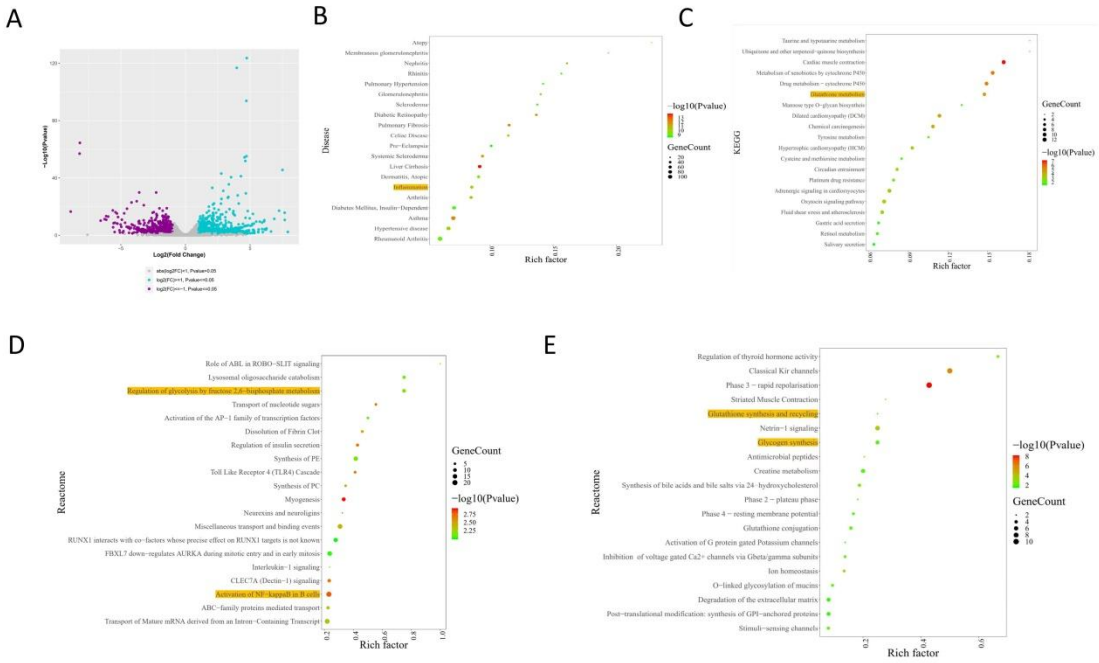
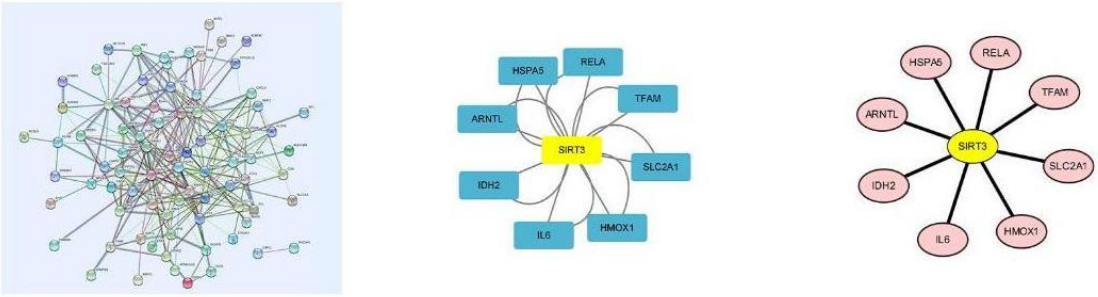
Supplementary Table S1. Primers used for qPCR

Primer name	Sequence
GAPDH Forward	5′-AGGTCGGTGTGAACGGATTTG-3′
GAPDH Reverse	5′-TGTAGACCATGTAGTTGAGGTCA-3′
IL-6 Forward	5′-CCAAGAGGTGAGTGCTTCCC-3′
IL-6 Reverse	5′-CTGTTGTTCAGACTCTCTCCCT-3′
IL-1β Forward	5′-GCAACTGTTCCTGAACTCAACT-3′
IL-1β Reverse	5′-ATCTTTTGGGGTCCGTCAACT-3′
TNF-α Forward	5′-GACGTGGAACTGGCAGAAGAG-3′
TNF-α Reverse	5′-TTGGTGGTTTGTGAGTGTGAG-3′

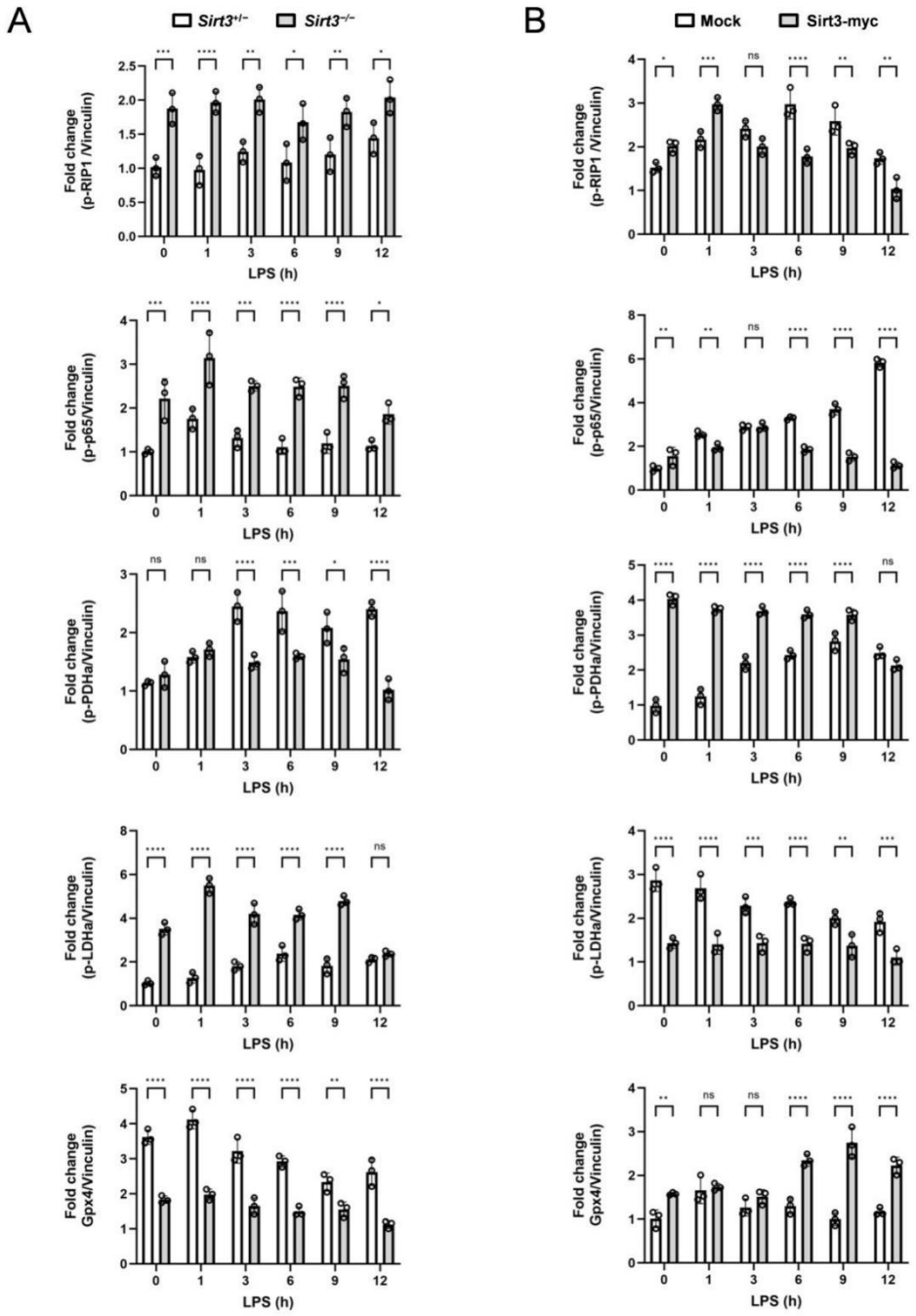


Supplementary Figure S1. RNA-seq sequencing the lung tissue of *Sirt3*$^{-/-}$ mice and *Sirt3*$^{+/-}$ mice. (A) Volcano plot, blue dots for up-regulated genes, and purple dots for down-regulated genes. (B) Disease enrichment analysis for upregulated genes was conducted using the DisGeNET database via the clusterProfiler R package. (C) KEGG enrichment analysis for down-regulated genes. (D) Reactome enrichment analysis for up-regulated genes. (E) Reactome enrichment analysis for down-regulated genes. RNA sequencing was performed on lung tissues from *Sirt3*$^{-/-}$ mice and *Sirt3*$^{+/-}$ mice

within the ALI model (n=3/per group). Pathway enrichment analysis in this study was performed by first identifying all differentially expressed genes (DEGs) between experimental and control groups using the DESeq2 R package, with screening thresholds set at $P \leq 0.05$ and $|\log2FoldChange| \geq 1$, yielding 1,926 significant DEGs. These DEGs were subsequently grouped by expression direction (upregulated/downregulated) and subjected to separate KEGG and Reactome enrichment analyses through the clusterProfiler R package. The core algorithm employed the hypergeometric test using all detected genes as background, with statistical significance ensured by Benjamini-Hochberg correction (FDR < 0.05).



Supplementary Figure S2. PPI network found that Sirt3, Gpx4 and RELA were involved in the protein interactions of ferroptosis in ALI PPI Network. GSE3037 from the GEO database was analyzed using the R project (R version 4.2.1), gene expression profiles of ALI and normal tissues were compared to identify DEGs, and *t*-test was used to determine *P*-value and adjusted *P*-value in DGE analysis. Genes retained from diverse tissues were selected using the following criteria: adjusted *P*-value < 0.05， and log2 FC > 1. 231 ferroptosis genes were obtained from the ferroptosis database (FerrDb; zhounan.org) and intersected with the above DEGs to identify ferroptosis-related DEGs. The STRING online database (https://string-db.org/) and Cytoscape software (version 3.9.1) were used to analyze the interaction among ferroptosis-related DEGs.



Supplementary Figure S3. Quantitative analysis of WB bands in Figure 2D-E (A) Subsequent quantitative analysis of p-RIP1, p-p65, p-LDHa, p-PDHa and Gpx4 expression in *Sirt3*$^{+/-}$ and *Sirt3*$^{-/-}$ AEC Ⅱ (Figure 2D). (B) Subsequent quantitative analysis of p-RIP1, p-p65, p-LDHa, p-PDHa and Gpx4 expression in Sirt3 overexpressed MLE-12 cells (Figure 2E). Results are expressed as protein/Vinculin ratio. Data were presented as mean ± SD of three independent experiments. ($^{*}P$ < 0.05, $^{**}P$ < 0.01, $^{***}P$ < 0.001, $^{****}P$ < 0.0001)