

Supplemental Table 1. Sample metadata. Pig ID, group, dose, weight, age, duration the pig was under anesthesia measured in minutes, and clinical measurements including heart rate, body temperature, blood oxygen saturation (SpO₂), and mean arterial pressure (MAP).

Supplemental Table 2. Differential expression analysis of the bulk brain transcriptome in septic versus control swine. Comprehensive output of bulk RNA sequencing (RNAseq) differential expression analysis comparing Yorkshire pigs receiving continuous intravenous *E. coli* infusion (n=4) against saline-infused controls (n=4). The dataset includes all expressed genes. Columns are defined as follows: Chr (Chromosomal location); width (Gene length); gene_id/GENEID (Ensembl/Internal identifiers); gene_name (HGNC/Symbol); gene_biotype (Functional classification); logFC (Log₂ fold change); CI.L/CI.R (95% Confidence Intervals); AveExpr (Average log₂-expression across all samples); t (Moderated t-statistic); P.Value (Nominal p-value); adj.P.Val (Benjamini-Hochberg false discovery rate); B (Log-odds of differential expression).

Supplemental Table 3. Bulk RNAseq metascape enrichment analysis of the porcine transcriptome during sepsis. Comprehensive functional enrichment of differentially expressed genes (DEGs) identified in the brain tissue of Yorkshire swine following *E. coli*-induced sepsis. Annotation tab provides detailed functional metadata for each DEG. The Enrichment tab summarizes the statistically significant biological clusters.

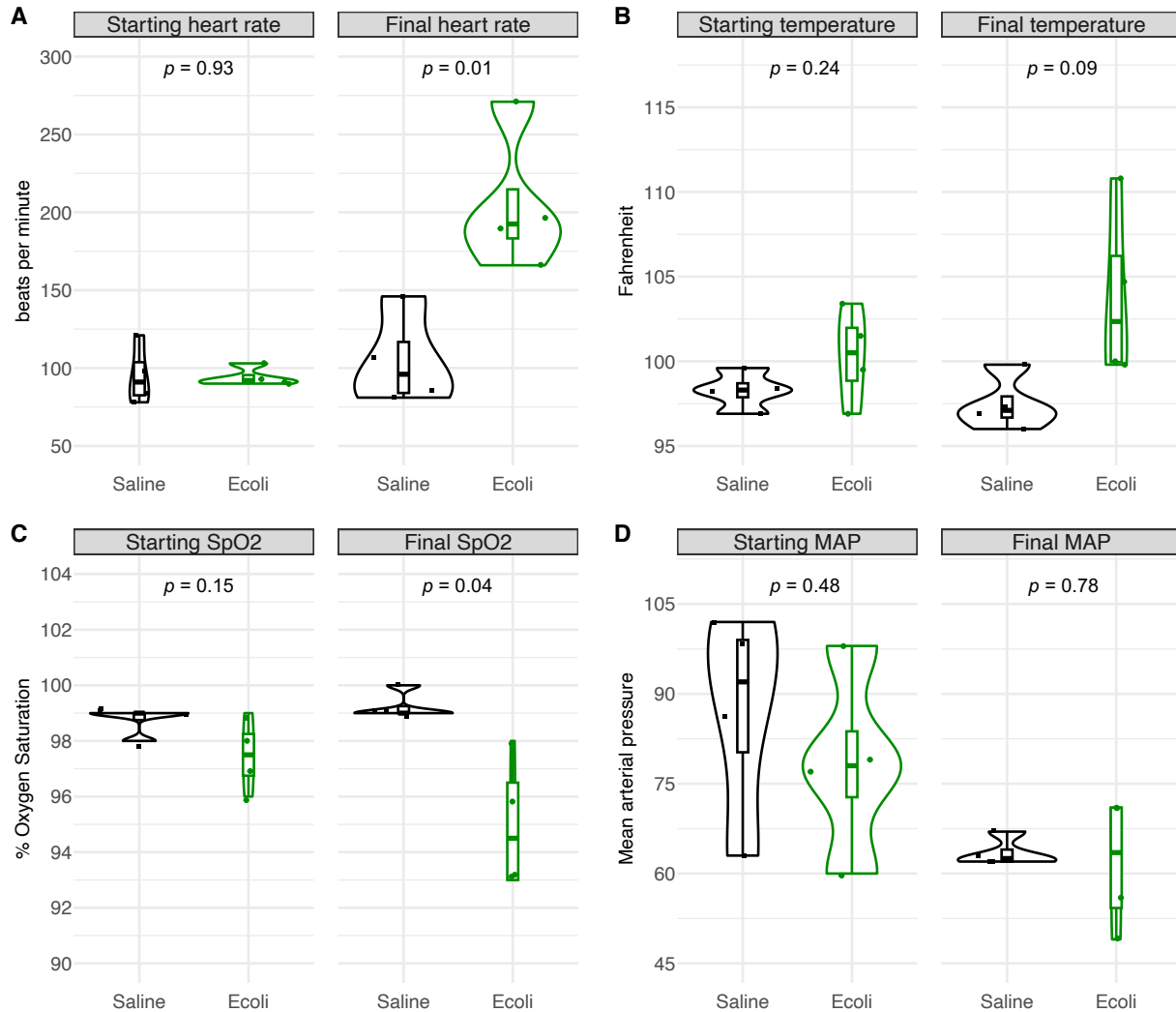
Supplemental Table 4. Differential expression analysis of single-nucleus (snRNAseq) in each brain cell population in septic versus saline control swine. Transcriptional response to *E. coli*-induced sepsis within each specific cell population of the porcine brain. Single-nucleus RNA sequencing was performed on nuclei harvested from the frontal cortex of Yorkshire pigs subjected to a continuous intravenous infusion of *E. coli* (n=4) or saline control (n=4). Cell expression data were aggregated for pseudobulk analysis and were limited to protein-coding genes. After aggregation, cell-type-specific gene filtering was applied only to genes with mean expression above the first quartile in each cell type. Following pseudobulk creation and

filtering out lowly expressed genes, differential expression analysis between *E. coli* and saline within each cell type was performed using the DESeq2 method.

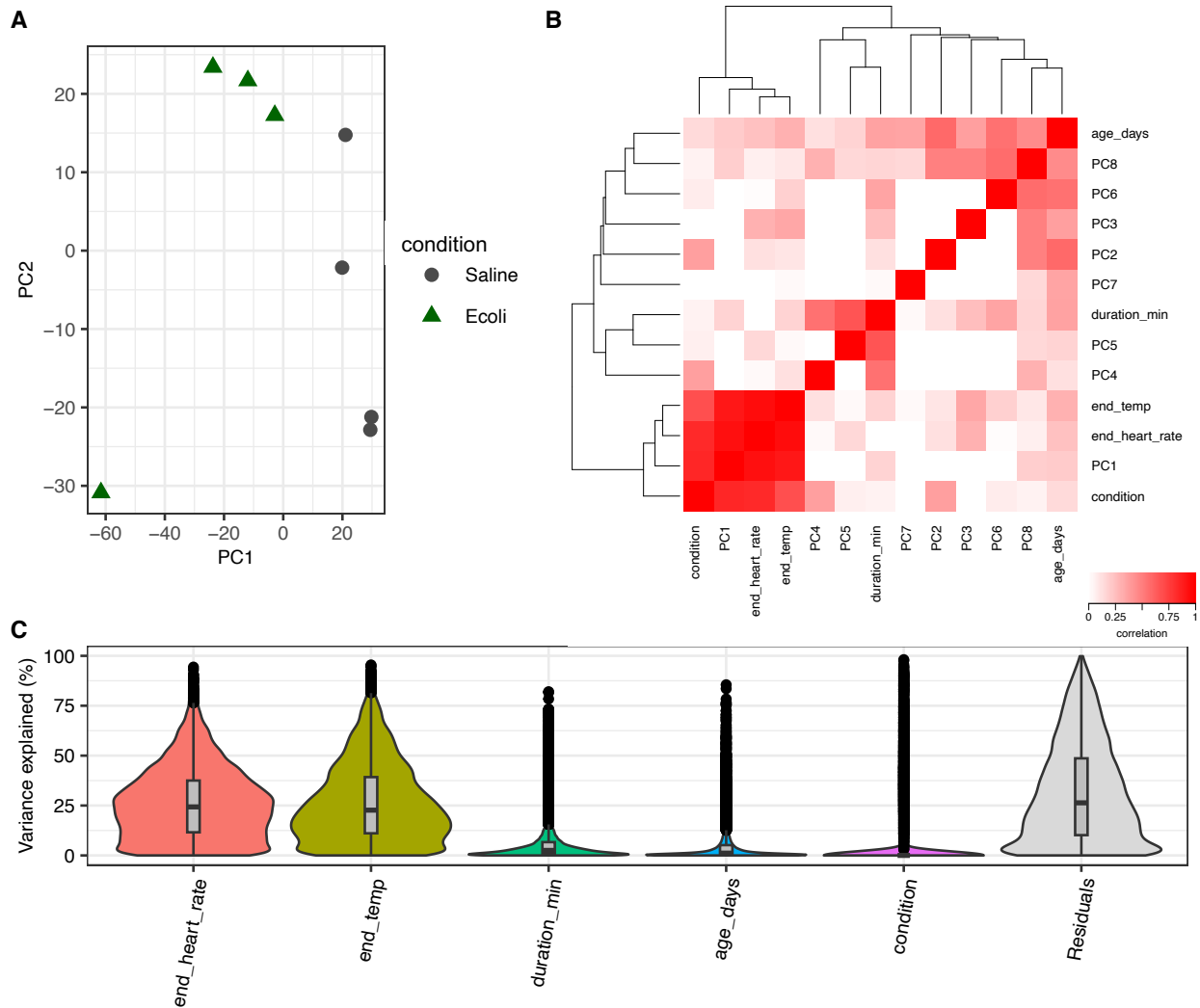
Supplemental Table 5. Comparative Analysis of differentially expressed genes (DEGs) between bulk tissue and single-nucleus RNA Sequencing. Comparative overview of the transcriptomic signatures identified through bulk RNAseq and single-nucleus RNAseq (snRNAseq) in the porcine brain during *E. coli*-induced sepsis. The analysis utilizes a significance threshold of $q < 0.05$ and absolute $\log_2$ fold change > 1 for both datasets. The table is partitioned into three tabs to categorize the overlap and divergence of these methodologies.

Supplemental Table 6. Metascape functional enrichment analysis of cell-type DEGs in sepsis. This table presents the biological processes, molecular functions, and canonical pathways significantly enriched within the differentially expressed genes (DEGs) of each major brain cell type. To identify these pathways, DEGs from each cell population (q -value < 0.05 and absolute $\log_2$ fold change > 1) were analyzed using Metascape. This analysis facilitates a granular understanding of how systemic *E. coli* infection alters the functional landscape of the neurovascular unit and glia populations. The table is organized by cell type, with each tab containing the enriched terms.

Supplemental Table 7. Statistical analysis of clinical and physiological parameters. Comparison of baseline (start) and terminal (end) measurements between Yorkshire pigs receiving continuous intravenous infusion of *E. coli* ($n = 4$) or saline ($n = 4$). For each parameter, the mean and standard deviation (stdv) are reported. Data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated via Levene's test. Significant differences between groups were determined using a two-sample t-test, with the Wilcoxon rank-sum test as a nonparametric alternative for non-normally distributed variables. Statistical significance was defined as $p < 0.05$.

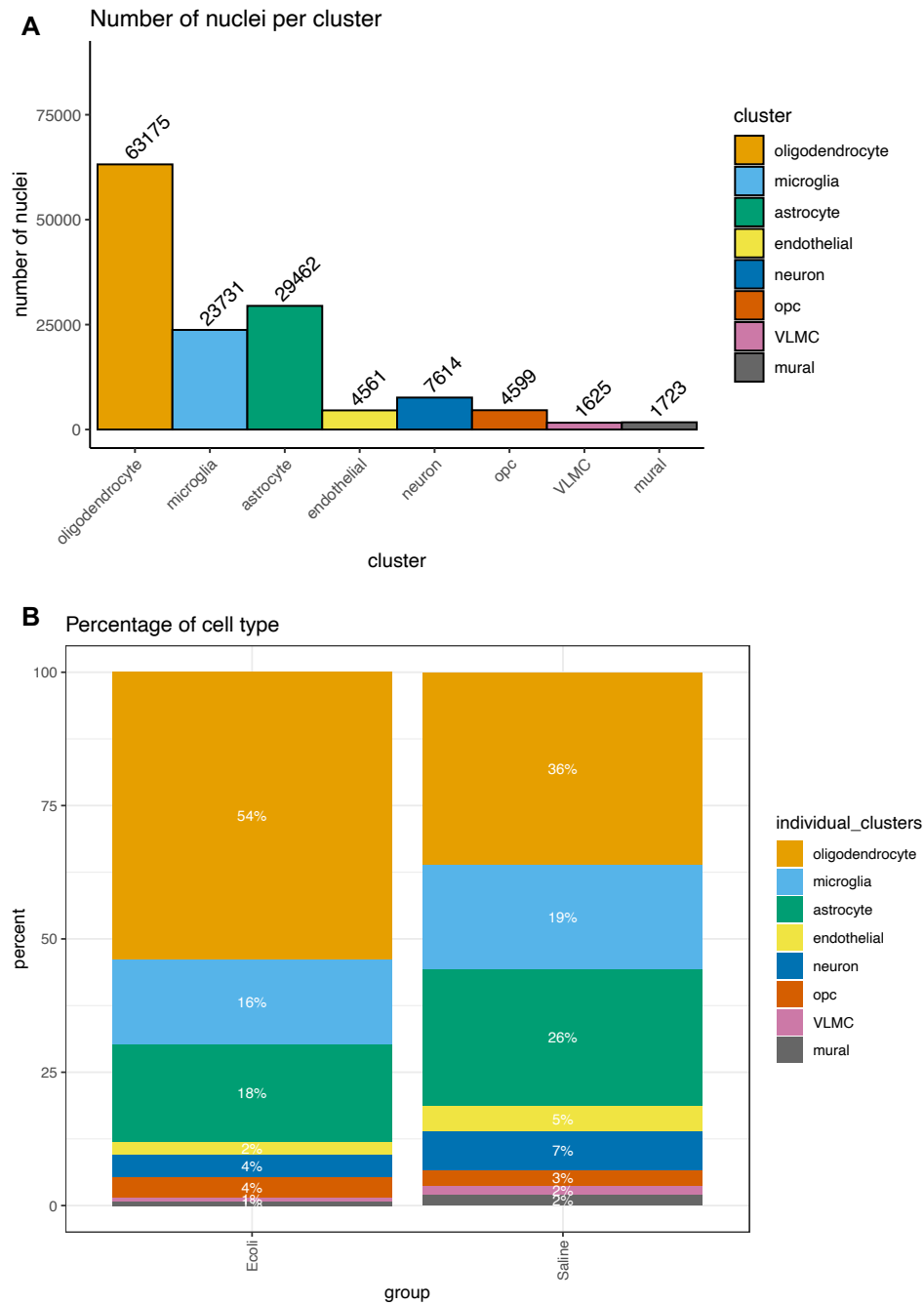


Supplemental Figure 1. Physiological changes in response to *E. coli* sepsis. Start refers to the beginning time point of the experiment when the pig either received *E. coli* or saline. The final time point is right before euthanasia. **(A)** Starting and final heart rate for saline and *E. coli* pigs. While initial heart rates were similar ($p = 0.93$), the final heart rate was significantly higher in the *E. coli* group ($p = 0.01$). **(B)** No significant difference was observed in starting ($p = 0.24$) or final ($p = 0.09$) body temperature. **(C)** Starting blood oxygen saturation (SpO2) for saline and *E. coli* pigs was similar ($p = 0.15$), but final SpO2 was significantly lower in the *E. coli* group ($p = 0.04$). **(D)** There was no significant difference in starting ($p = 0.48$) or final ($p = 0.78$) mean arterial pressure (MAP) between saline and *E. coli* pigs. P-values were determined using t-tests.

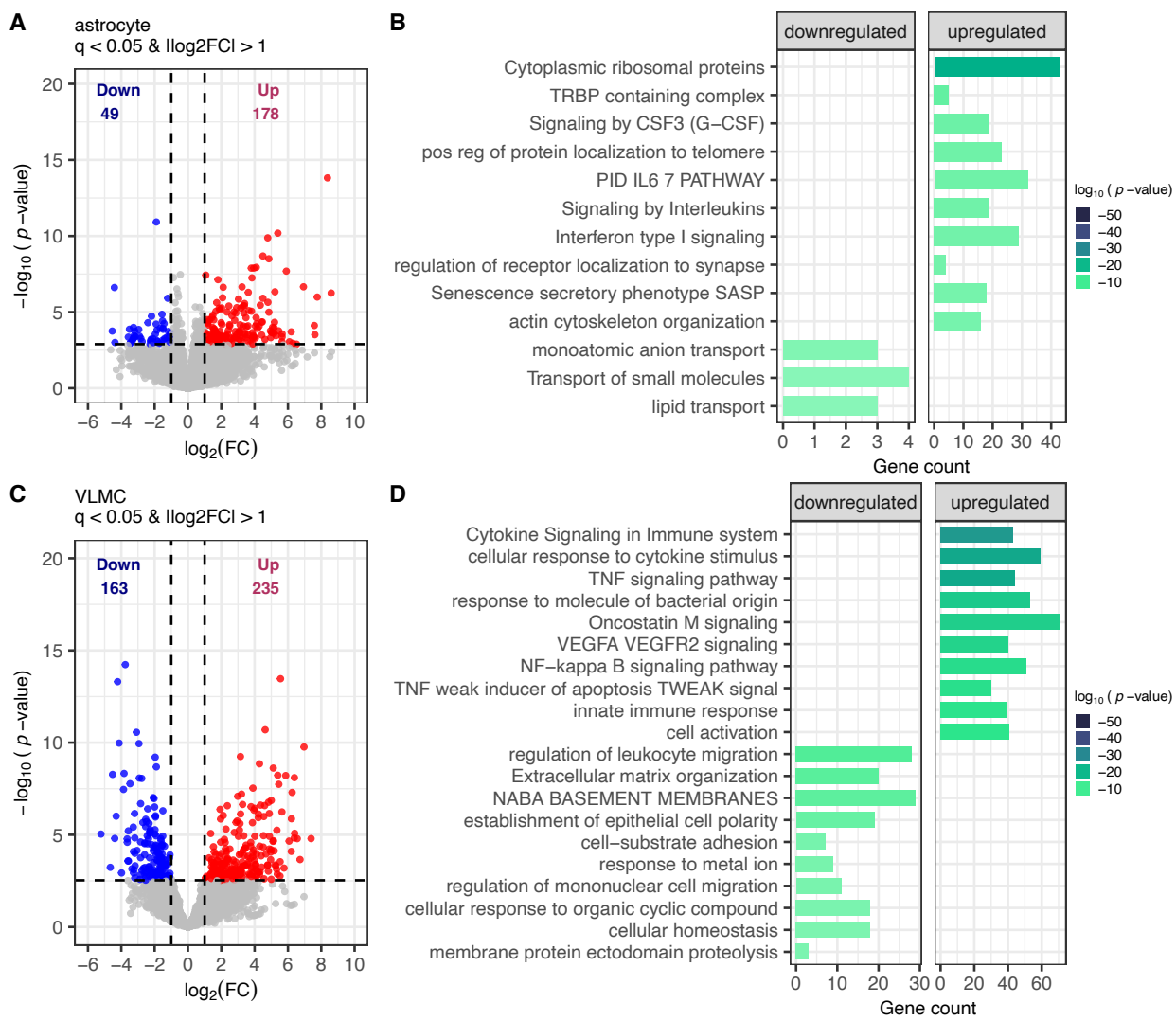


Supplemental Figure 2. Assessment of variation in bulk RNAseq expression data. (A) Principal Component Analysis (PCA) of bulk RNAseq samples. PC1 effectively separates the experimental groups, with *E. coli*-treated pigs (green triangles) clustering distinctly from saline controls (gray circles). (B) The Canonical Correlation Analysis (CCA) heatmap depicts correlations between the top principal components (PC1–PC8) and various biological or clinical metadata. The color scale indicates the strength of the correlation (r), ranging from 0 (white) to 1 (red). Hierarchical clustering (left and top) groups variables with similar correlation profiles. PC1 shows a strong correlation with condition (*E. coli* vs. saline), end heart rate, and end temperature, suggesting that the primary source of transcriptional variation is the physiological response to septic shock. (C) Variance partitioning of bulk gene expression illustrates the percentage of total gene expression variance explained by specific experimental variables: end heart rate, end

temperature, duration (minutes), age (days), and condition (*E. coli* or saline). Residuals represent the variance not explained by these covariates. The clinical indicators of sepsis (end heart rate and end temperature) account for a substantial portion of the observed transcriptional variance across the dataset.

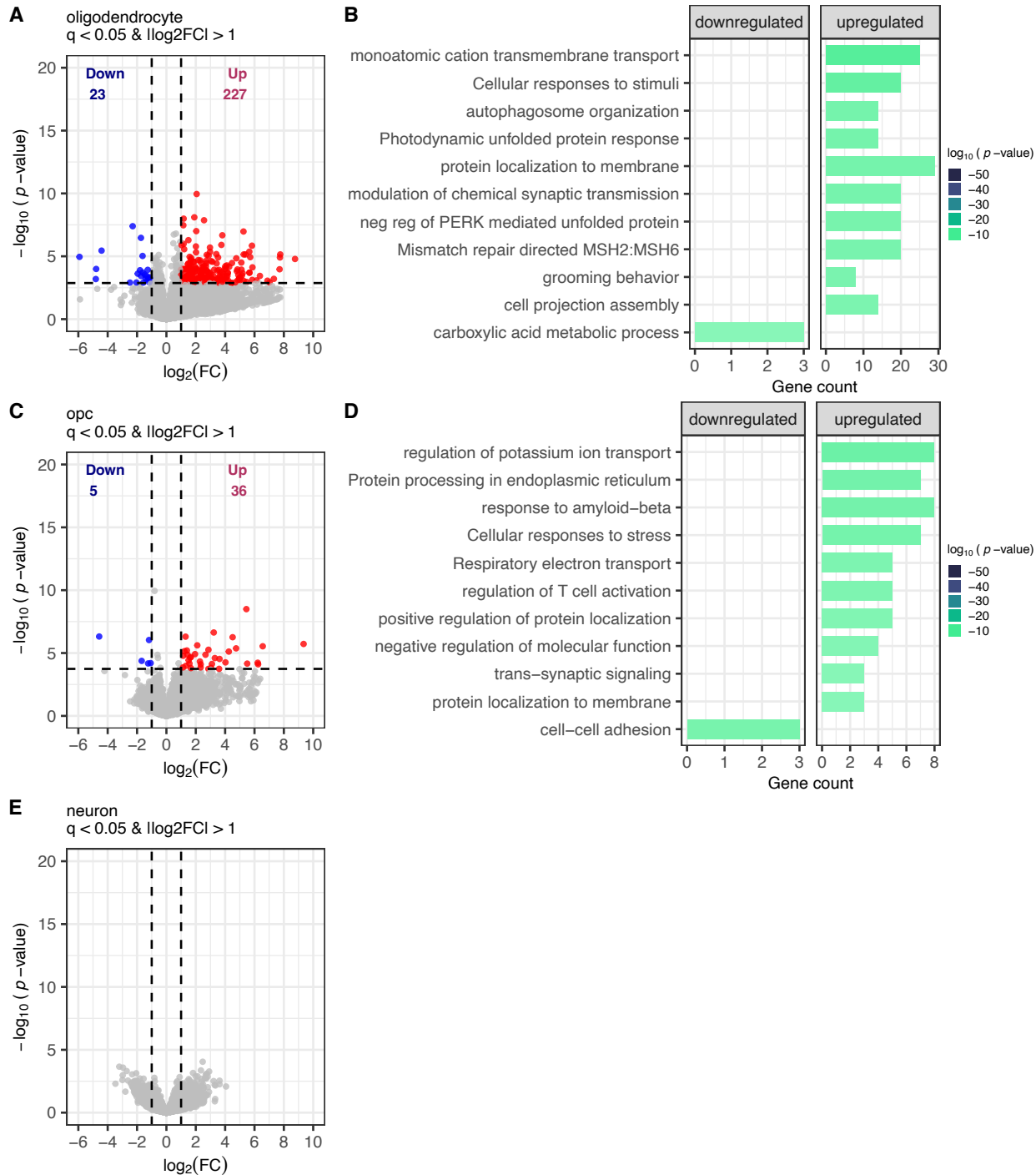


Supplemental Figure 3. Nuclei count per cell type and relative abundance. (A) Number of nuclei in each cell type. **(B)** Relative abundance of cell type within the *E. coli* and the saline control pigs.

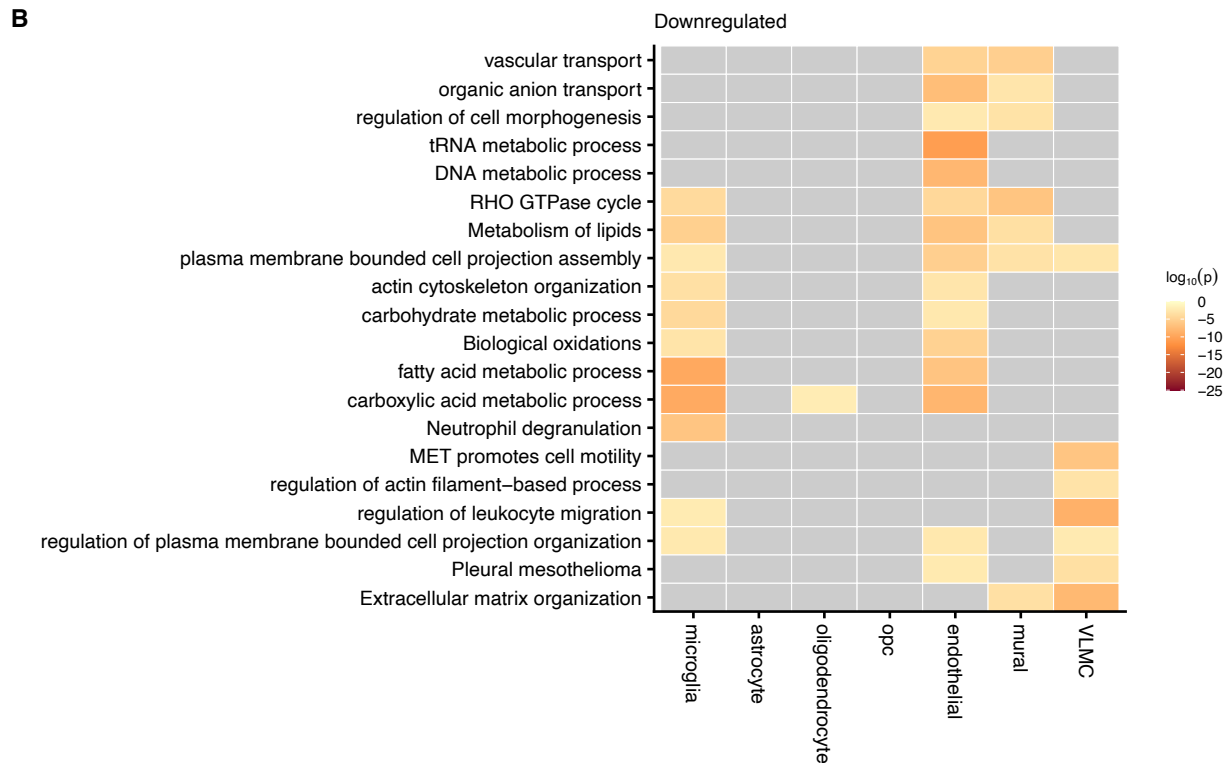
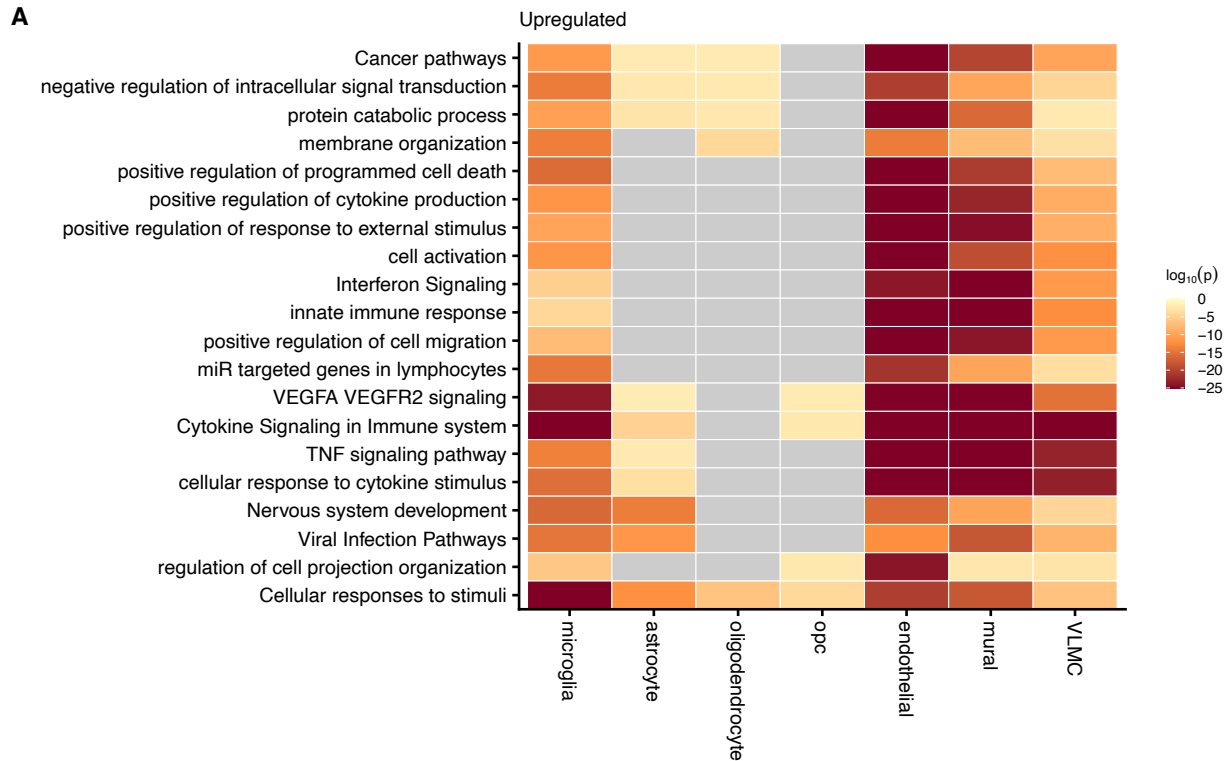


87 **Supplemental Figure 4. Transcriptional changes in brain astrocytes and vascular leptomeningeal**
88 **cells (VLMC) during early sepsis.** Volcano plots illustrating differential gene expression in brain astrocytes
89 **(A)** and VLMCs **(C)** from *E. coli* septic pigs compared to saline controls. The x-axis represents the $\log_2$ fold
90 change, and the y-axis shows the negative $\log_{10}$ of the p-value. Each point represents a single gene. Genes
91 with a false discovery rate (q-value) < 0.05 and an absolute $\log_2$ fold change > 1 are considered statistically
92 significant and are highlighted in blue (downregulated) or red (upregulated). Metascape pathway analysis
93 bar graphs showing the top 10 most significantly enriched pathways identified by Metascape for both
94 downregulated (left panel) and upregulated (right panel) genes in astrocytes **(B)** and VLMCs **(D)**. The length

of each bar indicates the gene count within that pathway. The color intensity of the bars represents the significance of the pathway, with darker shades corresponding to lower p-values.

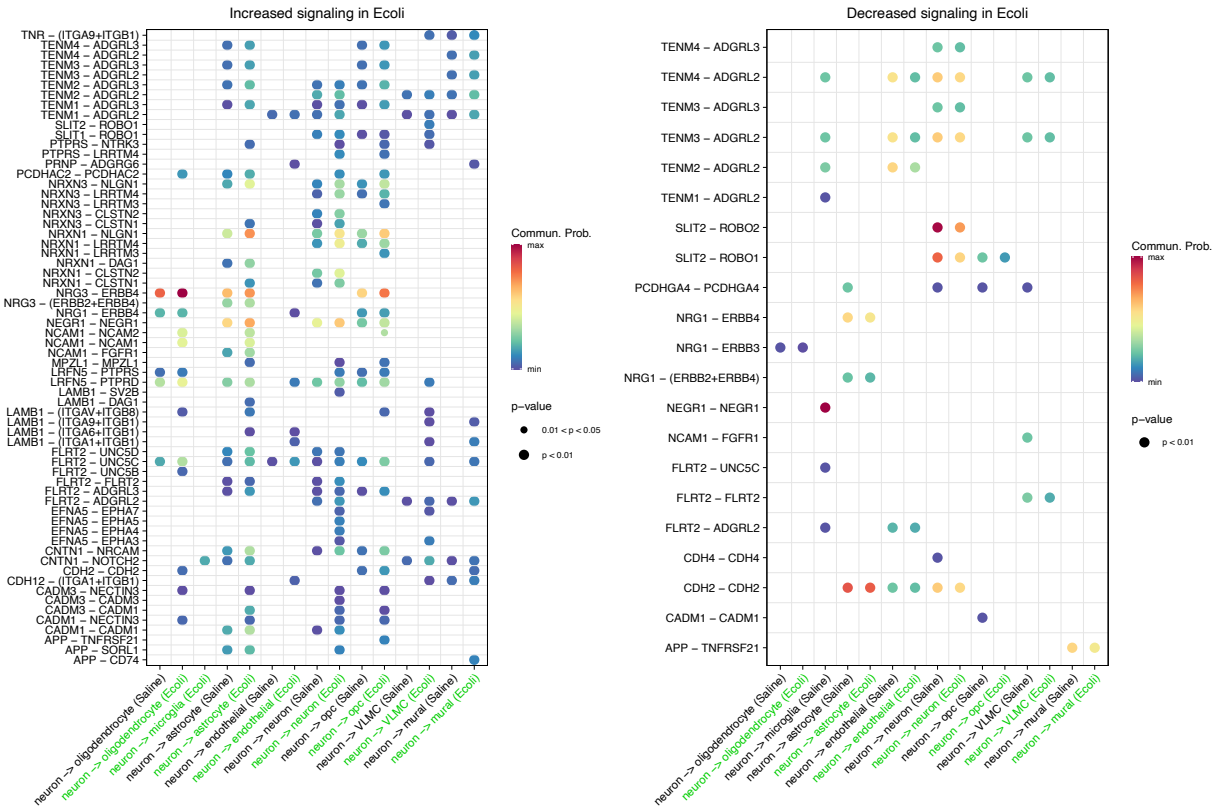


Supplemental Figure 5. Robust transcriptional changes in brain oligodendrocytes, and minimal and no changes in oligodendrocyte precursor cells (OPCs), and neurons during early sepsis. Volcano plots illustrating differential gene expression in brain oligodendrocytes **(A)**, OPCs **(C)**, and neurons **(E)** from *E. coli* septic pigs compared to saline controls. Genes with a false discovery rate (q-value) < 0.05 and an absolute log₂ fold change > 1 are considered statistically significant and are highlighted in blue (downregulated) or red (upregulated). Horizontal dashed lines represent the maximum raw p-value corresponding to a q < 0.05. In oligodendrocytes, there were 23 downregulated and 227 upregulated genes. For OPCs, 5 genes were downregulated, and 36 were upregulated. Neurons showed no significant differential gene expression, and no false discovery threshold line is shown for the neuron population, as no genes reached the significance cutoff of q < 0.05. Metascape pathway analysis bar graphs showing the top 10 most significantly enriched pathways identified by Metascape for both downregulated (left panel) and upregulated (right panel) genes in oligodendrocytes **(B)** and OPCs **(D)**. The length of each bar indicates the gene count within that pathway. The color intensity of the bars represents the significance of the pathway, with darker shades corresponding to lower p-values.



Supplemental Figure 6. Shared and unique cell type pathway alterations in the brain during early sepsis. Heatmaps generated from Metascape multi-gene list analysis showing the most significant **(A)**

upregulated and **(B)** downregulated pathways across all cell types in the brains of septic compared to saline control pigs. The columns represent different cell types, including vascular leptomeningeal cells (VLMC), mural cells, astrocytes, microglia, endothelial cells, oligodendrocytes, and oligodendrocyte precursor cells (OPCs). No differentially expressed genes were detected in the neurons; therefore, neurons are not shown. The rows represent specific biological pathways. The color intensity of each cell in the heatmap indicates the significance of the pathway enrichment, as measured by the negative $\log_{10}$ of the p-value, with darker orange signifying a more significant p-value. This approach helps to identify consistent biological signals among cell types. VLMCs, mural, endothelial, and microglial cells exhibit significant upregulation of pathways related to inflammation and immune response, including cytokine signaling in the immune system, interferon signaling, innate immune response, and cellular responses to cytokine stimuli. There is a broad downregulation of metabolic and structural pathways across several cell types, but with minimal overlap among the cell types. VLMCs and endothelial cells show downregulation of the extracellular matrix organization pathway. Microglia and endothelial cells share a few downregulated pathways, largely involved in metabolic processes.



Supplemental Figure 7. Alterations in neuronal ligand-receptor (L-R) interaction networks during sepsis. Each dot represents a specific ligand-receptor interaction. The y-axis lists individual L-R pairs, while the x-axis indicates the communication direction from neurons to specific target cell types. The color gradient represents the strength of the interaction probability, ranging from low (blue) to high (red). The left plot shows the L-R pairs with significantly higher communication probabilities in the septic group. Notable increases are observed in pathways such as APP–CD74 (associated with neuroinflammation) and various integrin-based interactions (ITGA+ITGB). Decreased signaling in *E. coli* (Right) displays L-R pairs where communication is significantly attenuated during sepsis. This includes the downregulation of structural and synaptic organizing molecules, such as TENM (Teneurin) and ADGRL (Latrophilin) interactions.