

Supplementary information

Supplementary figures

Supplementary figure legend

Figure S1 Characterization of hCOs and hBVOs induction from hESCs, related to Figure 1.

- (A) Schematic diagram illustrating the differentiation process of hESCs into hCOs.
- (B) Representative bright-field images of hCOs at distinct developmental stages, highlighting morphological maturation over time. Scale bars: 200 μ m.
- (C) Quantitative analysis of hCO diameter across different time points. Data are presented as mean $\pm$ SEM (n = 6). Statistical significance between groups was determined by one-way ANOVA test (*p < 0.05, **p < 0.01, ***p < 0.001).
- (D) Schematic diagram depicting the stepwise differentiation protocol of hESCs into hBVOs.
- (E) Bright-field images showing morphological progression of BVOs at key developmental time points, demonstrating structural maturation. Scale bars: 200 μ m.
- (F) Quantitative measurement of organoid diameter across sequential culture stages. Data are presented as mean $\pm$ SD (n = 5 organoids per group). Statistical significance was analyzed by one-way ANOVA test (*p < 0.05, **p < 0.01, ***p < 0.001).

Figure S2. Characterization of single-cell transcriptome in vhCOs, related to Figure 3.

- (A-C) Violin plots displaying the distributions of (A) total read counts, (B) detected genes per cell (C) mitochondrial gene content across each sample after quality control.
- (D-H) Transcriptional analyses of normoxic, hypoxic and reoxygenated vhCOs, mapping onto the developing and adult human brain (age 4 pcw to >60 years) using Spearman's rank correlation coefficient. Related to Figure 3D. pcw, post-conceptual weeks; mos, months; yrs, years. CGE, caudal ganglionic eminence; MGE, medial ganglionic eminence; HIP, hippocampus; M1C-S1C, primary motor-sensory cortex (samples); MFC, anterior cingulate (medial prefrontal) cortex; Ocx, occipital neocortex; OFC, orbital frontal cortex; PCx, parietal neocortex; STC, posterior (caudal) superior temporal cortex; VFC, ventrolateral prefrontal cortex.

Figure S3. Cell type annotation of single-cell transcriptome in vhCOs, related to Figure 3.

- (A) UMAP visualization of the single-cell transcriptome of vhCOs (n=46,418 cells), colored by clusters. Related to Figure 3E-3F.
- (B) Violin plots displaying cell type-specific gene expression to each cell cluster. oRG, outer radial glia; vRG, ventricular radial glia, related to Figure 3G.

Figure S4. Feature plots showing cell-type-specific marker expression in vhCOs, related to Figure 3G.

Figure S5. Gene ontology enrichment analysis of DEGs across the 16 cell clusters in vhCOs, related to Figure 3H.

oRG, outer radial glia; vRG, ventricular radial glia.

Figure S6. Transcriptional analyses of each cell type in normoxic, hypoxic and reoxygenated vhCOs, mapping onto the developing and adult human brain, related to Figure 3D.

pcw, post-conceptual weeks; mos, months; yrs, years; oRG, outer radial glia; vRG, ventricular radial glia.

Figure S7. Transcriptional characteristics of each cell type in normoxic, hypoxic and reoxygenated vhCOs, related to Figure 5 and 6.

(A and B) The number of DEGs in indicated (A) neural-lineage and non-neural lineage (B) cell type of hypoxic and reoxygenated vhCOs compared to that of normoxic vhCOs. GABANs, GABAergic neurons; AstPs, astrocyte precursors; GlutNs, glutamatergic neurons; IPs, intermediate progenitors; oRG, outer radial glia; vRG, ventricular radial glia; ECs, endothelial cells; MSCs, mesenchymal stromal cells; SMCs, smooth muscle cells.

(C) Bar plots showing neurodegenerative disease-associated genes expression within indicated neural-lineage cell types of hypoxic and reoxygenated vhCOs compared to that of normoxic vhCOs, related to Figure 5A.

Figure S8. Ligand-receptor pairs prediction of each cell type in vhCOs during hypoxia-reoxygenation, related to Figure 7.

(A) CellChat-generated network plots visualizing ligand-receptor interactions (color-coded) between each indicated cell type in vhCOs under normoxic (normoxia_48h/7d), hypoxic (hypoxia_48h), and reoxygenated (hypoxia_7d) conditions. Node size scales with interaction strength, while line thickness reflects communication probability.

(B) CellChat-predicted top 10 ligand-receptor interactions between neural lineages in vhCOs under normoxic, hypoxic (hypoxia_48h), and reoxygenated (hypoxia_7d) conditions, visualized with sizes and colors corresponding to cell-cell communication (CCC) scores.

Figure S9. Characteristics of GABAergic neuron subtypes during hypoxia-reoxygenation, related to Figure 8.

(A) Violin plots displaying subtype-specific gene expression to each cell cluster, related to Figure 8A-8C.

(B) Feature plots displaying subtype-specific gene expression within GABAergic neurons, related to Figure 8A-8C.

(C-E) Gene ontology enrichment analysis of DEGs across the 3 subtypes within GABAergic neurons, related to Figure 8A-8C.

(F-H) Heatmap displaying the expression level of TOP 100 DEGs in each branch with pseudo- time, related to Figure 8D-8E.

Figure S10. SCENIC analyses of each cell type during hypoxia-reoxygenation within vhCOs, related to Figure 10.

(A) Dot plots displaying RSS and Z score of transcription factors (TFs) within each indicated cell type in normoxic (normoxia_48h/7d), hypoxic (hypoxia_48h), and reoxygenated (hypoxia_7d) vhCOs, related to Figure 10A. RSS, regulon specificity scores. GABAN I, GABAergic neurons Subtype I; GABAN II, GABAergic neurons Subtype II.

(B-O) SCENIC plots displaying RSS of top 5 TFs within each indicated cell type in normoxic (normoxia_48h/7d), hypoxic (hypoxia_48h), and reoxygenated (hypoxia_7d) vhCOs. GABAN, GABAergic neurons.

Figure S11. TF regulatory network alterations during hypoxia-reoxygenation within vhCOs, related to Figure 10.

(A-C) TF regulatory networks of specific cell types in hypoxic (hypoxia_48h) and

reoxygenated (hypoxia_7d) vHCOs. Color-coded diamonds denote upregulated (red), unchanged (green), and downregulated (blue) TF activities compared to normoxic (normoxia_48h/7d) vHCOs. Arrows indicate TF target genes, with expression levels color-coded by fold change (hypoxia_48h vs. normoxia_48h; hypoxia_7d vs. normoxia_7d), red and blue circles representing upregulation and downregulation respectively.

Supplementary Tables

Table S1. Key resources.

Table S2. GO enrichment of each cell type.

Table S3. pseudo-bulk gene expression and GSEA (vs normoxia).

Table S4. DEG of each cell type (hypoxia vs normoxia).

Table S5. GSEA of each cell type (hypoxia vs normoxia).

Table S6. GO enrichment of each GABAergic neuron subtype.

Table S7. GABAergic neuron subtype DEG & GSEA (vs normoxia).

Table S8. DEG and GSEA of GABAergic neuron subtype I vs II.

Table S1. Key resources.

Antibodies				
Name	Application / Dilution	Source	RRID	Cat#
anti-SOX1	IF (1:100)	Bio-Techne	AB_2239879	AF3369-SP
anti-PAX6	IF (1:350)	Abcam	AB_2750924	AB195045
anti-MAP2	IF (1:500)	Abcam	AB_2895301	AB183830
anti-SOX2	IF (1:350)	Abcam	AB_10710406	AB79351
Anti-TBR1	IF (1:100)	Abcam	AB_2936859	AB183032
Anti-TBR2	IF (1:100)	Abcam	AB_778267	AB23345
Anti-HIF-1 α	IF (1:100)	Cell Signaling Technology	AB_2799095	36169T
Anti-CD31 (mouse)	IF (1:200)	Abcam	AB_307284	AB9498
Anti-CD31 (goat)	IF (1:100)	R&D systems	AB_2161028	AF3628-SP
Anti-ACTA2	IF (1:200)	Abclonal	AB_2861755	A17910
Anti-PDGFR β	IF (1:100)	Bio-Techne	AB_355339A	F385-SP
Anti-GFAP	IF (1:100)	Abclonal	AB_2757050	A0237
Anti-S100B	IF (1:100)	Proteintech	AB_2254244	15146-1-AP
Anti-CLDN5	IF (1:100)	Santa Cruz Biotechnology	AB_10988234	sc-374221
Anti-ZO-1	IF (1:100)	Abclonal	-	A0659
Alexa Fluor 488-Conjugated anti-mouse	IF (1:500)	Abcam	AB_2732856	AB150105
Alexa Fluor 594-conjugated anti-rabbit	IF (1:500)	Abcam	AB_2782993	AB150076
Alexa Fluor 647-conjugated anti-goat	IF (1:500)	Abcam	AB_2687955	AB150135

Figure S1

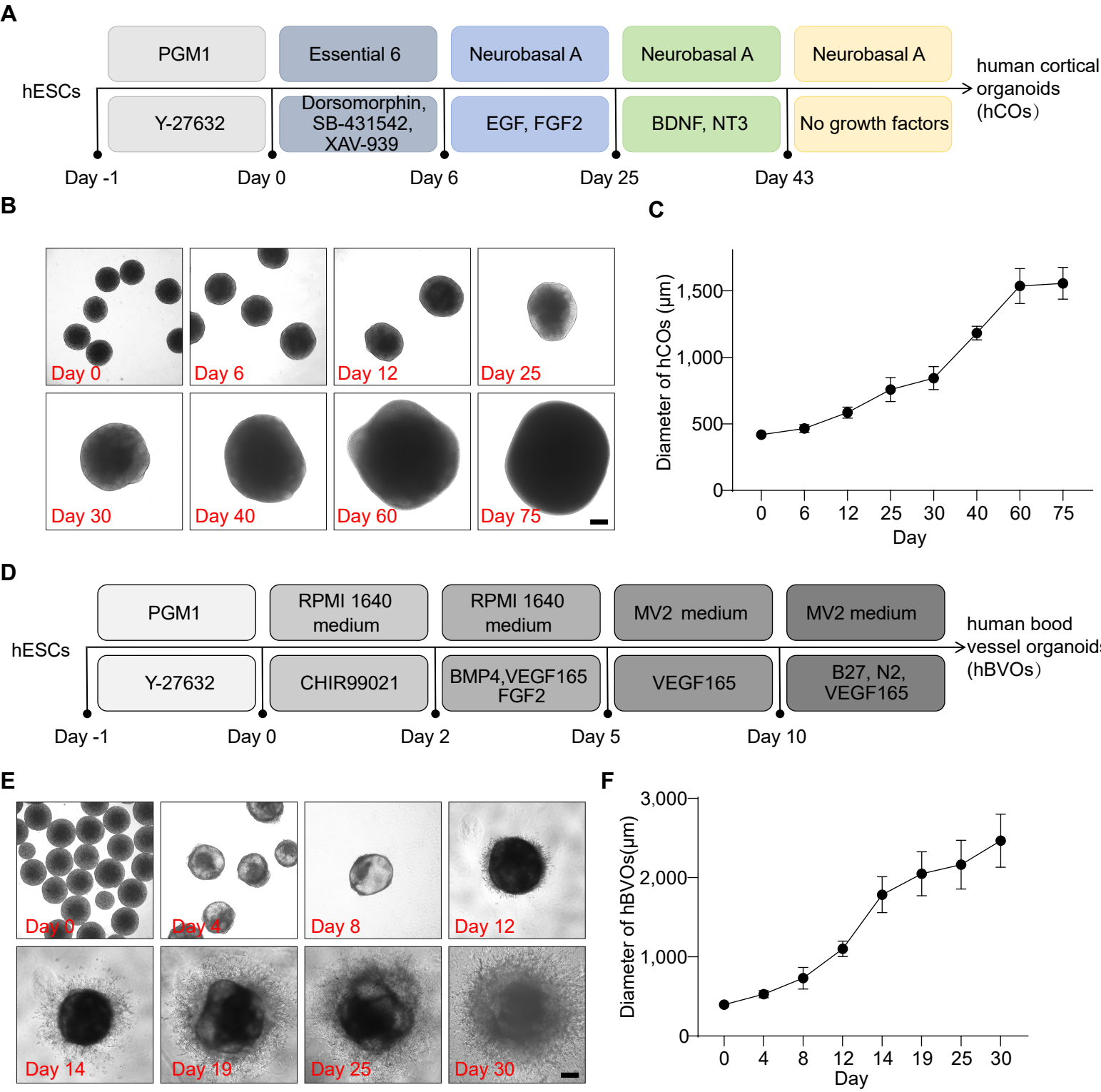


Figure S2

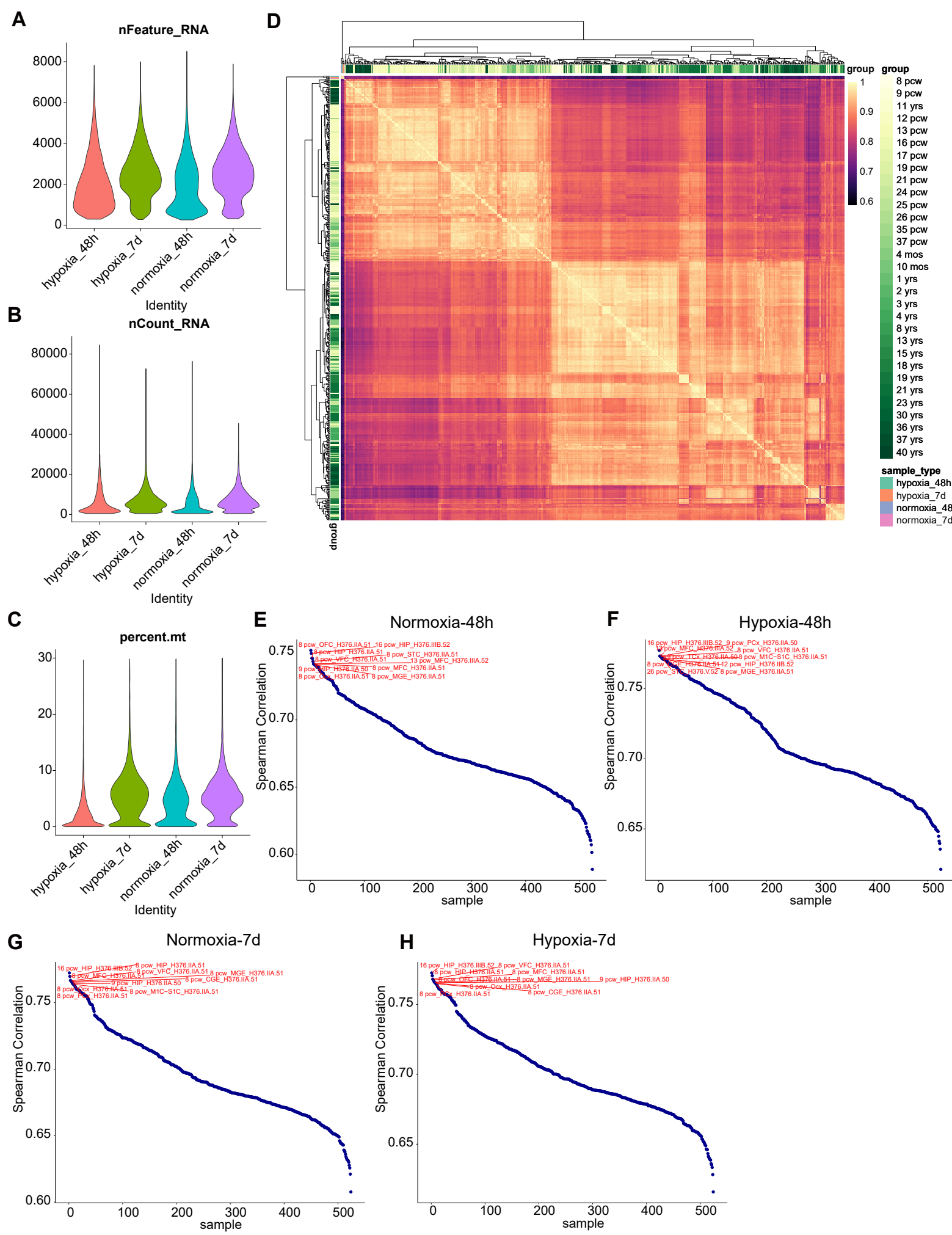
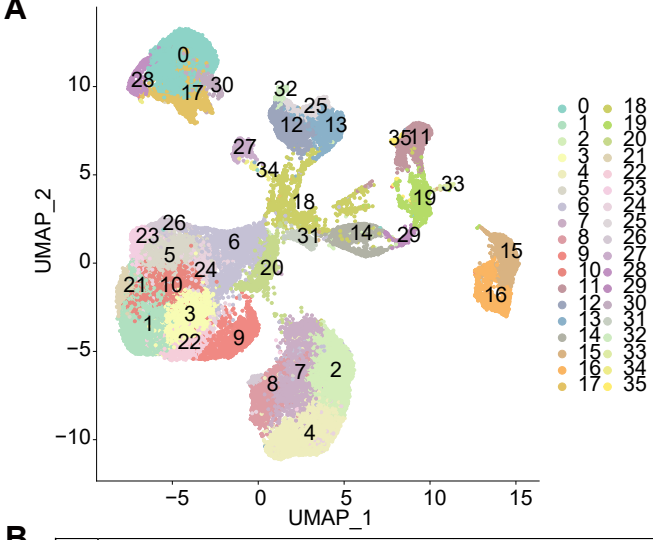


Figure S3

A



B

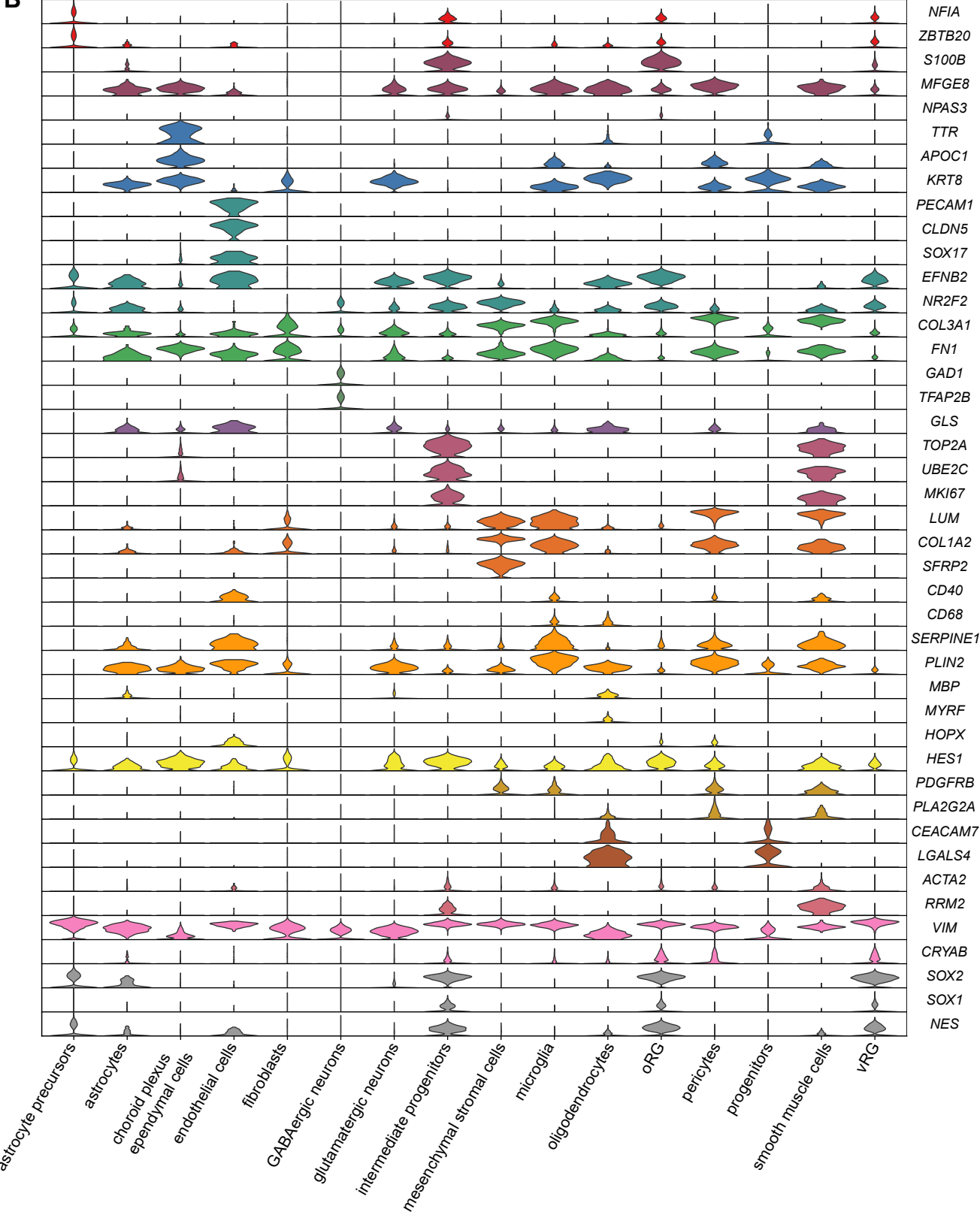


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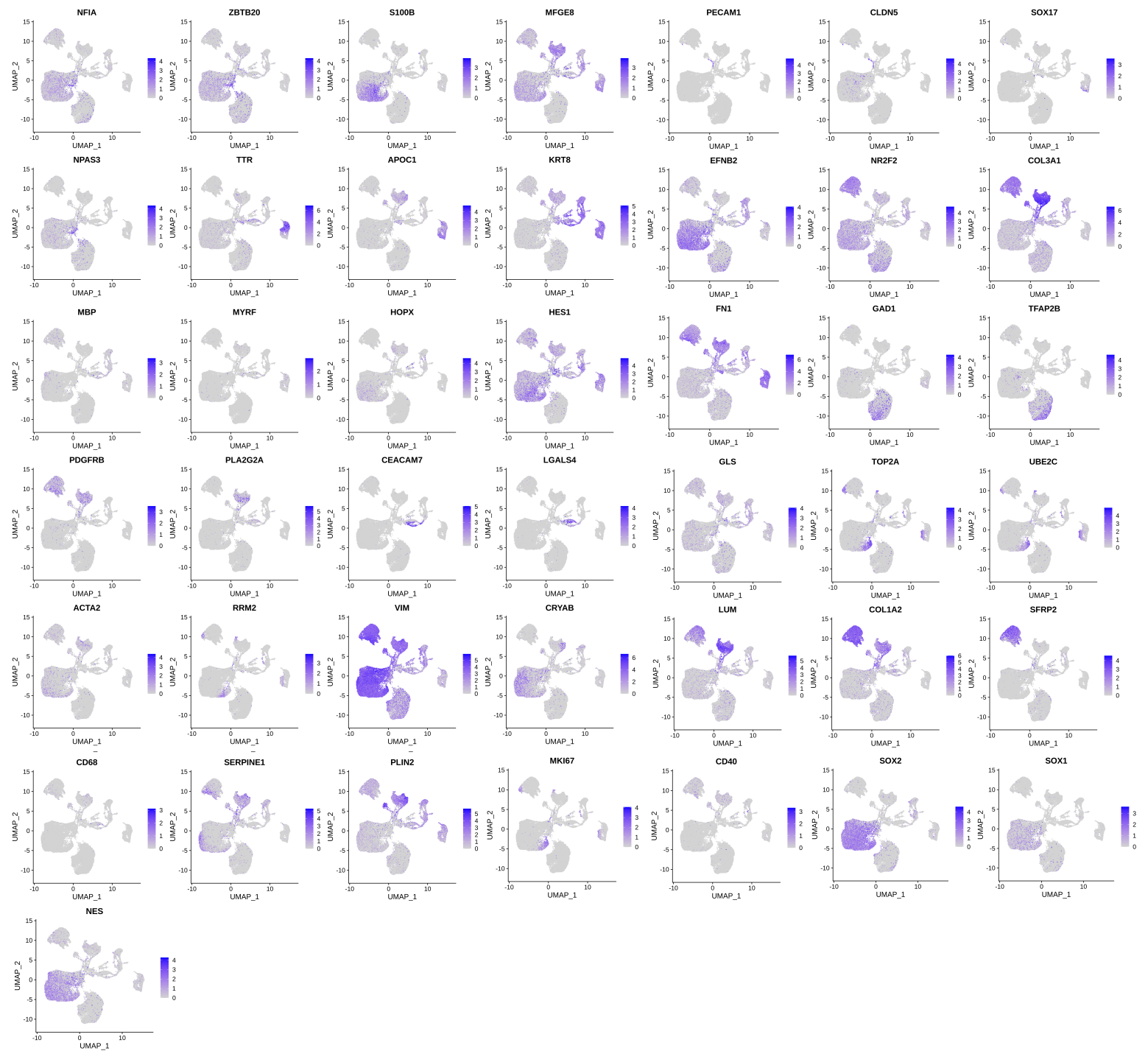


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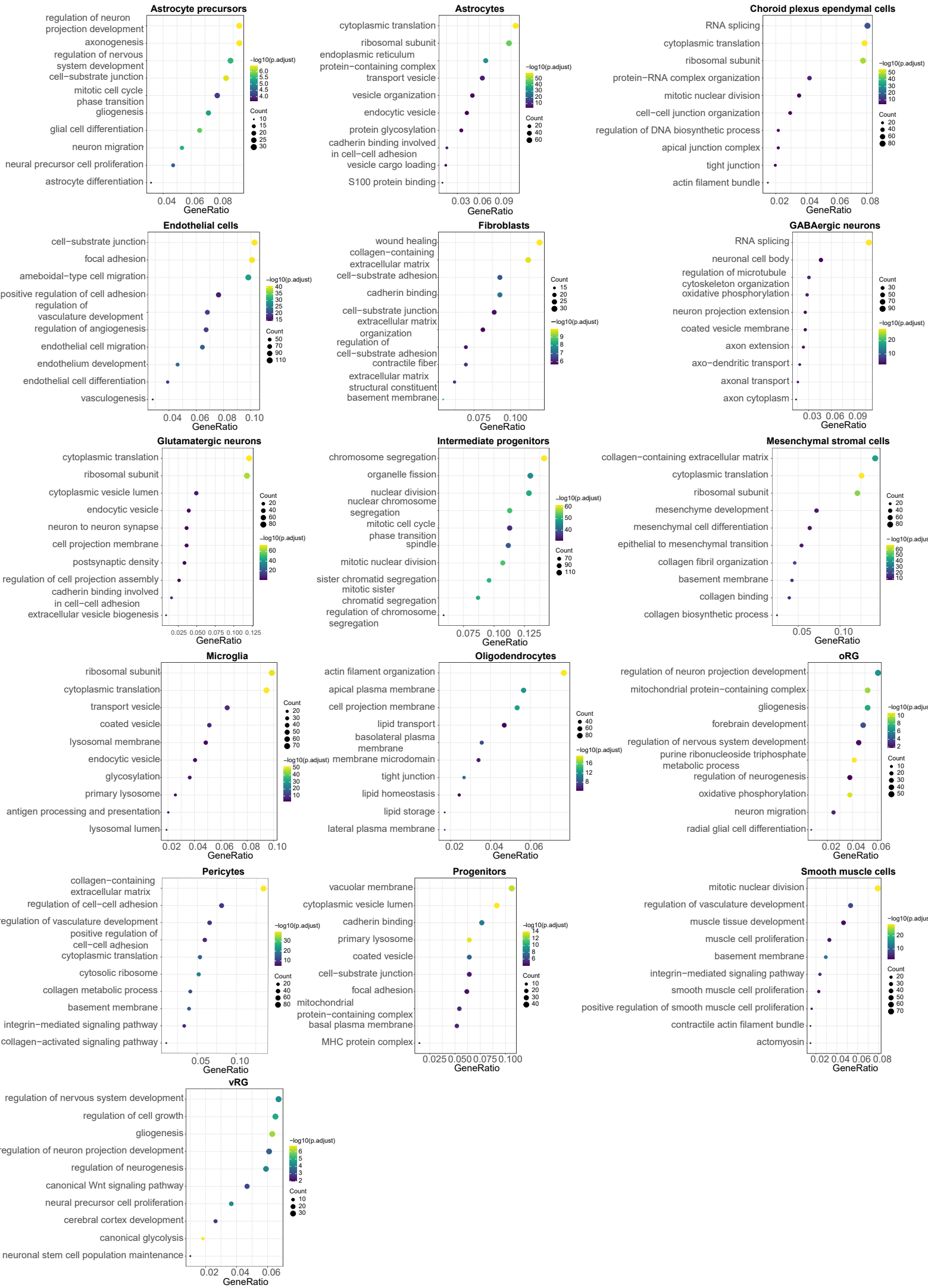
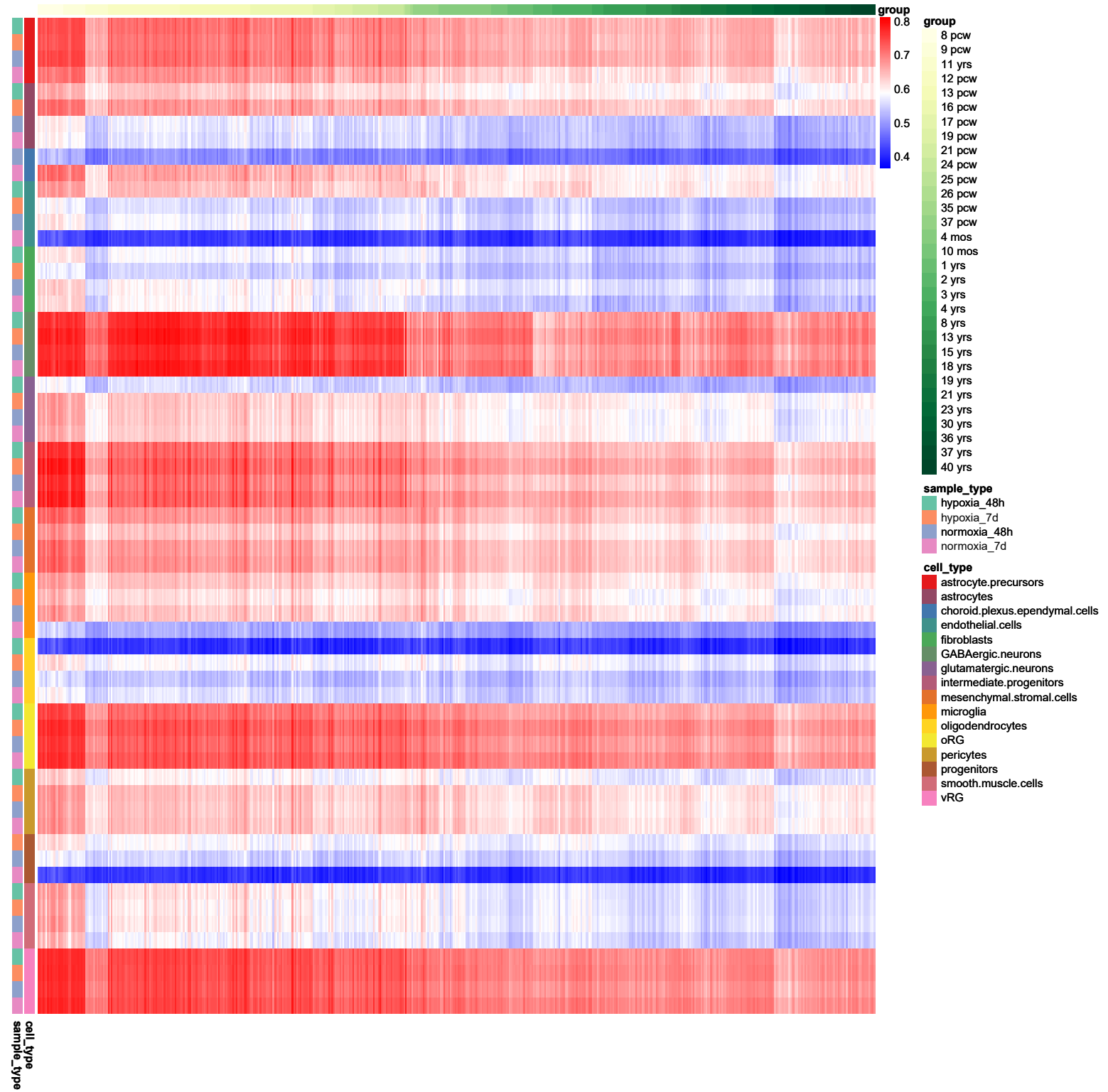


Figure S6



A

A

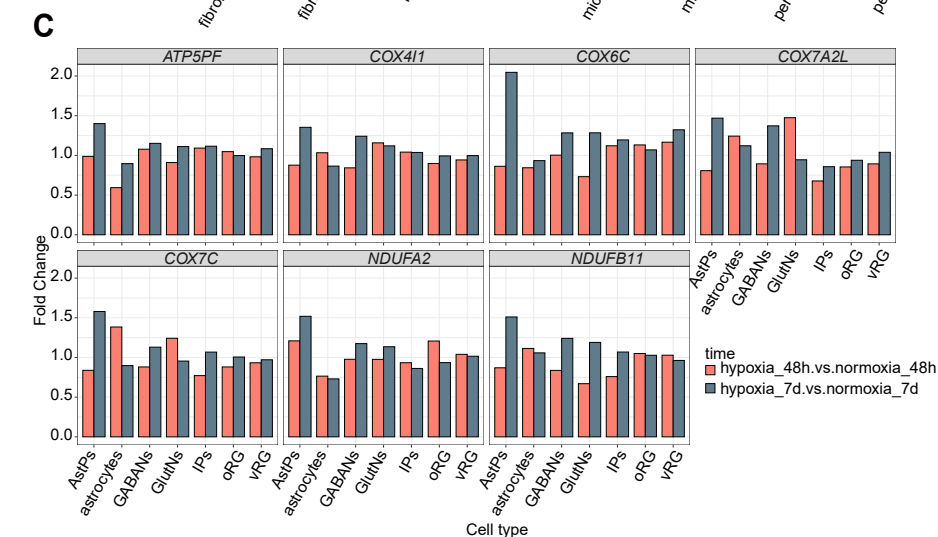
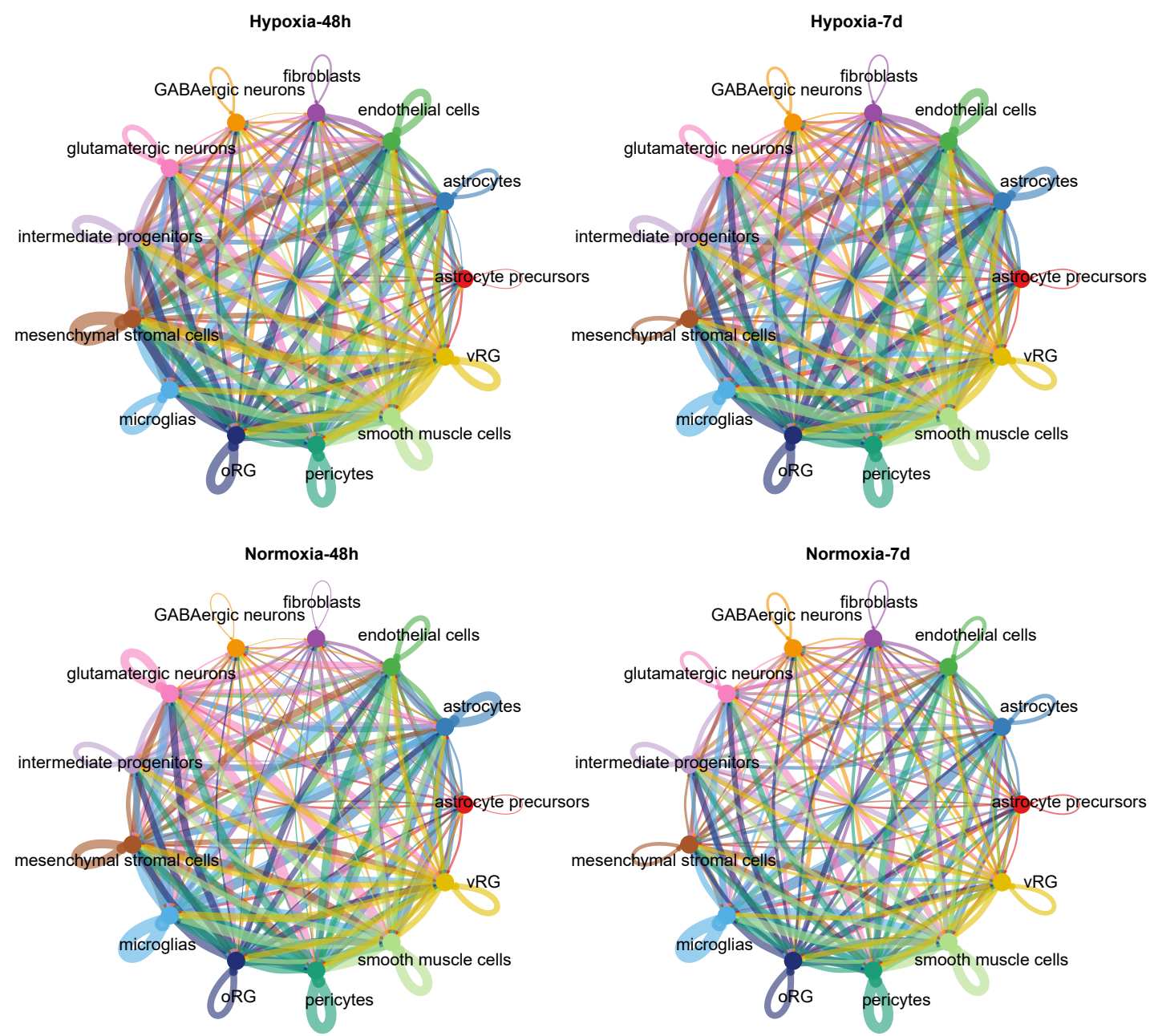


Figure S8

A



B

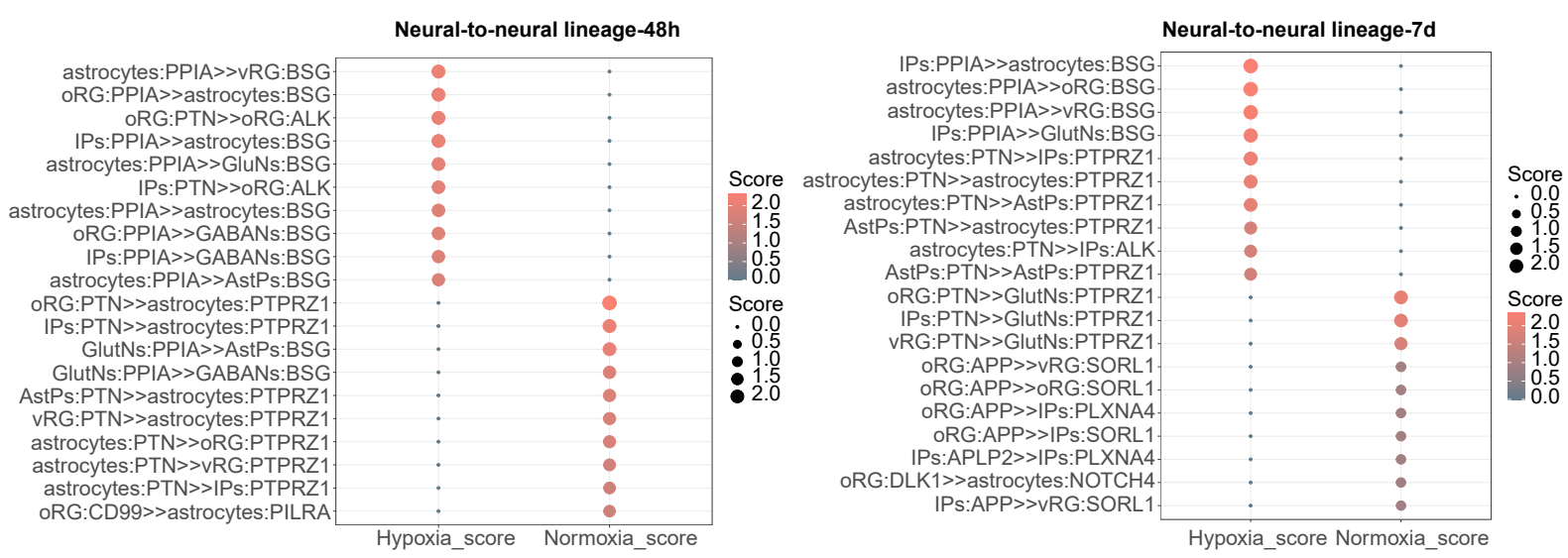
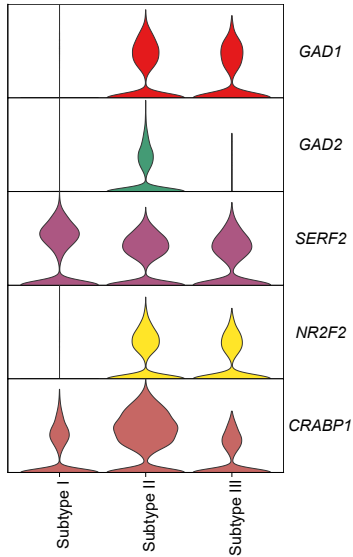
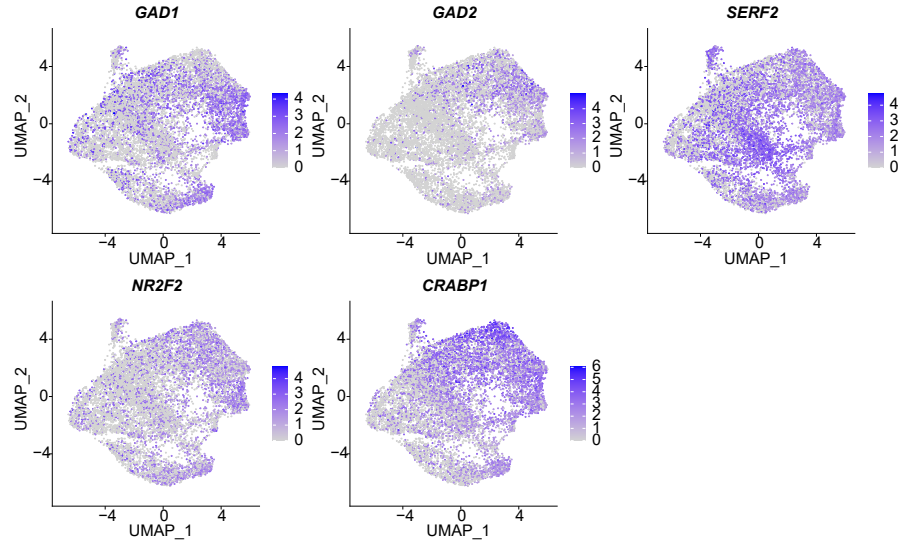


Figure S9

A

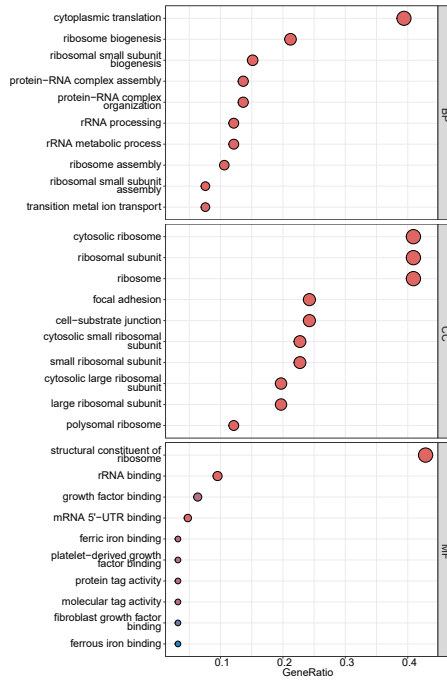


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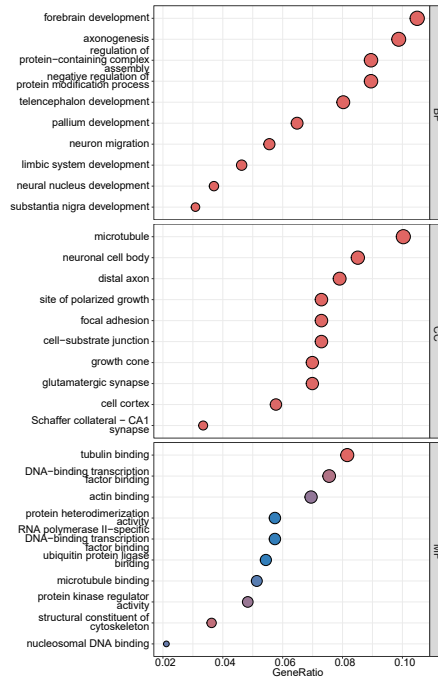
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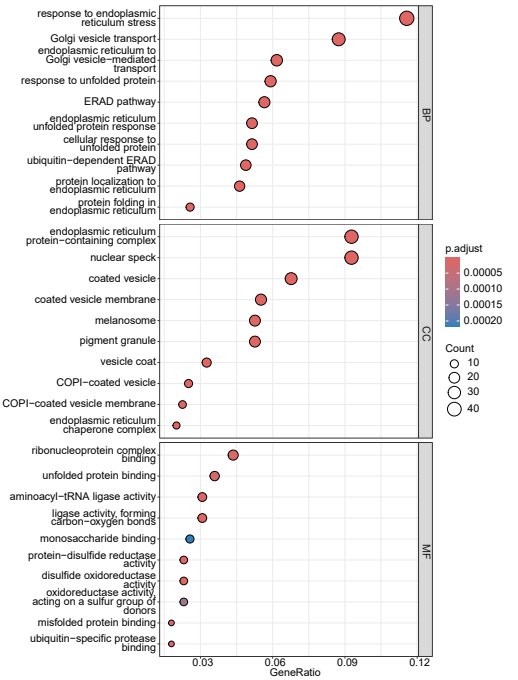
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Subtype II



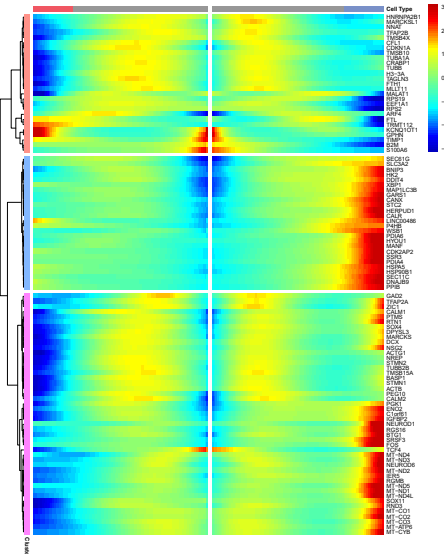
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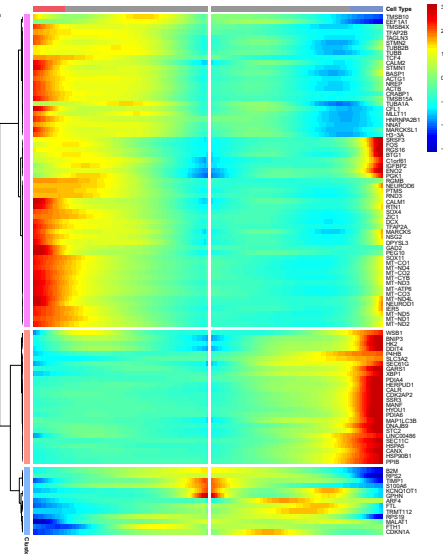
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Sub-branch 1



G

Sub-branch 2



H

Sub-branch 3

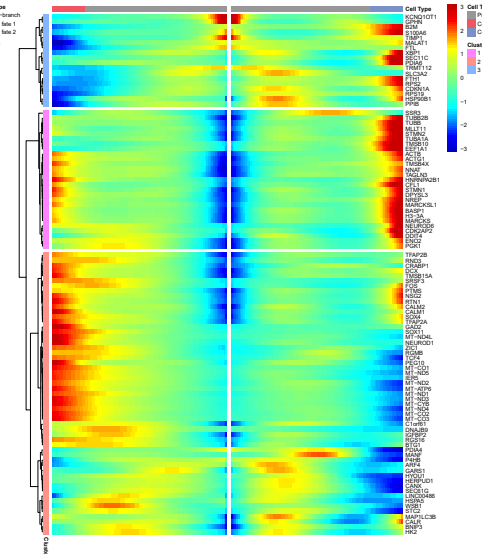
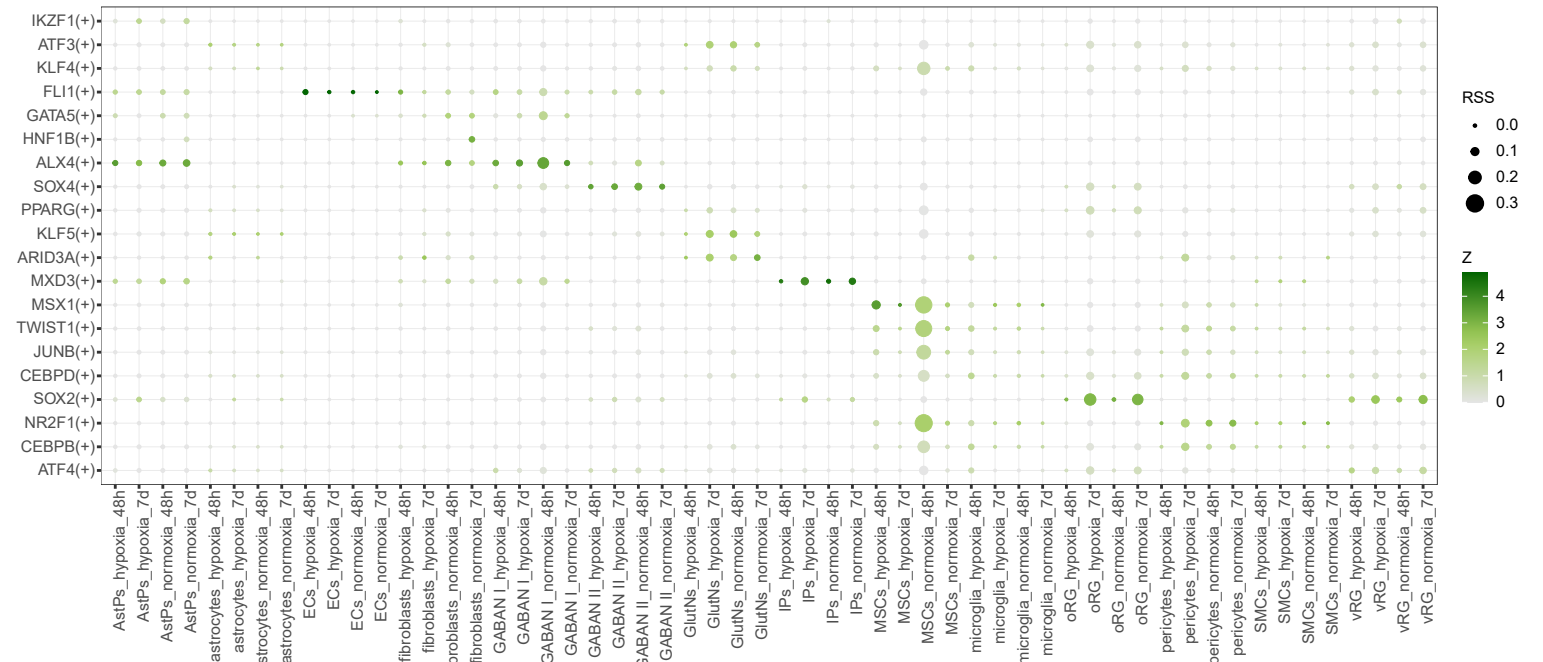
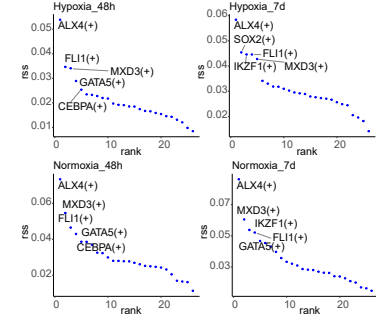


Figure S10

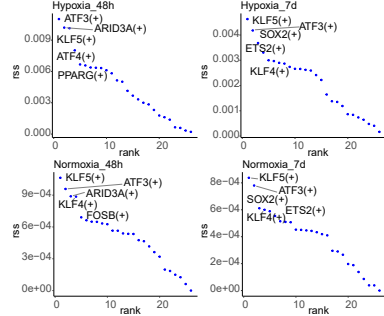
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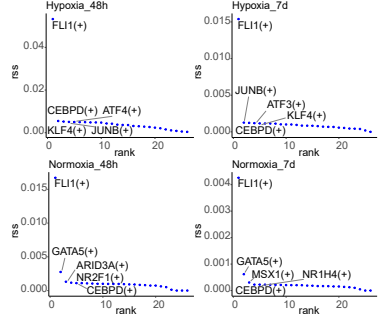
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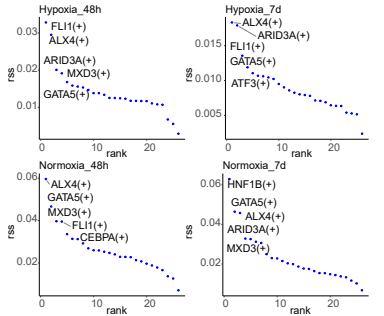
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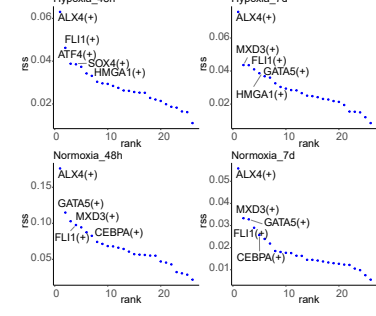
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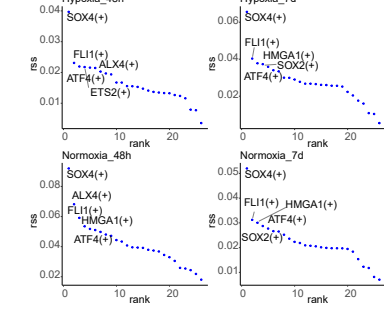
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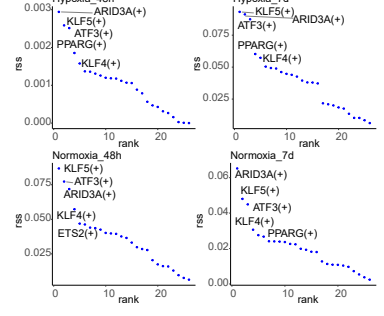
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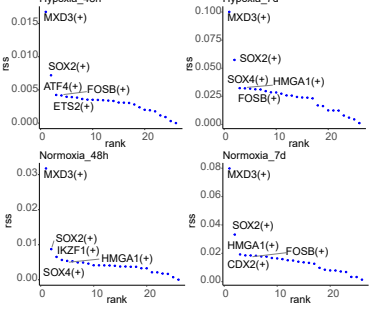
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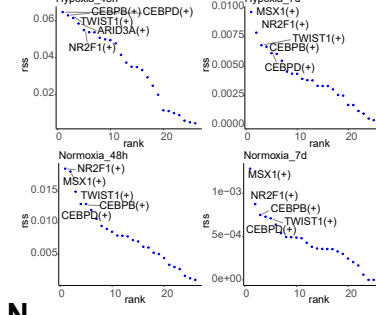
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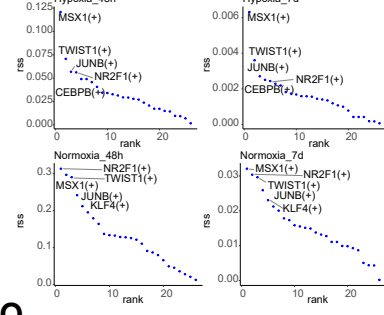
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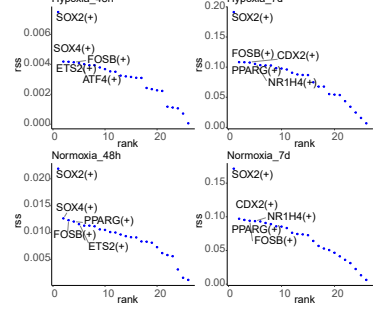
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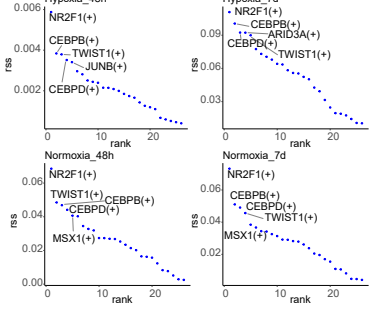
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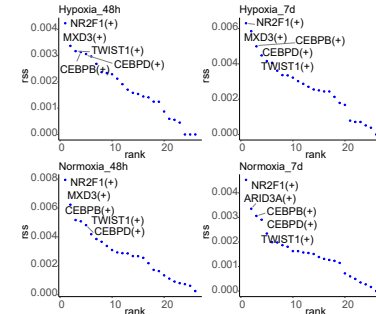
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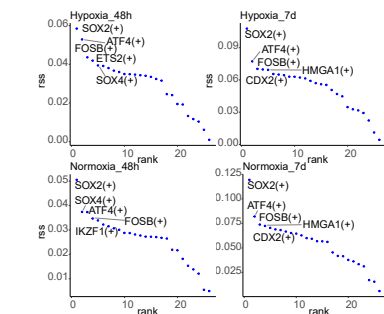
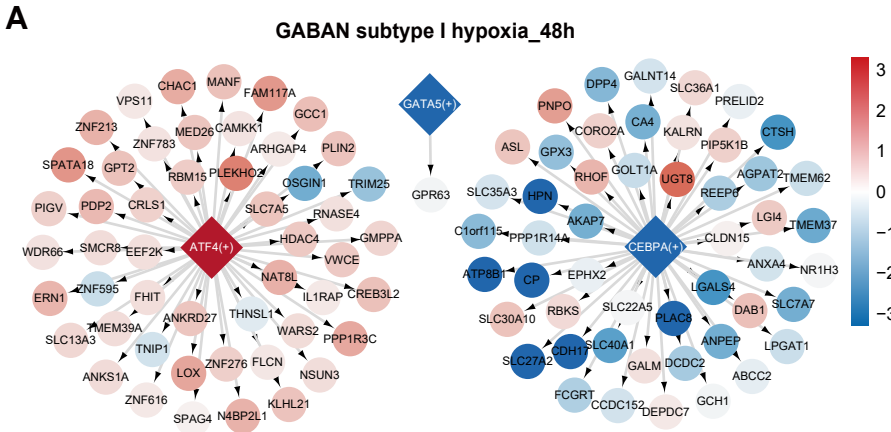
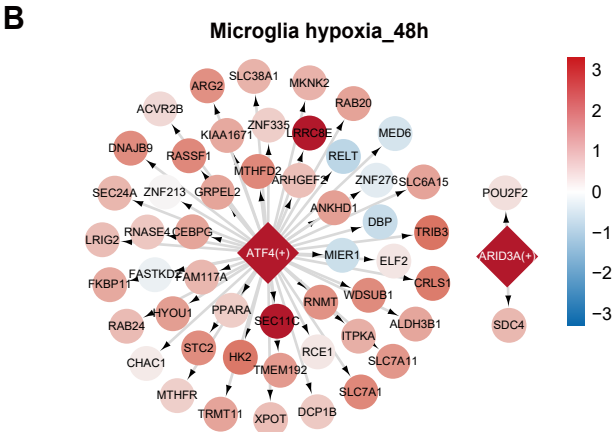


Figure S11

A



B



C

