFC)
Eosinophils (DFC)
DC (DFC)
iDC (DFC)
pDC (DFC)
cDC (DFC)
aDC (DFC)
Monocytes (DFC)
Macrophages (DFC)
Macrophages_M1 (DFC)
Macrophages_M2 (DFC)
DS vs Ctrl expression difference
4
2
0
-2
-4
-6
5-8
6-9
8-10
9-11
10-12
11-13
12-14
Periods (sliding window)

Figure S5. Differential expression patterns of myeloid cell-related genes in the DFC, related to Figure 1 and Discussion

Differential expression patterns of myeloid cell genes between DS and control in the DFC are displayed on a log2 scale across developmental stages. A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. Genes tended to be less expressed in DS than in control during early stages and more expressed in DS than in control during late stages. Dendritic cell genes were highly expressed in DS. *, $P < 0.05$ (paired *t*-test).

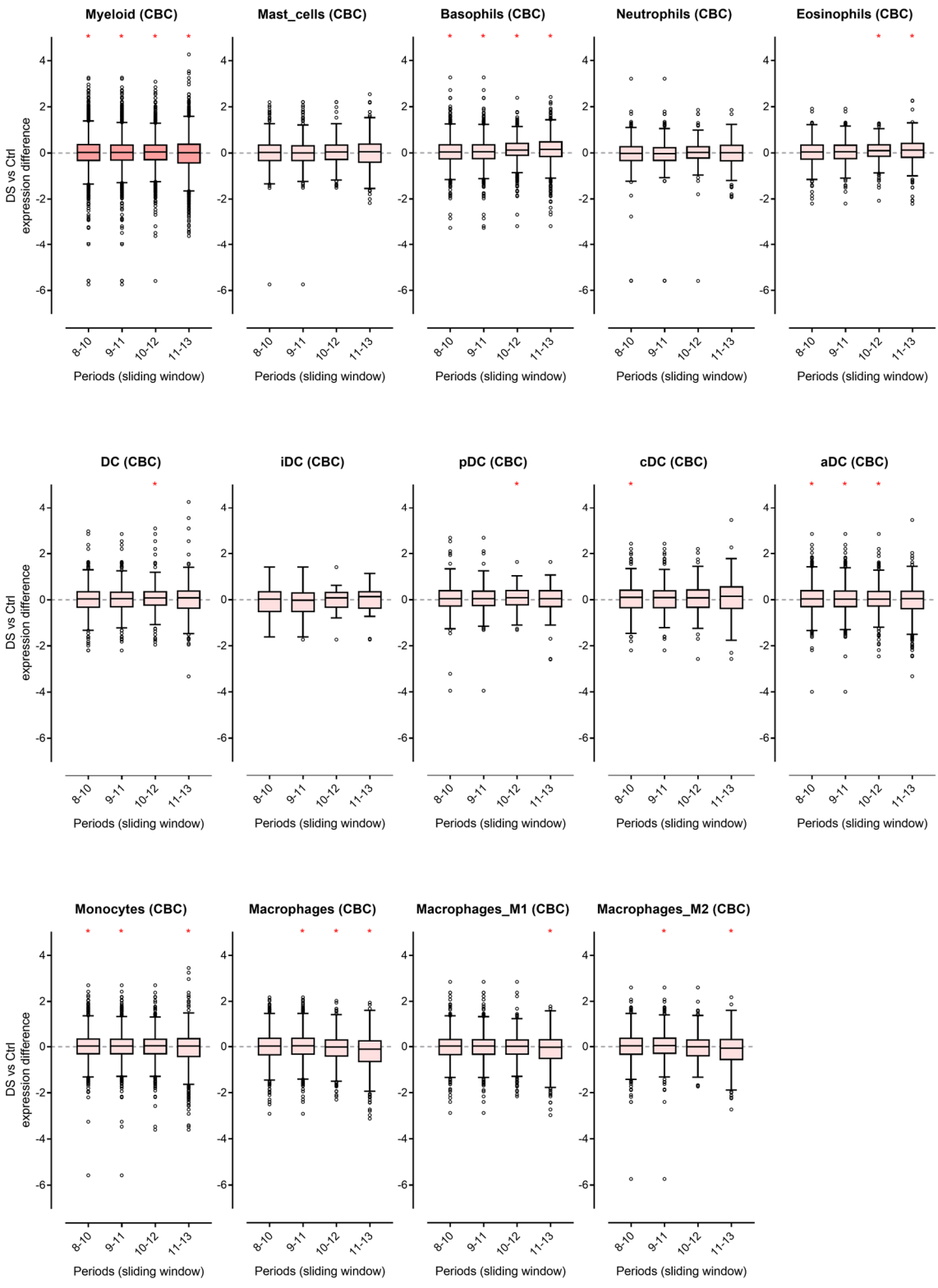
Myeloid (CBC)
Mast_cells (CBC)
Basophils (CBC)
Neutrophils (CBC)
Eosinophils (CBC)
DC (CBC)
iDC (CBC)
pDC (CBC)
cDC (CBC)
aDC (CBC)
Monocytes (CBC)
Macrophages (CBC)
Macrophages_M1 (CBC)
Macrophages_M2 (CBC)
DS vs Ctrl expression difference
Periods (sliding window)
8-10
9-11
10-12
11-13

Figure S6. Differential expression patterns of myeloid cell-related genes in the CBC, related to Figure 1 and Discussion

Differential expression patterns of myeloid cell genes between DS and control in the CBC are displayed on a log2 scale across developmental stages. A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. Genes tended to be more expressed in DS than in control during former stages and less expressed in DS than in control during the last sliding window. Basophil genes were highly expressed in DS. Macrophage genes were more downregulated in DS than in control during the last sliding window. *, $P < 0.05$ (paired *t*-test).

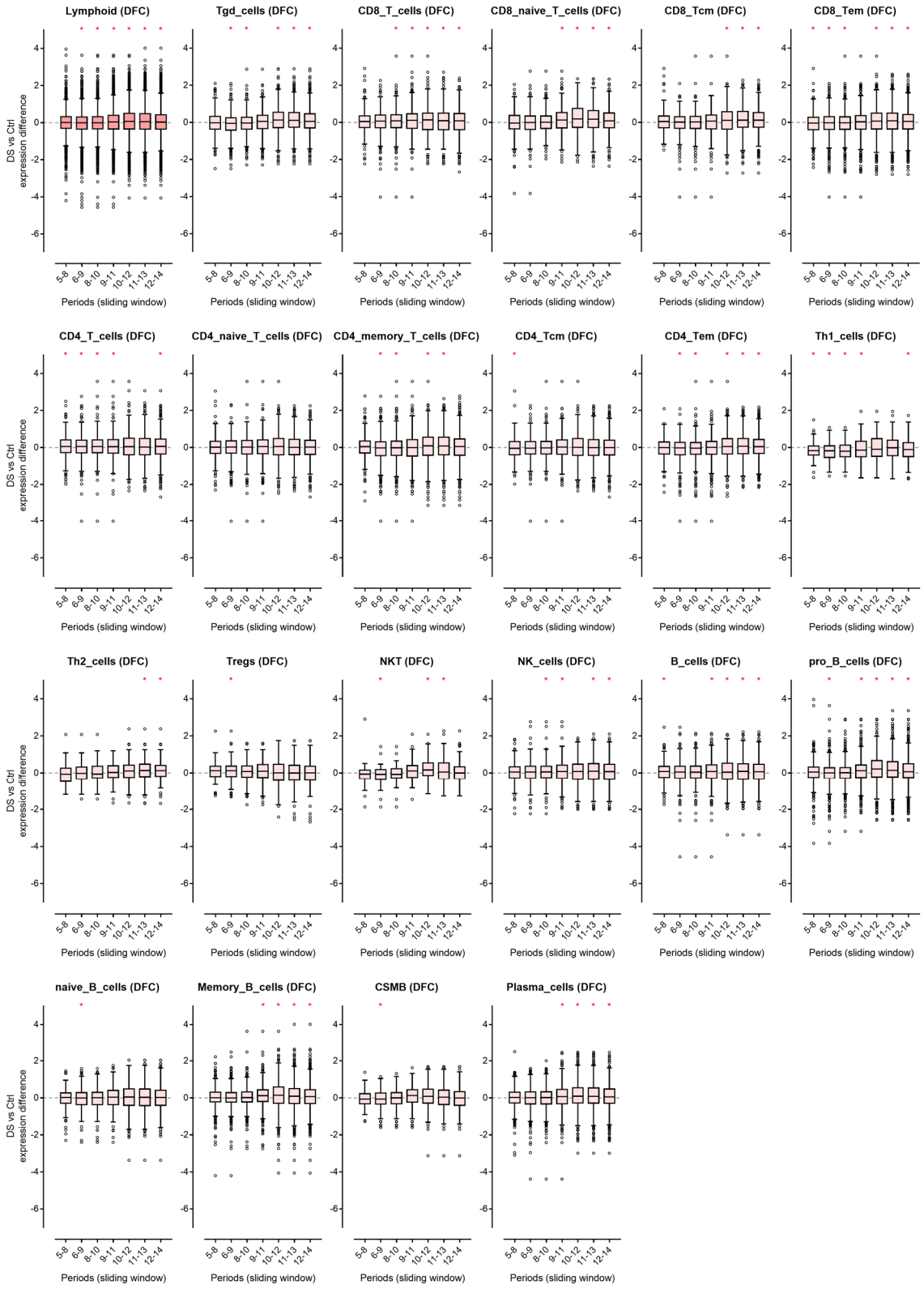
Lymphoid (DFC)
Tgd_cells (DFC)
CD8_T_cells (DFC)
CD8_naive_T_cells (DFC)
CD8_Tcm (DFC)
CD8_Tem (DFC)
CD4_T_cells (DFC)
CD4_naive_T_cells (DFC)
CD4_memory_T_cells (DFC)
CD4_Tcm (DFC)
CD4_Tem (DFC)
Th1_cells (DFC)
Th2_cells (DFC)
Tregs (DFC)
NKT (DFC)
NK_cells (DFC)
B_cells (DFC)
pro_B_cells (DFC)
naive_B_cells (DFC)
Memory_B_cells (DFC)
CSMB (DFC)
Plasma_cells (DFC)
DS vs Ctrl expression difference
Periods (sliding window)
5-8
6-9
8-10
9-11
10-12
11-13
12-14

Figure S7. Differential expression patterns of lymphoid cell-related genes in the DFC, related to Figure 1 and Discussion

Differential expression patterns of lymphoid cell genes between DS and control in DFC are displayed on a log2 scale across developmental stages. A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. Genes tended to be less expressed in DS than in control during early stages and more expressed in DS than in control during late stages. Type 1 helper T cell (Th1) genes were highly downregulated in DS. *, $P < 0.05$ (paired *t*-test).

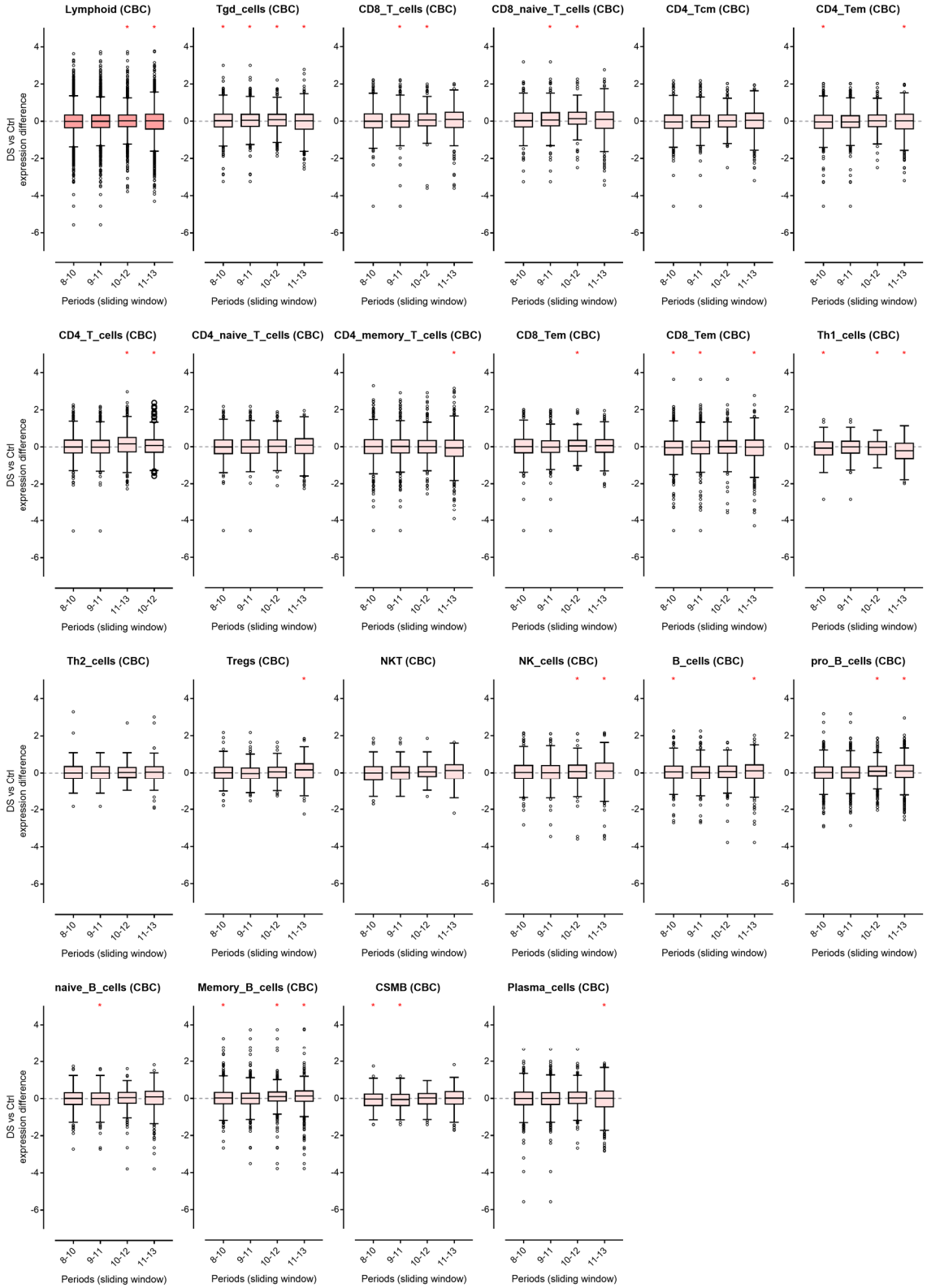
Lymphoid (CBC)
Tgd_cells (CBC)
CD8_T_cells (CBC)
CD8_naive_T_cells (CBC)
CD4_Tcm (CBC)
CD4_Tem (CBC)
CD4_T_cells (CBC)
CD4_naive_T_cells (CBC)
CD4_memory_T_cells (CBC)
CD8_Tem (CBC)
CD8_Tem (CBC)
Th1_cells (CBC)
Th2_cells (CBC)
Tregs (CBC)
NKT (CBC)
NK_cells (CBC)
B_cells (CBC)
pro_B_cells (CBC)
naive_B_cells (CBC)
Memory_B_cells (CBC)
CSMB (CBC)
Plasma_cells (CBC)
DS vs Ctrl expression difference
Periods (sliding window)
8-10
9-11
10-12
11-13

Figure S8. Differential expression patterns of lymphoid cell-related genes in the CBC, related to Figure 1 and Discussion

Differential expression patterns of lymphoid cell genes between DS and control in the CBC are displayed on a log2 scale across developmental stages. A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. Genes tended to be more expressed in DS than in control during former stages and less expressed in DS than in control during the last sliding window. Basophil genes were highly expressed in DS. Th1 genes were the most downregulated genes in DS. *, $P < 0.05$ (paired *t*-test).

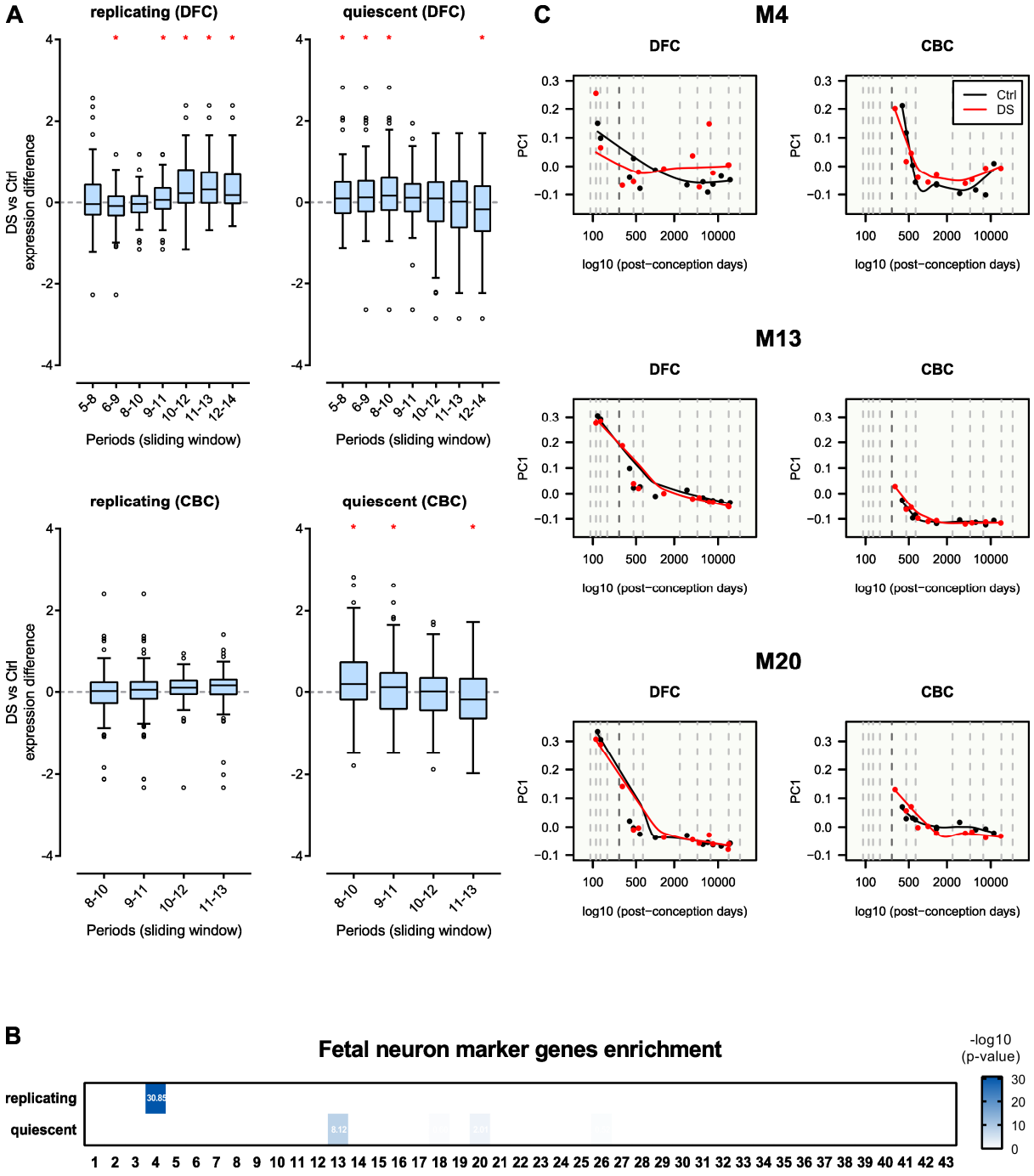

Figure S9. Cell-type analysis with fetal neuron genes, related to Figures 1-6 and Discussion

Differential expression patterns of replicating and quiescent fetal neuron genes between DS and control are displayed on a log2 scale across developmental stages (**A**). A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. *, $P < 0.05$ (paired *t*-test). Cell-type enrichment analysis with replicating and quiescent fetal neuron genes (**B**) was conducted on 43 co-expressed gene modules constructed by WGCNA. The -log10 (*P*-value) value is shown (BHA $P < 0.05$, Fisher's exact test). Gene expression patterns of fetal neuron modules (M4, M13, and M20) are visualized in a line graph of module eigengenes (**C**).

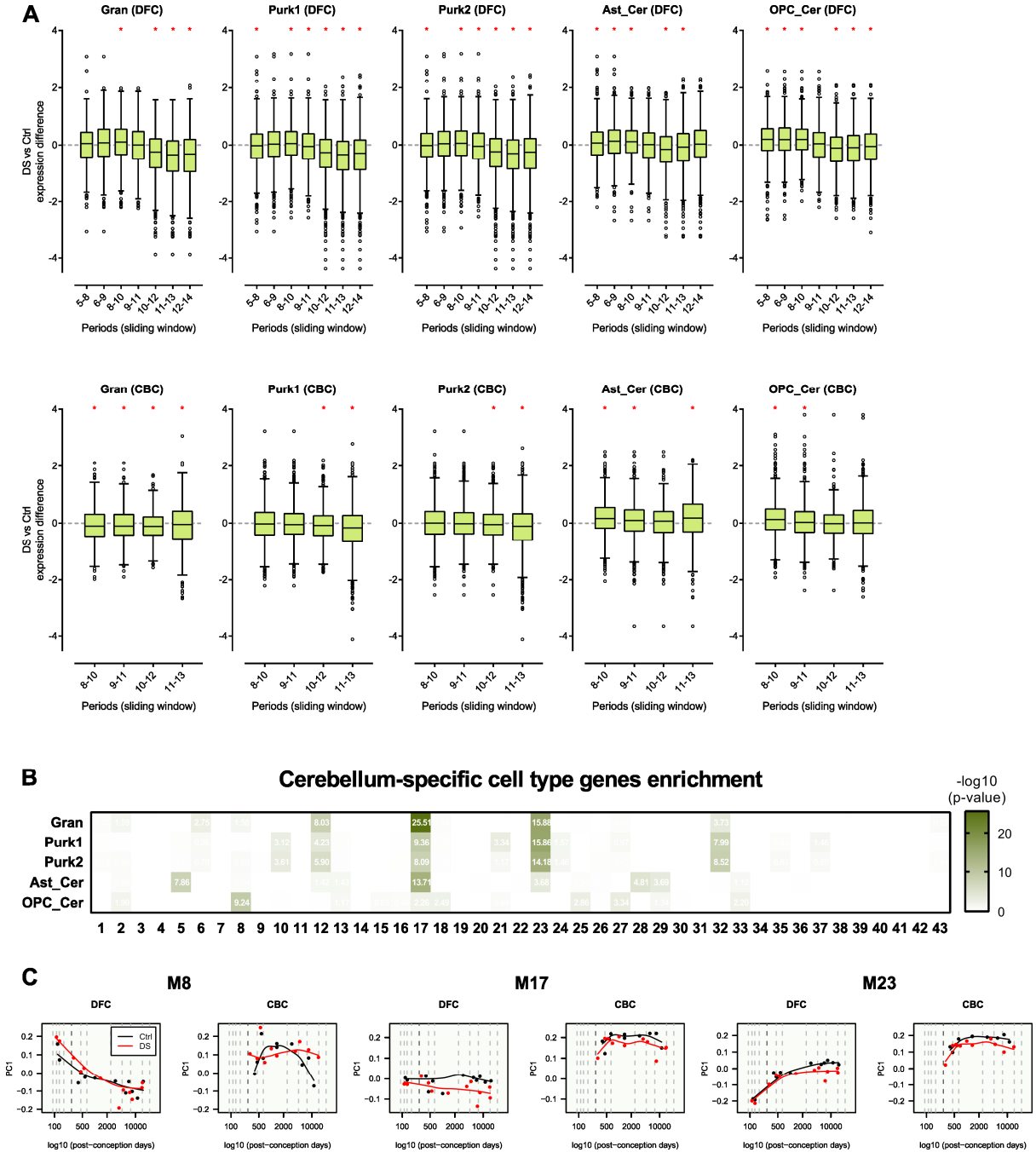

Figure S10. Cell-type analysis with cerebellum-specific cell-type genes, related to Figures 1-6 and Discussion

Differential expression patterns of cerebellum-specific cell-type genes between DS and control are displayed on a log2 scale across developmental stages (**A**). A sliding window approach was used to investigate the temporal expression pattern. Human brain developmental periods are described in Table S1. *, $P < 0.05$ (paired *t*-test). Cell-type enrichment analysis with cerebellum-specific cell-type genes (**B**) was conducted on 43 co-expressed gene modules constructed by WGCNA. The -log10 (*P*-value) value is shown (BHA $P < 0.05$, Fisher's exact test). Gene expression patterns of cerebellum-specific cell-type modules (M8, M17, and M23) are visualized in a line graph of module eigengenes (**C**).

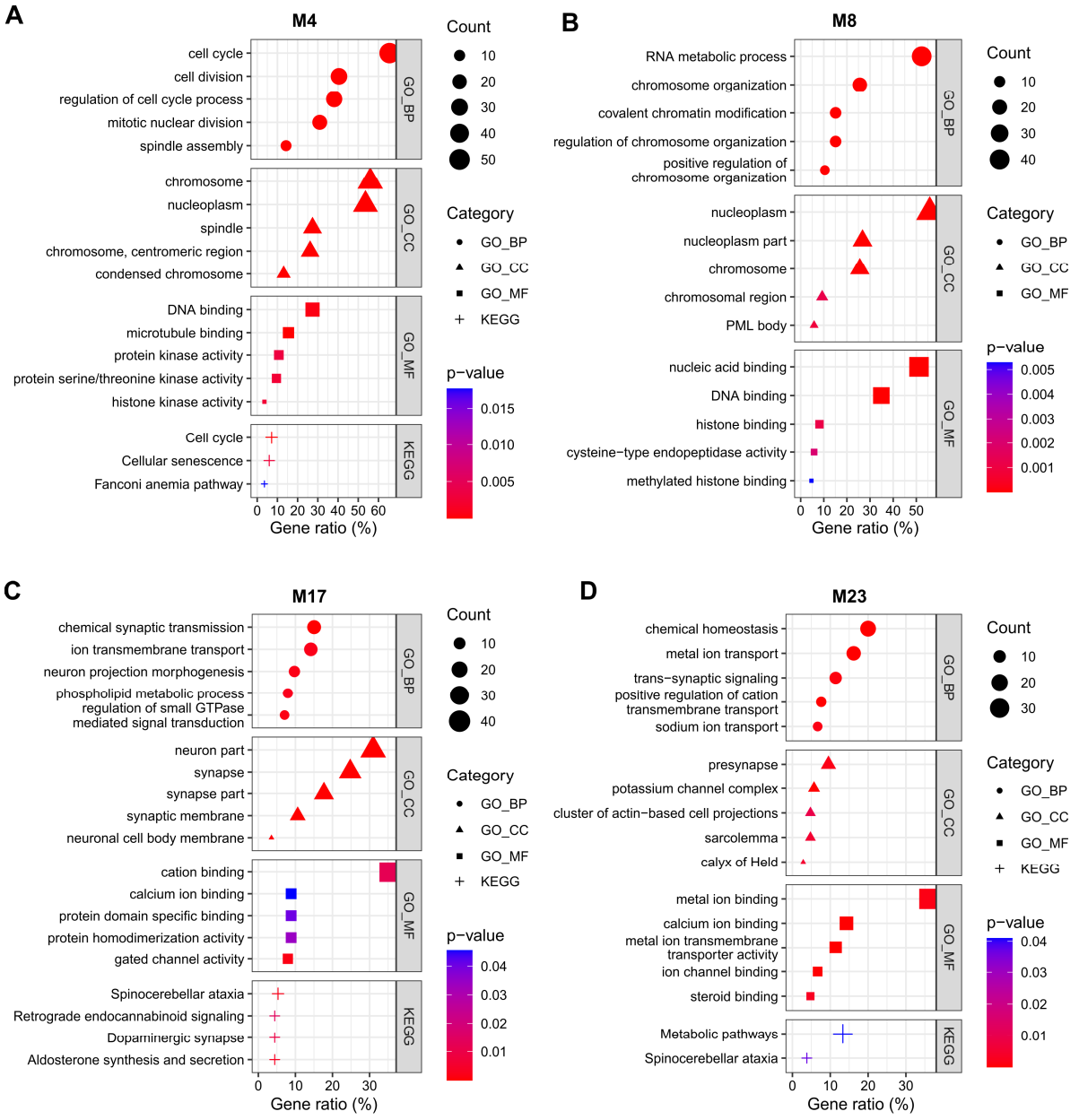

Figure S11. Functional annotation of fetal neuron and cerebellum-specific cell type-related modules, related to Figures 7, 8, and Discussion

Functional enrichment analysis with genes related to GO terms and KEGG pathways on the fetal neuron (**A**) and cerebellum-specific cell-type (**B-D**) modules. The five most significant terms ($P < 0.05$) are shown in each category. GO: gene ontology, BP: biological process, CC: cellular component, MF: molecular function, KEGG: Kyoto Encyclopedia of Genes and Genomes pathway.

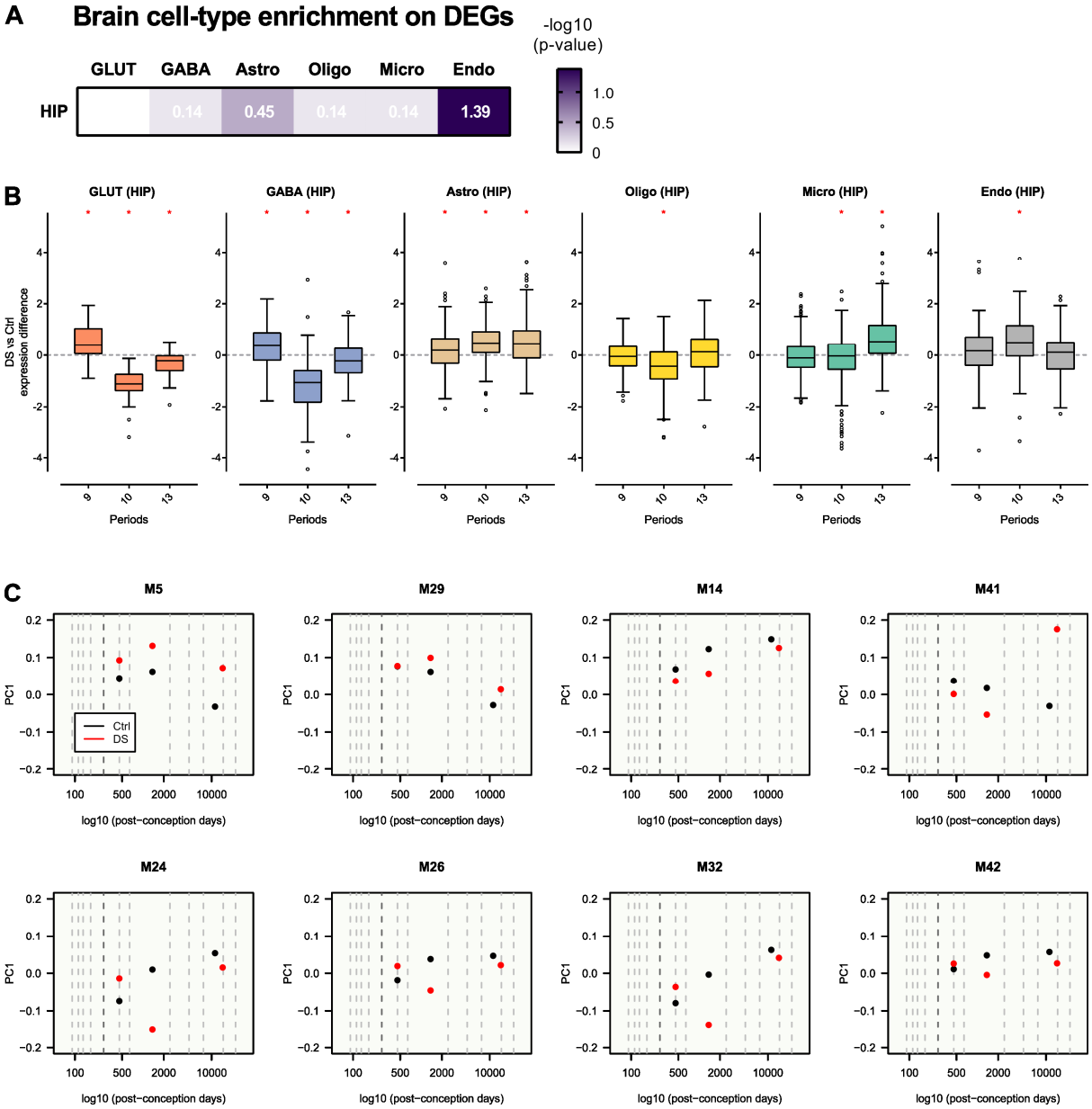

Figure S12. Cell type analysis using hippocampus data, related to Figures 1, 3-6, and Discussion

Cell-type enrichment analysis with cell type-enriched genes of brain cell types was conducted on the DEGs of the hippocampus (**A**). The -log10 (BHA *P*-value) value is shown in the enriched cell types (Fisher's exact test). Differential expression patterns of each cell type-related gene between DS and control are displayed on a log2 scale across brain regions and developmental stages (**B**). Human brain developmental periods are described in Table S1. *, $P < 0.05$ (paired *t*-test). Gene expression patterns of cell-type modules are visualized in a dot plot of module eigengenes (**C**).