Supplementary Material

Screening of Differentially Expressed Genes

We utilized the normalizeBetweenArrays function to normalize RNA sequencing (RNA-seq) data and visualized data distribution using boxplots (**Figure S1**A, E). The principal Components Analyses (PCA) indicated a significant difference between sepsis and control groups in both two gene sets (Supplemental **Figure S1**C, G). Differential gene expression analysis showed 2132 DEGs in GSE100159, including 891 upregulated and 1241 downregulated DEGs (**Figure S1**B, D). While in GSE80496, a total of 947 DEGs were obtained, including 481 upregulated and 466 downregulated DEGs (**Figure S1**H, F).

WGCNA analysis and identification of module eigengenes in GSE80496

The gene clustering tree and trait heatmap was plotted based on RNA Seq data of GSE80496 (**Figure S2** A). β was a soft-thresholding power and set at 10 (**Figure S2**B, C). With the minimum module size of 30 and a threshold merging of 0.25, seventeen modules were generated by the average linkage hierarchical clustering (**Figure S2**D, E). Next, Heatmap of module-trait correlation was used to visualize the correlation between modules eigengenes and the disease state (**Figure S2**F). There were four modules strongly correlated associated with sepsis (**Figure S2**; $p < 0.01$, $|r| > 0.7$). The scatter plot of the correlation between module membership (MM) and gene significance (GS) for state illustrated that blue, red, and light green modules had the highest correlations with sepsis (Supplemental Figure 2 G, H, I; $p < 0.01$, $|r| > 0.8$). A total of 2143 candidate genes were used for the subsequent analysis.

WGCNA analysis and identification of module eigengenes in GSE100159

The RNA-seq data of GSE10095 were processed with the help of a bioinformatics pipeline and processing workflow in the same way as previously described (**Figure S3**A-H). A total of 1244 candidate genes were used for the subsequent analysis.

The test in search of U-shaped associations

A U-shaped relationship was examined using the following formula: $y = β + β_1 x + β_2 x^2$ Note: a significant and positive β2 indicates a U-shaped relationship. In our study, the quadratic regression model showed HIF-1α (β= -0.034, p<0.05) and HIF-1α2 (β=0.00007, p<0.01, Table S1). The results showed that there

was a U-shaped association in septic patients.
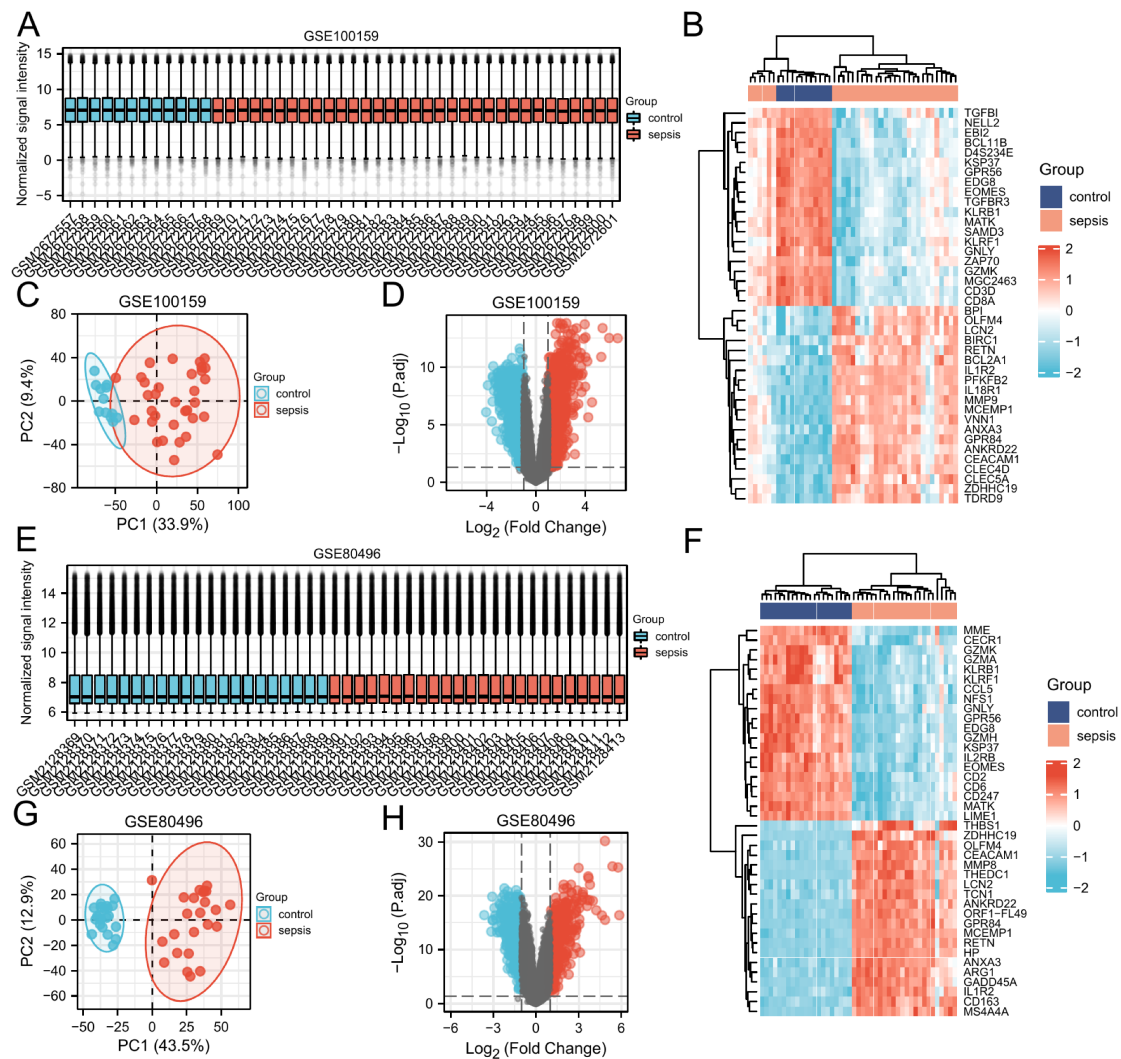
Supplemental Figure 1. Differential gene expression screening. A-D The visualization of GSE100159 gene expression analysis results. (A) The boxplots of normalized gene expression. (B) DEG expression heat map of differentially expressed genes. (C) PCA score plot in control and sepsis groups. (D) A volcano plot of differentially expressed genes. E-F, The visualization of GSE80496 gene expression analysis results. (E) The boxplots of normalized gene expression. (F) DEG expression heat map of differentially expressed genes. (G) PCA score plot in control and sepsis groups. (H) A volcano plot of differentially expressed genes.
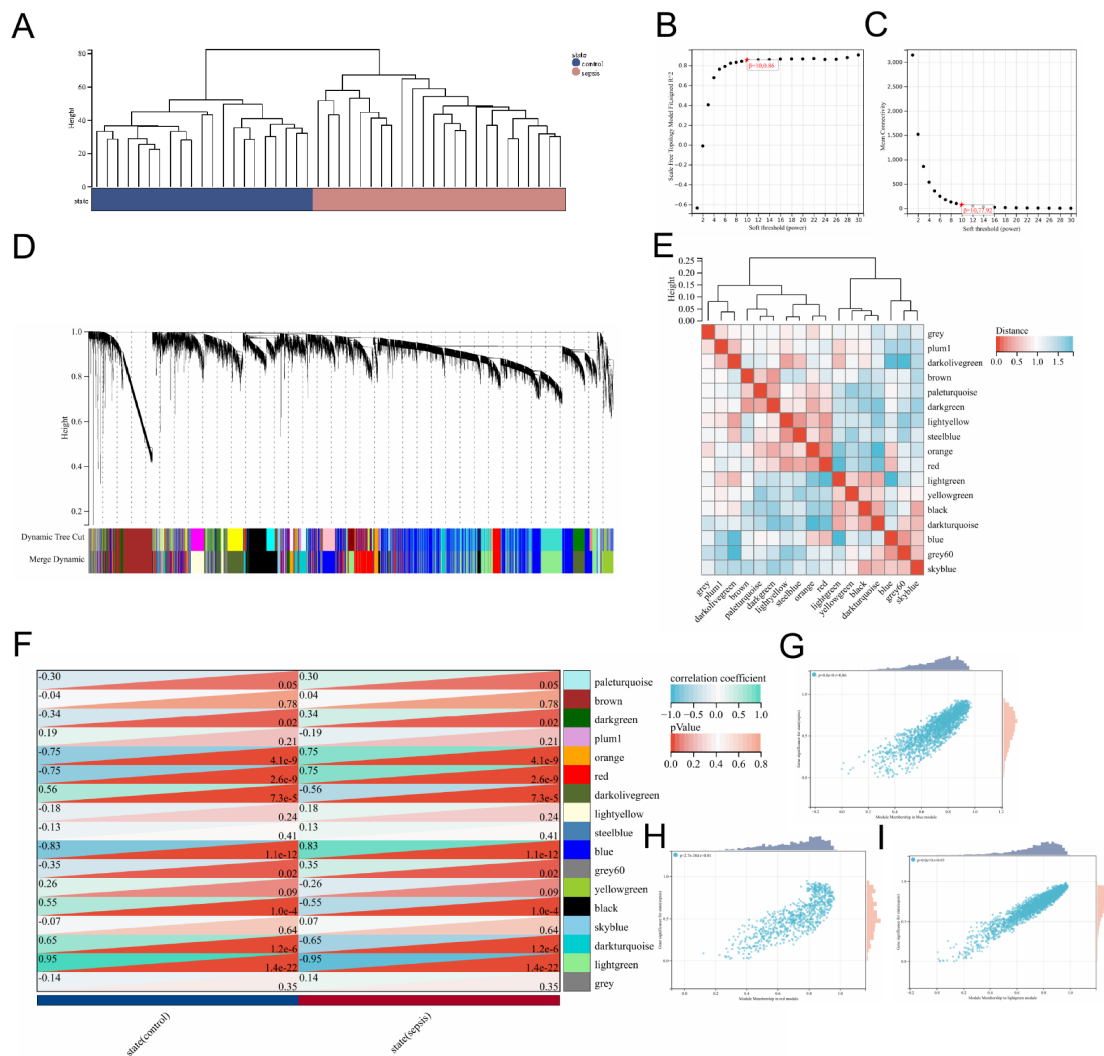
Supplemental Figure 2. The visualization of GSE80496 gene expression WGCNA analysis. (A) The Clustering dendrogram between sepsis and non-sepsis. (B) The plot exhibits a scale-free topology for select soft-thresholding power (β). (C) The mean connectivity under different soft-thresholding power. (D) The cluster dendrogram of RNA-seq data. Branches in the Fig represented genes, and colors below represented co-expression modules. (E) The correlation between module eigengenes and disease state was visualized as a heatmap. Each column shows the correlation and p value. (G-I) A scatter plot of the GS for sepsis and the MM in different modules, including the blue, red, and light green modules (p < 0.01, |r| > 0.8).
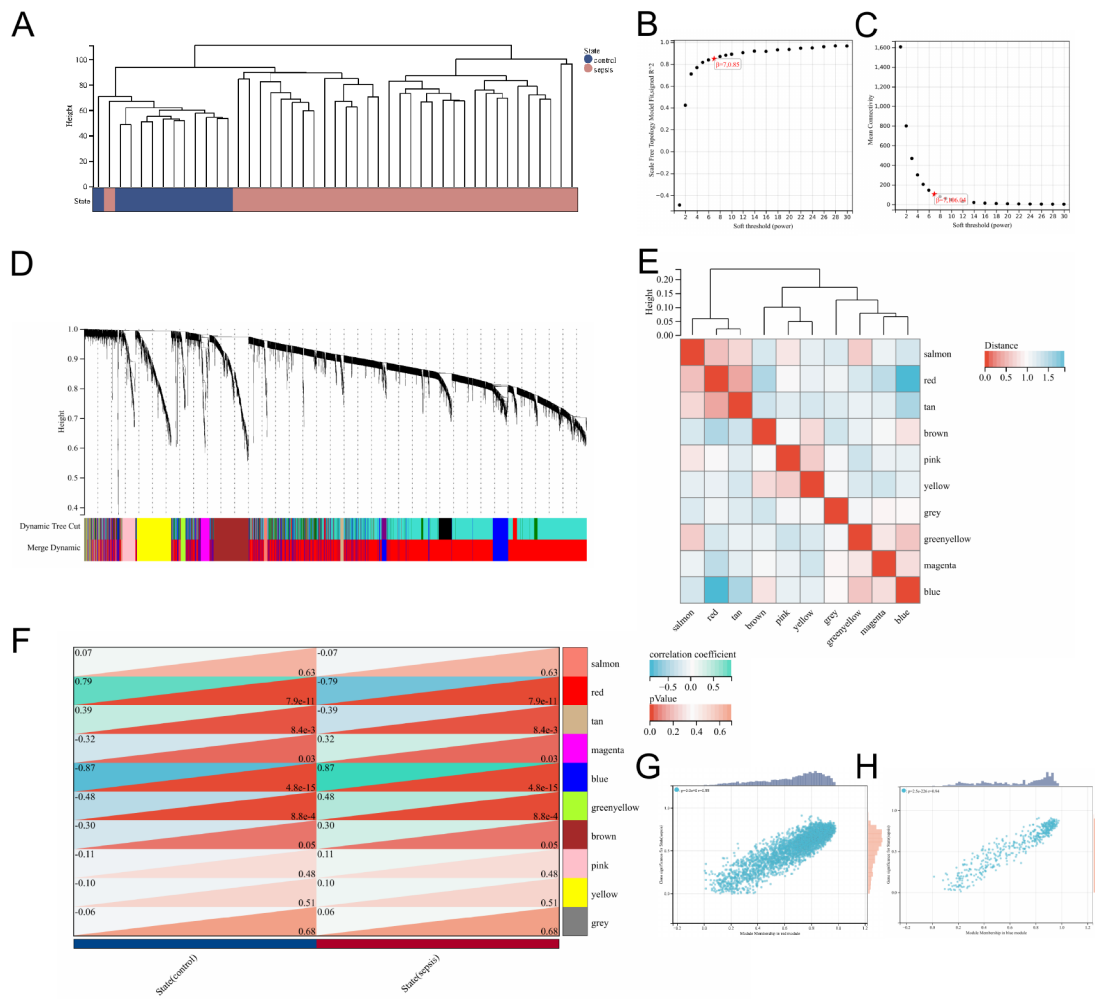
Supplemental Figure 3. The visualization of GSE10095 gene expression WGCNA analysis. (A) The Clustering dendrogram between sepsis and non-sepsis. (B) The plot exhibits a scale-free topology for select soft-thresholding power (β). (C) The mean connectivity under different soft-thresholding power. (D) The cluster dendrogram of RNA-seq data. Branches in the Fig represented genes, and colors below represented co-expression modules. (E) The correlation between module eigengenes and disease state was visualized as a heatmap. Each column shows the correlation and p value. (G-H) A scatter plot of the GS for sepsis and the MM in different modules, including the blue, and red modules (p < 0.01, |r| > 0.8).

Table S1. Tests for U-shaped relationships were performed by including quadratic terms.

Variables	β	SD	Z	p-Value	95% CI
HIF-1α	-0.034	0.141	-2.42	0.015	-0.062 ~ -0.006
HIF-1α^2	0.000	0.000	2.77	0.006	0.000~0.0001

Note: β = coefficient. HIF-1α^2= HIF-1α* HIF-1α