Supporting Materials

Integrative transcriptomics and metabolomics explore the mechanism of kamepferol on improving non-alcoholic steatohepatitis

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Results:

Kaempferol altered genes in the liver of HFD-NASH mice

The DEGs were identified significant variations in transcriptional expression with more than 2-fold change (P<0.05; Supplementary Figure S2). Many DEGs among three groups were annotated with GO terms. Among three GO functional categories including biological process (BP), cellular component (CC) and molecular function (MF), the proportions of DEGs in corresponding subcategories were mainly consistent with each other (Supplementary Figure S3).

According to the defined filtering criteria, 159 genes and 758 genes were detected to be up-regulated and down-regulated in model group compared with control group respectively, while in kaempferol group 632 up-regulated genes and 37 down-regulated genes were achieved compared with the model group. Compared with the control group, 422 genes were up-regulated and 172 genes were down-regulated(P<0.05; Supplementary Tables S1).

The GO database was used to understand the effect of kaempferol on gene expression in the HFD-induced NASH models. The top 10 significant GO classification of DEGs among three groups were presented in Supplementary Figure S4-5 and Supplementary Table S2. Most of the enriched BP terms of up-regulated DEGs were involved in cellular response to chemical stimulus (Supplementary Figure S4A and S5A) while the down-regulated DEGs were mainly categorized into small molecule metabolic process (Supplementary Figure S5B). Interestingly, this is consistent with the up-regulated functions in the kaempferol group (Supplementary Figure S4B and S5G). Respectively, the down-regulated DEGs were mainly involved in response to stilbenoid, long-chain fatty acid metabolic process and epoxygenase P450 pathway in kaempferol group (Supplementary Figure S5J). Meanwhile, the up-regulated DEGs in CC were mainly involved in endoplasmic reticulum (ER) (Supplementary Figure S5B) and the down-regulated DEGs were mainly involved in cytoplasm, cytoplasmic part and intracellular (Supplementary Figure S5E). Interestingly, this is similar to the up-regulated functions in the kaempferol group

(Supplementary Figure S5H). The down-regulated DEGs were mainly involved in membrane-bounded organelle, intracellular part and intracellular in kaempferol groups (Supplementary Figure S4A and S5C), respectively. The same regulatory effect is also reflected in MF(Supplementary Figure S5I). The up-regulated DEGs were mainly involved in oxidoreductase activity (Supplementary Figure S4A), which is the main function of down-regulated DEGs in kaempferol group (Supplementary Figure S4B). The down-regulated DEGs were mainly involved in catalytic activity and were also function of up-regulated DEGs in kaempferol groups (Supplementary Figure S4B). Another interesting phenomenon is that the GO function of the DEGs whatever up-regulated and down-regulated in the K and C groups is the same(Supplementary Figure S4C and S5M-R).

Kaempferol altered gene pathways in the liver of HFD-NASH mice

According to the KEGG database, biological pathways were classified into different items, and specific functional items related to DEGs. 59 differential gene pathways in the model group were annotated including 24 up-regulated and 35 down-regulated pathways compared with the control group were shown in Supplementary Figure S5 and Supplementary Table S3. The up-regulated differential pathways were mainly involved in protein processing in ER, steroid hormone biosynthesis and retinol metabolism (Supplementary Figure S5A), Interestingly, this is consistent with main down-regulated pathways in the kaempferol group. Down-regulated differential pathways were mainly involved in carbon metabolism, peroxisome and fatty acid degradation (Supplementary Figure S5B). Compared with the model group, 53 differential gene pathways in the kaempferol group were annotated including 36 up-regulated and 17 down-regulated pathways. The up-regulated differential pathways were mainly involved in ribosome, insulin signaling pathway and glucagon signaling pathway (Supplementary Figure S5C), while down-regulated differential pathways were mainly involved in steroid hormone biosynthesis, retinol metabolism and chemical carcinogenesis (Supplementary Figure S5D).

Kaempferol restored the serum metabolic perturbations of NASH

There was a slight separation but not significant trend in the PCA score plots

among three groups (Supplementary Figure S6A and D). In order to find out different metabolites related to NASH and the improvement of kaempferol, the supervised OPLS-DA was used for pattern recognition analysis of serum samples between two groups (Supplementary Figure S6B and E). Results from cross validation suggested the model was robust and with good predictabilities (Supplementary Figure S6C and F). Combined with the Wilcox test analysis, 32 serum metabolites were revealed to show significant differences between control and model group (P<0.001 and FC>1.5, Supplementary Tables S4). Finally, 6 metabolic pathways (impact value>0.1) were involved in phenylalanine, tyrosine and tryptophan biosyntheses, methane metabolism, arachidonic acid metabolism, glycine, serine and threonine metabolism, tyrosine metabolism, aminoacyl-tRNA biosynthesis (Supplementary Figure S7A, Table S5). Similarly, 27 metabolites showed significant differences between model group and kaempferol group (P<0.05 and FC>1.5, Supplementary Tables S4). Finally, 2 metabolic pathways (impact value>0.1) were involved in alpha linolenic acid and linoleic acid metabolism and citric acid cycle (Supplementary Figure S7B, Table S5). Meanwhile,17 metabolites showed significant differences between kaempferol group and control group (P<0.05 and FC>1.5, Supplementary Tables S4). Finally, 12 metabolic pathways (impact value>0.1) were involved in phenylalanine metabolism (Supplementary Figure S7C, Table S5).

Kaempferol restored the liver metabolic perturbations of NASH

The PCA, OPLS-DA and cross validation based on liver metabolomics were shown in Supplementary Figure S8. In the same way, 47 metabolites showed significant differences between model group and kaempferol group based on Wilcox test analysis (P<0.001 and FC>1.5, Supplementary Tables S4) and 10 metabolic pathways (impact value >0.1) are found as follows: glycine, serine and threonine metabolism, phenylalanine, tyrosine and tryptophan, β-alanine metabolism, phenylalanine metabolism, methane metabolism, valine, leucine and isoleucine biosynthesis, alanine, aspartate and glutamate metabolism, aminoacyl-RNA biosynthesis, cysteine and methionine metabolism, tryptophan metabolism (Supplementary Figure S9A, Table S5). Similarly, 5 metabolites showed significant

differences between model group and kaempferol group (P<0.05 and FC>1.5, Supplementary Tables S4) and 2 metabolic pathways (impact value>0.1) are as follows: phenylalanine, tyrosine and tryptophan biosynthesis, phenylalanine metabolism (Supplementary Figure S9B, Table S5). 57 metabolites also showed significant differences between model group and kaempferol group (P<0.05 and FC>1.5, Supplementary Tables S4) and 37 metabolic pathways (impact value>0.1) are as follows: ubiquinone and other terpenoid-quinone biosynthesis (Supplementary Figure S9B, Table S5).

Figure S1. Liver Status and weight differences of Mice in three groups**Figure S2.** Comparison of gene expression among three groups

Figure S3. Gene ontology (GO) classification of differential expressed genes (DEGs) among three groups

Figure S4. The top 10 regulated gene ontology (GO) analysis of differentially expressed genes (DEGs) among three groups

Figure S5. Regulated pathways of differentially expressed genes (DEGs) among three groups through Kyoto encyclopedia of genes and genomes (KEGG) analysis

Figure S6. The principal components analysis (PCA), orthogonal partial least square discriminant analysis (OPLS-DA) and cross validation of serum metabolic profiles among three groups

Figure S7. Metabolic pathway analysis of serum samples among three groups

Figure S8. The principal components analysis (PCA), orthogonal partial least square discriminant analysis (OPLS-DA) and cross validation of liver metabolic profiles among three groups

Figure S9. Metabolic pathway analysis of liver samples among three groups

Figure S10. Comparison of characteristic serum metabolites significantly among three groups

Figure S11. Comparison of characteristic liver metabolites significantly among three groups

Table S1. List of differentially expressed genes (DEGs) among three groups

Table S2. The top 10 regulated gene ontology (GO) classification among three groups

Table S3. The regulated pathways of differentially expressed genes (DEGs) among three groups through Kyoto encyclopedia of genes and genomes (KEGG) analysis

Table S4. Regulated metabolites among three groups in serum and liver

Table S5. Result from pathway analysis with Metaboanalyst Pathways Analysis (MetPA) on metabolic pathway among three groups

Figure Legends

Figure S1. Liver Status and weight differences of Mice in three groups

C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S2. Comparison of gene expression among three groups.

(A)Scatterplot matrix comparison expression among three groups. Red dot represents up-regulated gene while green dot represents down-regulated gene. (B) Volcano plot among three groups. (C) Clustering analysis among three groups. C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S3. Gene ontology (GO) classification of differential expressed genes (DEGs) among three groups.

The DEGs were classified into 3 functional categories: biological process, cellular component and molecular function. (A) Differential genes between M and C group. (B) Differential genes between M and K groups. (C) Differential genes between K and C group. C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S4. The top 10 regulated gene ontology (GO) analysis of differential expressed genes (DEGs) among three groups.

(A) The up-regulated differential genes in BP between C group and M group. (B) The up-regulated differential genes in CC between C group and M group. (C)The up-regulated differential genes in MF between C group and M group. (D) The down-regulated differential genes in BP between C group and M group. (E) The down-regulated differential genes in CC between C group and M group. (F) The down-regulated differential genes in MF between C group and M group. (G) The up-regulated differential genes in BP between M group and K group. (H) The up-regulated differential genes in CC between M group and K group. (I) The up-regulated differential genes in MF between M group and K group. (J) The down-regulated differential genes in BP between M group and K group. (K) The down-regulated differential genes in CC between M group and K group. (L) The down-regulated differential genes in MF between M group and K group. (M) The up-regulated differential genes in BP between K group and C group. (N) The up-regulated differential genes in CC between K group and C group. (O) The up-

regulated differential genes in MF between K group and C group. (P) The down-regulated differential genes in BP between K group and C group. (Q) The down-regulated differential genes in CC between K group and C group. (R) The down-regulated differential genes in MF between K group and C group. C: Control Group; M: Model Group; K: Kaempferol Group; BP, biological process; CC, cellular component; MF, molecular function.

Figure S5. Regulated pathways of the top 10 differential expressed genes (DEGs) among three groups through Kyoto encyclopedia of genes and genomes (KEGG) analysis.

(A) Up-regulated KEGG pathway of DEGs between C group and M group. (B) Down-regulated KEGG pathway of DEGs between C group and M group. (C) Up-regulated KEGG pathway of DEGs between M group and K group. (D) Down-regulated KEGG pathway of DEGs between M group and K group. (E) Up-regulated KEGG pathway of DEGs between K group and C group. (F) Down-regulated KEGG pathway of DEGs between K group and C group. C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S6. The principal components analysis (PCA), orthogonal partial least square discriminant analysis (OPLS-DA) and cross validation of serum metabolic profiles among three groups.

(A)The PCA between C group and M group; (B) the OPLS-DA between C group and M group. (C)The cross validation between C group and M group. (D) The PCA between C group and M group; (E) The OPLS-DA between C group and M group. (F) The cross validation between C group and M group. C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S7. Metabolic pathway analysis of serum samples among three groups

(A) Metabolic pathway analysis of serum sample between C group and M group. (B) Metabolic pathway analysis of liver sample between M group and K group. (1) Phenylalanine, tyrosine and tryptophan biosynthesis (2) Methane metabolism (3) Arachidonic acid metabolism (4) Glycine, serine and threonine metabolism (5) Tyrosine metabolism (6) Aminoacyl-tRNA biosynthesis (7) Alpha Linolenic Acid and

Linoleic Acid Metabolism (8) Citric Acid Cycle (9) Arachidonic acid metabolism (10) Phenylalanine metabolism (11) Glycine, serine and threonine metabolism (12) Aminoacyl-tRNA biosynthesis C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S8. The principal components analysis (PCA), orthogonal partial least square discriminant analysis (OPLS-DA) and cross validation of liver metabolic profiles among three groups.

(A)The PCA between C group and M group. (B) The OPLS-DA between C group and M group. (C)The cross validation between C group and M group. (D)The PCA between C group and M group. (E) The OPLS-DA between C group and M group. (F)The cross validation between C group and M group. C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S9. Metabolic pathway analysis of liver samples among three groups.

(A)Metabolic pathway analysis of liver sample between C group and M group. (B) Metabolic pathway analysis of liver sample between M group and K group. (1) Glycine, serine and threonine metabolism (2) Phenylalanine, tyrosine and tryptophan (3) beta-Alanine metabolism (4) Phenylalanine metabolism (5) Methane metabolism (6) Valine, leucine and isoleucine biosynthesis (7) Alanine, aspartate and glutamate metabolism (8) Aminoacyl-tRNA biosynthesis (9) Cysteine and methionine metabolism (10) Tryptophan metabolism (11) Phenylalanine, tyrosine and tryptophan biosynthesis (12) Phenylalanine metabolism. (13) Ubiquinone and other terpenoid-quinone biosynthesis (14) Glycine, serine and threonine metabolism (15) Starch and sucrose metabolism (16) Alanine, aspartate and glutamate metabolism (17) Pyruvate metabolism (18) Arginine biosynthesis (19) Cysteine and methionine metabolism (20) Aminoacyl-tRNA biosynthesis (21) Glyoxylate and dicarboxylate metabolism (22) Pentose and glucuronate interconversions (23) Glycolysis / Gluconeogenesis. C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S10. Comparison of characteristic serum metabolites significantly among three groups. Values represent the mean ± SD. One-way ANOVA was used, * P<0.05, ** P<0.01, *** P<0.001, ****P<0.0001, represents vs control group. #

P<0.05, ## *P*<0.01, ### *P*<0.001, represents vs model group. C: Control Group; M: Model Group; K: Kaempferol Group.

Figure S11. Comparison of characteristic liver metabolites significantly among three groups. Values represent the mean ± SD. One-way ANOVA was used, * *P*<0.05, ** *P*<0.01, *** *P*<0.001, ****P*<0.0001, represents vs control group. # *P*<0.05, ## *P*<0.01, ### *P*<0.001, represents vs model group. C: Control Group; M: Model Group; K: Kaempferol Group.

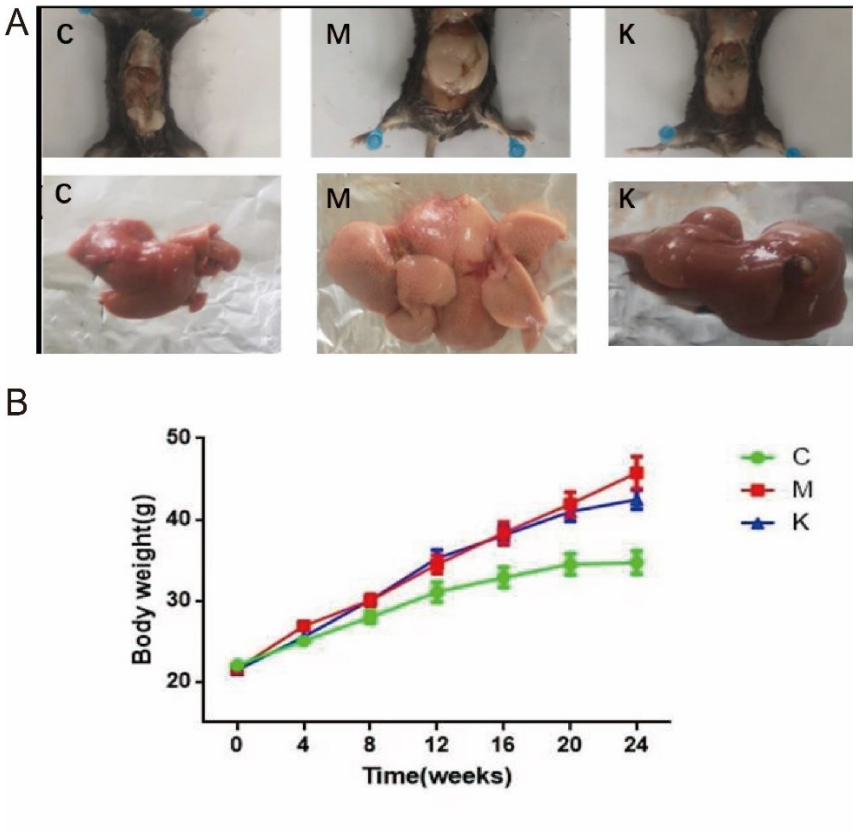

Figure S1. Liver Status of Mice in three groups

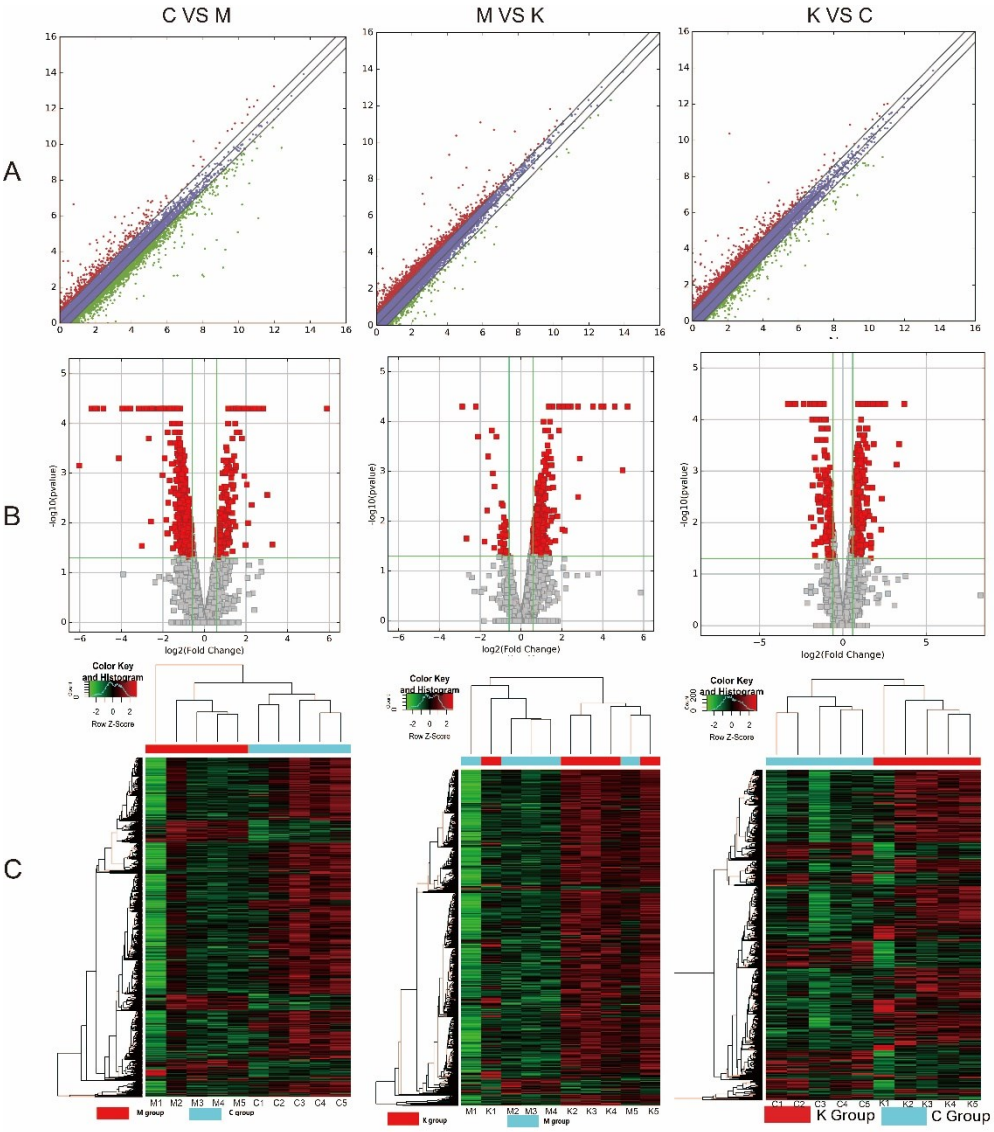

Figure S2. Comparison of gene expression among three groups

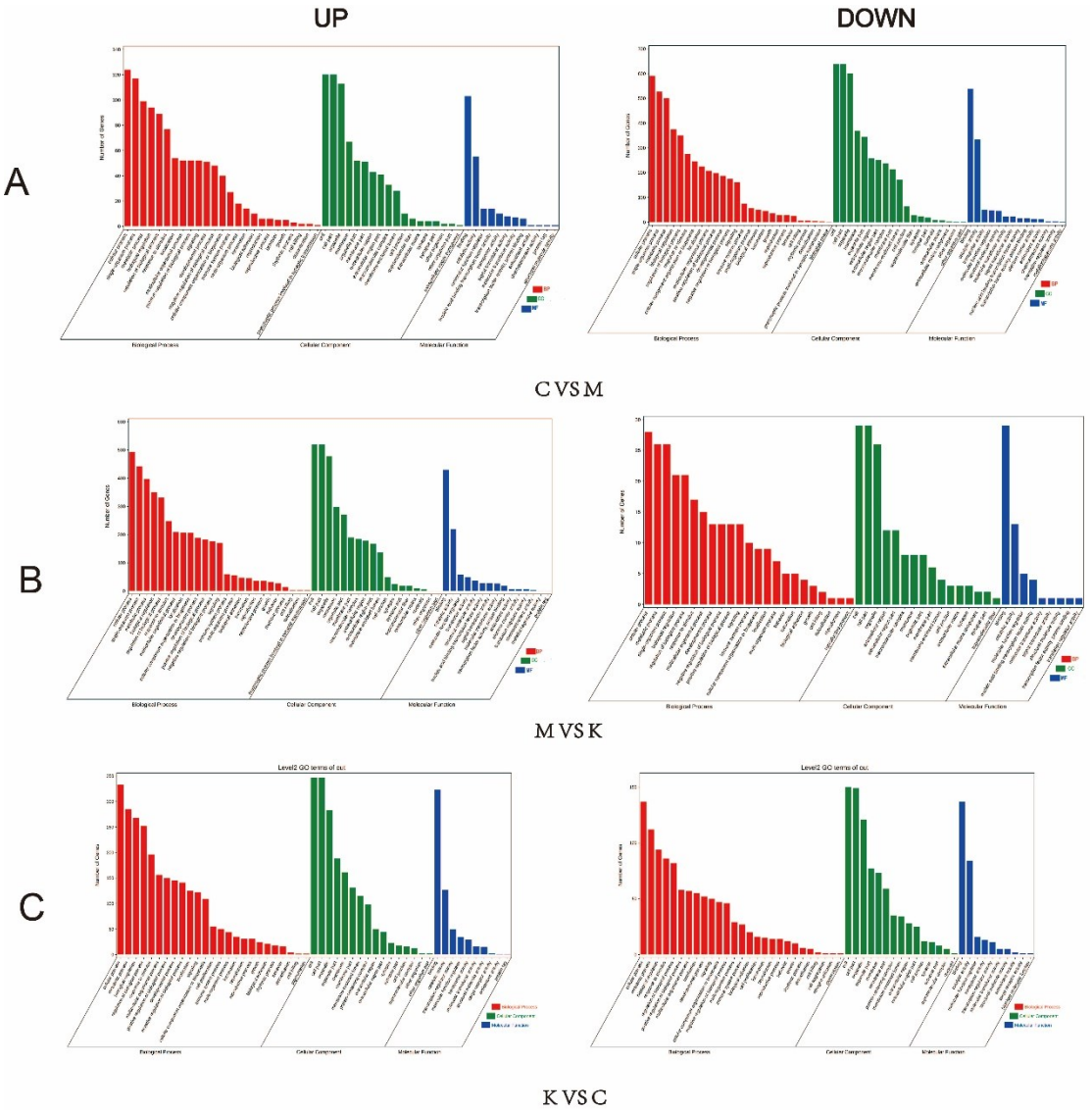

Figure S3. Gene ontology (GO) classification of differential expressed genes (DEGs) among three groups

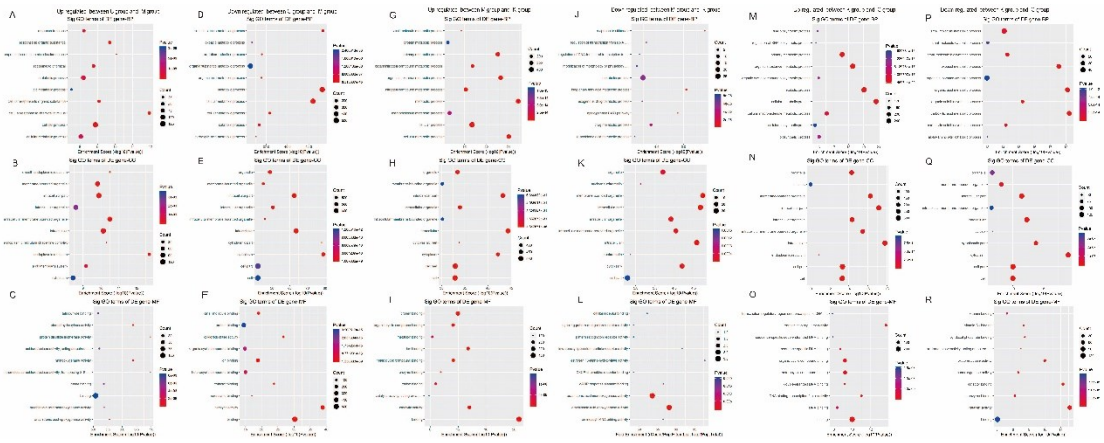

Figure S4. The top 10 regulated gene ontology (GO) analysis of differential expressed genes (DEGs) among three groups

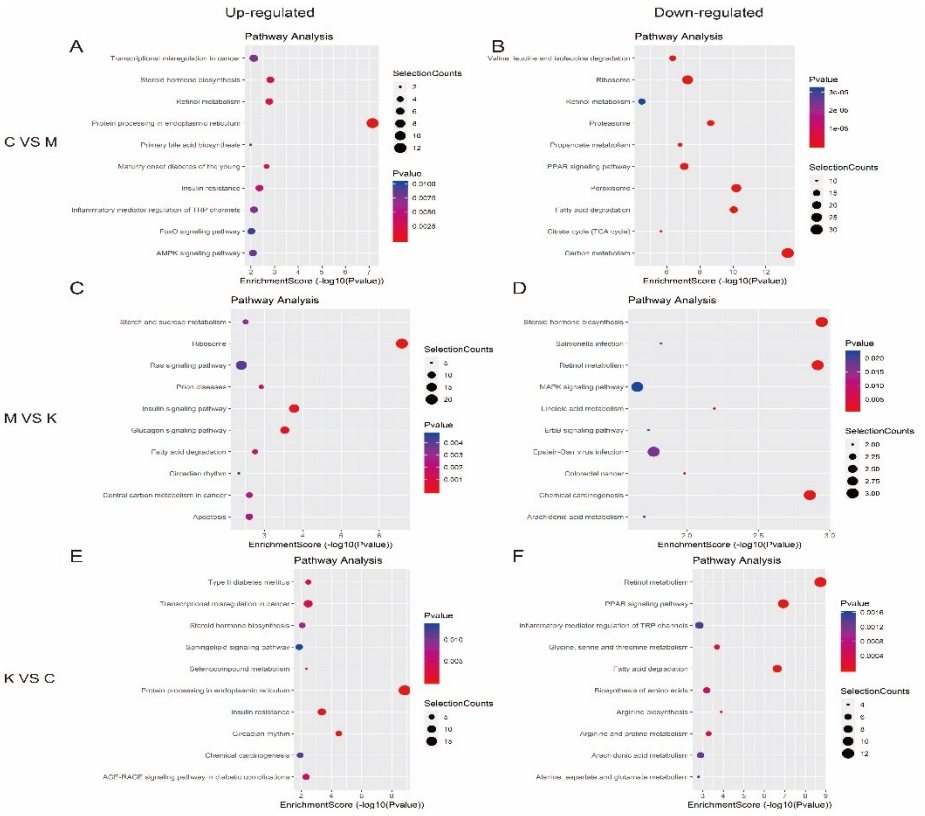

Figure S5. Regulated pathways of the top 10 differential expressed genes (DEGs) among three groups through Kyoto encyclopedia of genes and genomes (KEGG) analysis

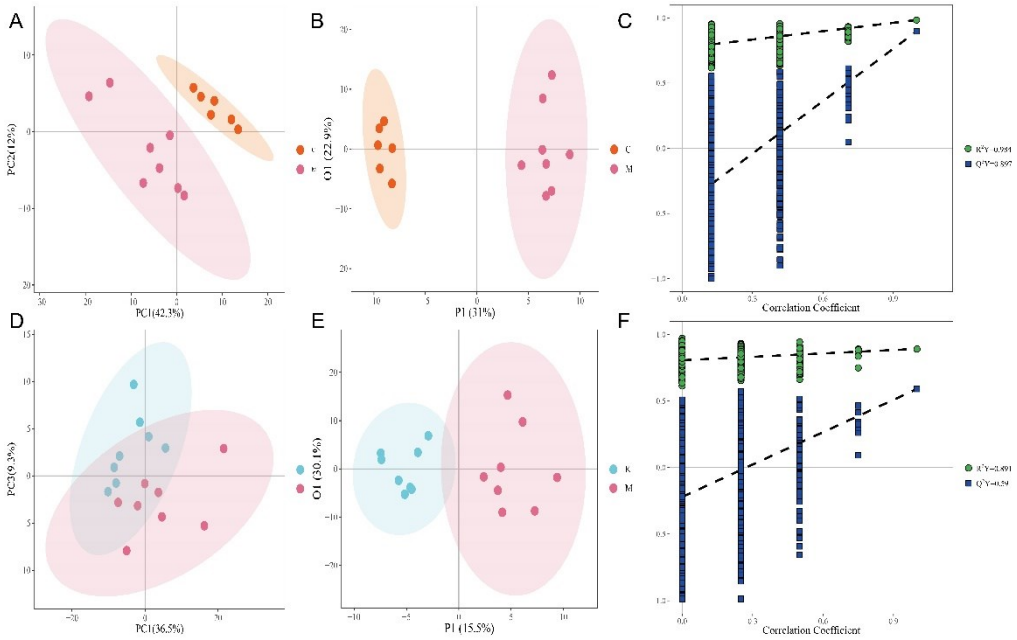

Figure S6. The principal components analysis (PCA), orthogonal partial least square discriminant analysis (OPLS-DA) and cross validation of serum metabolic profiles among three groups

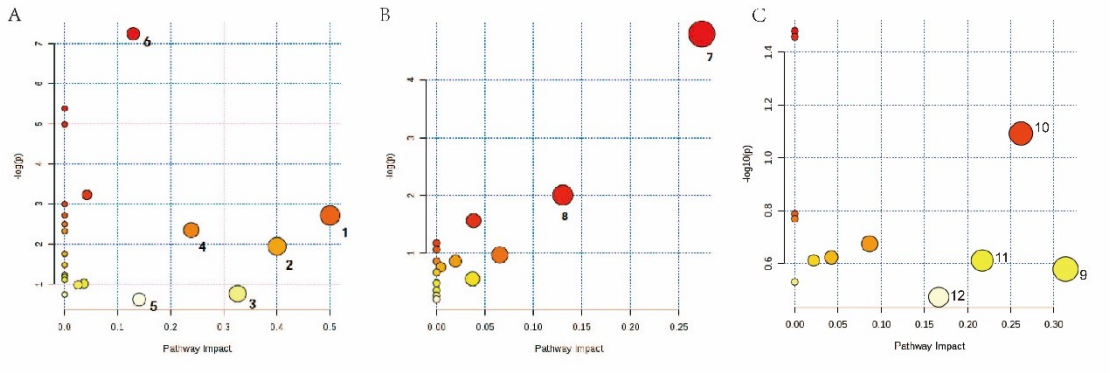

Figure S7. Metabolic pathway analysis of serum samples among three groups

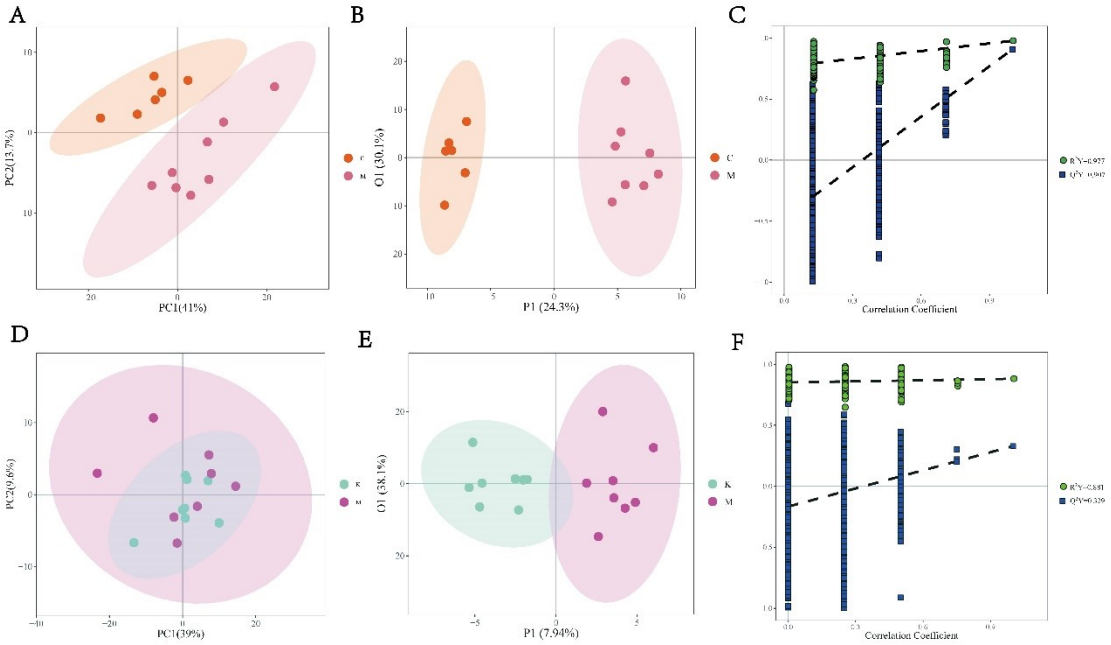

Figure S8. The principal components analysis (PCA), orthogonal partial least square discriminant analysis (OPLS-DA) and cross validation of liver metabolic profiles among three groups

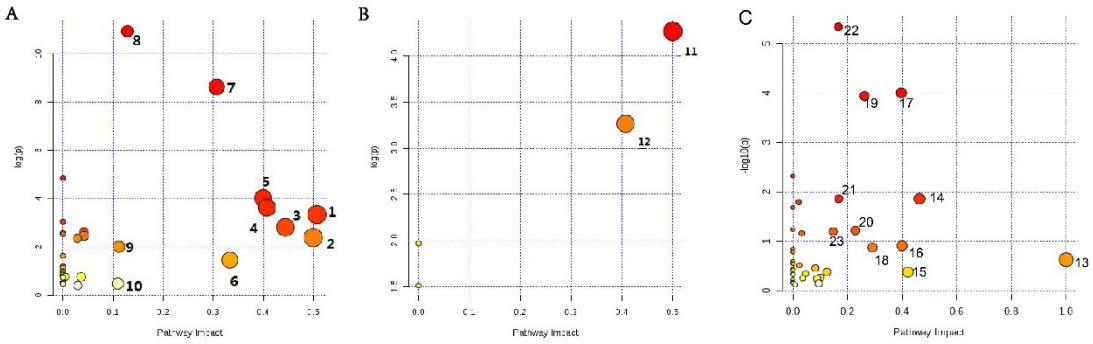

Figure S9. Metabolic pathway analysis of liver samples among three groups

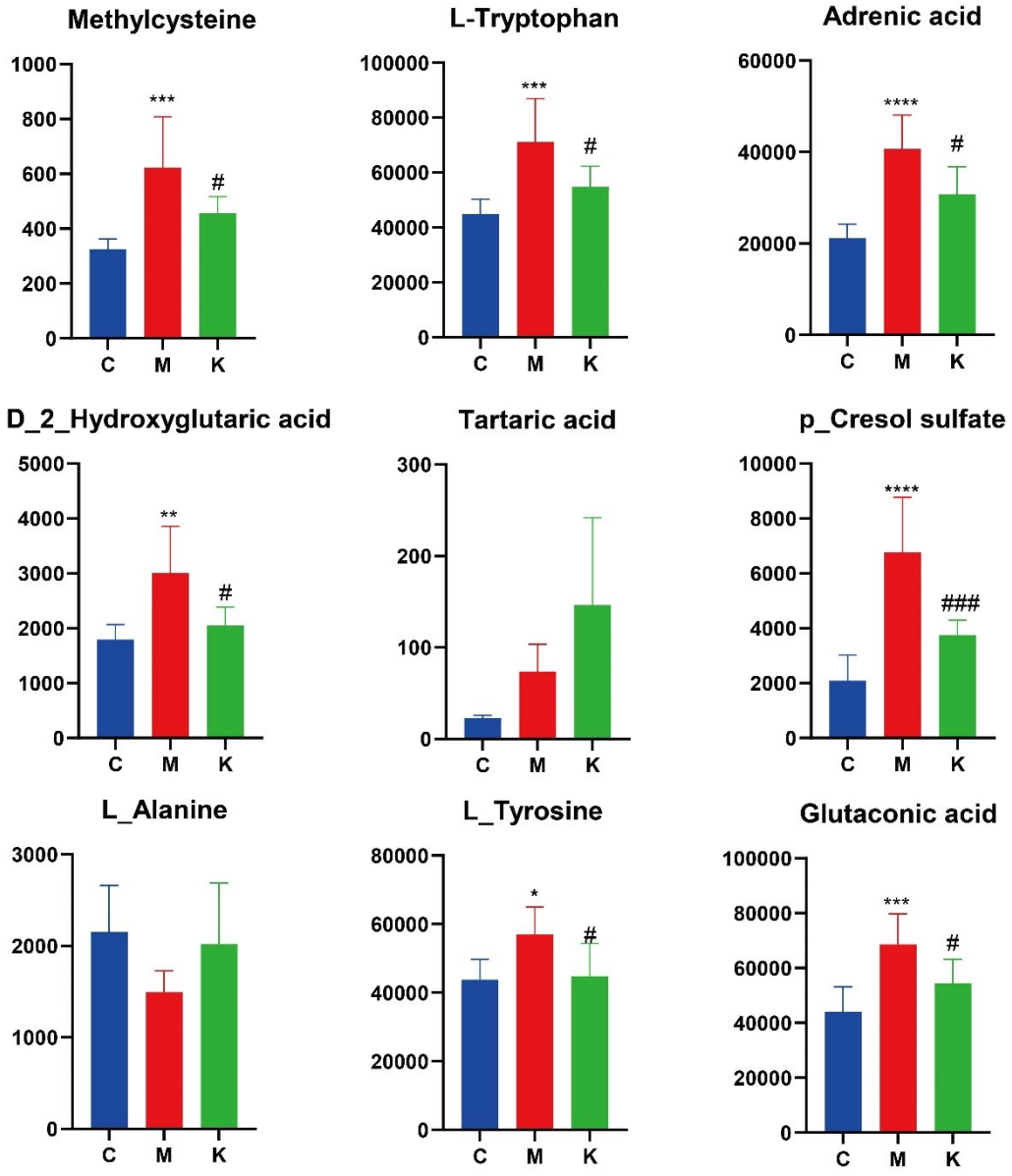

Figure S10. Comparison of characteristic serum metabolites significantly among

three groups

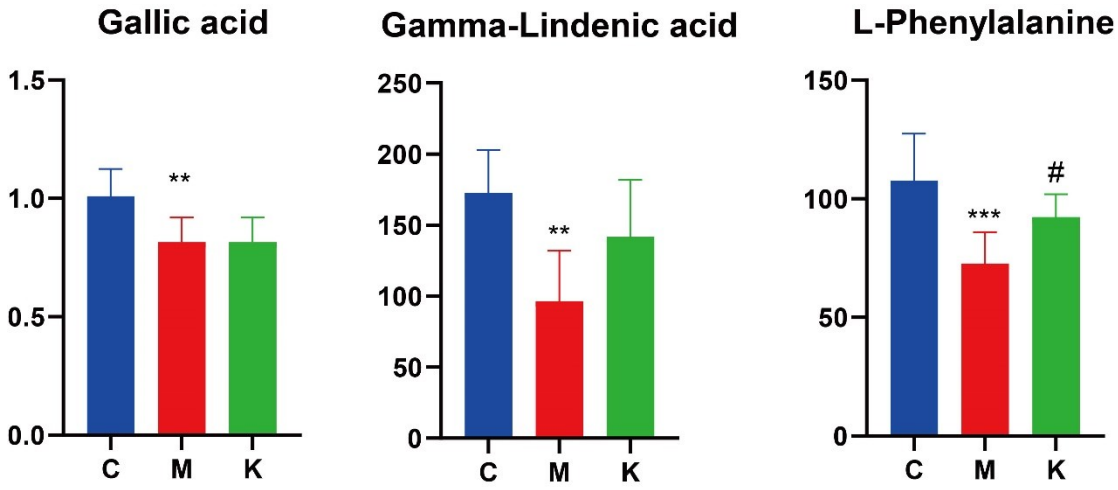

Figure S11. Comparison of characteristic liver metabolites significantly among three groups