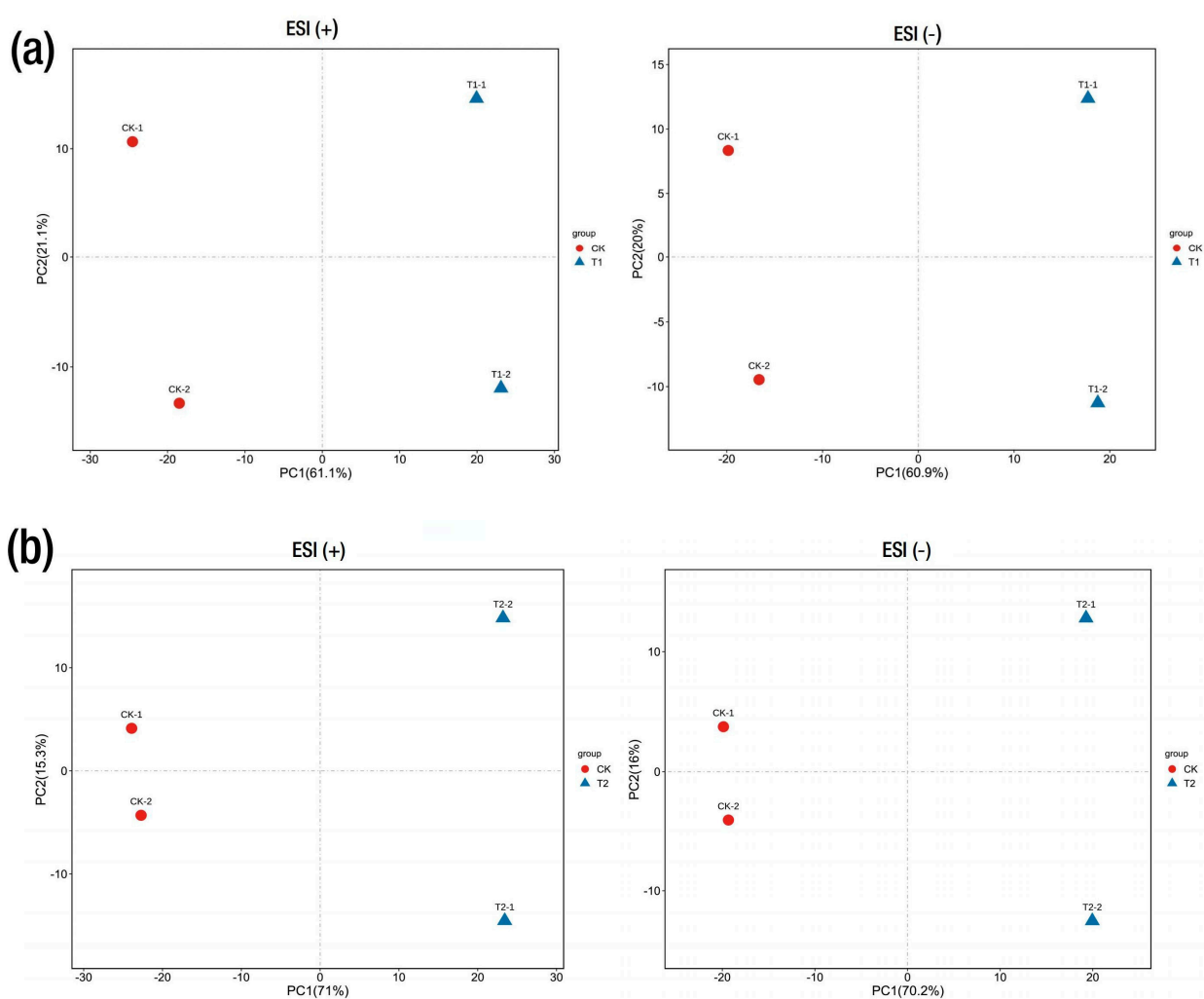


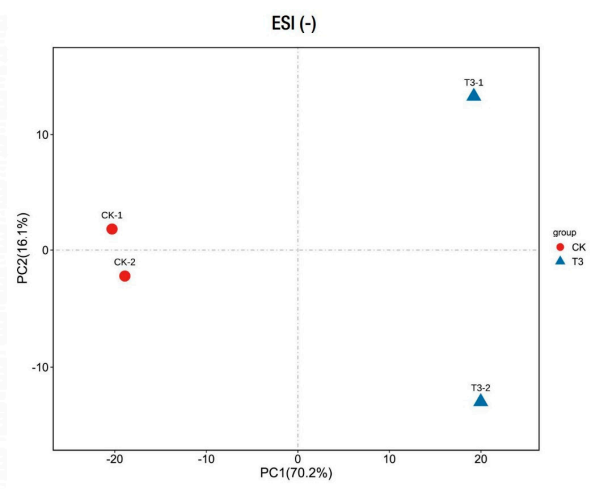
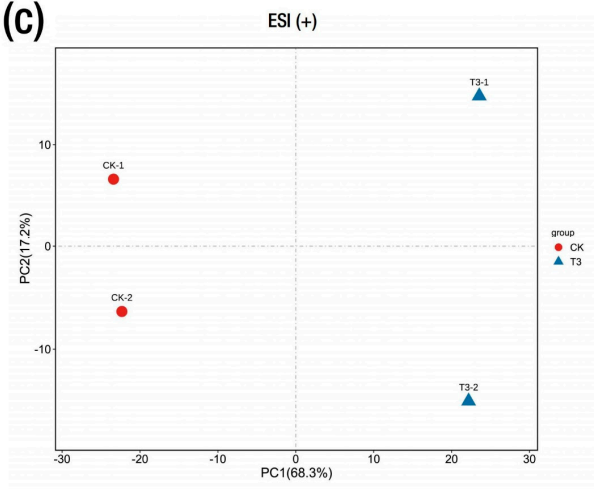
# Magnesium Nutrient Application Induces Metabolomics and Physiological Responses in Mulberry (*Morus alba*) Plants

Xin Jin<sup>1</sup>, Michael Ackah<sup>1,2\*</sup>, Lei Wang<sup>1</sup>, Frank Kwarteng Amoako<sup>3</sup>, Yisu Shi<sup>1</sup>, Lionnelle Gyllye Essoh<sup>1</sup>, Jianbin Li<sup>1</sup>, Qiaonan Zhang<sup>1</sup>, Haonan Li<sup>1</sup>, Weiguo Zhao<sup>1\*</sup>

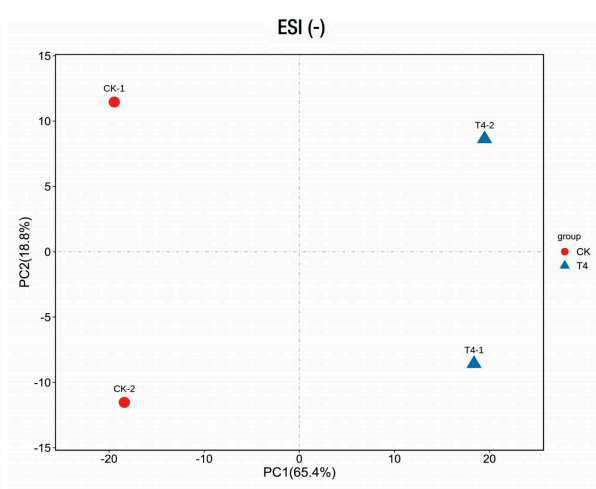
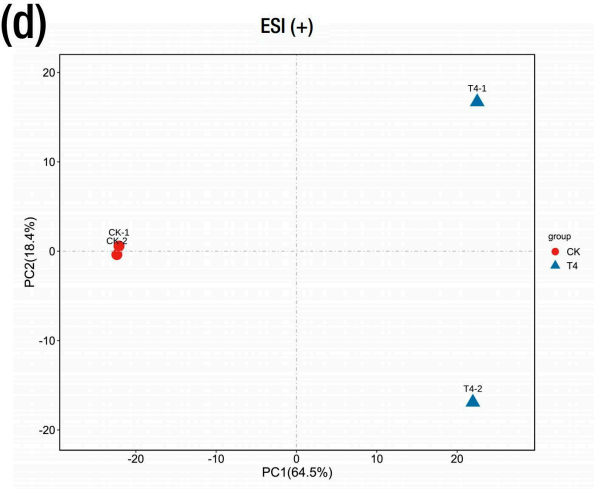
## Supplementary figures



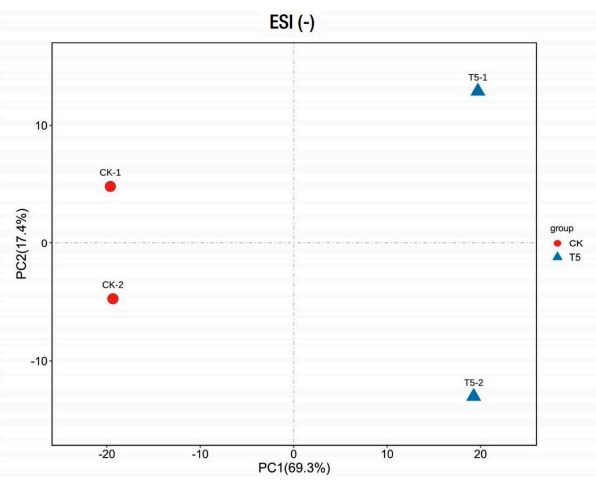
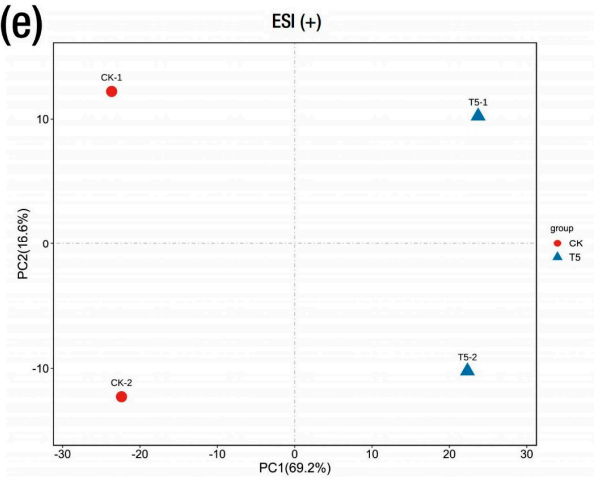
(c)



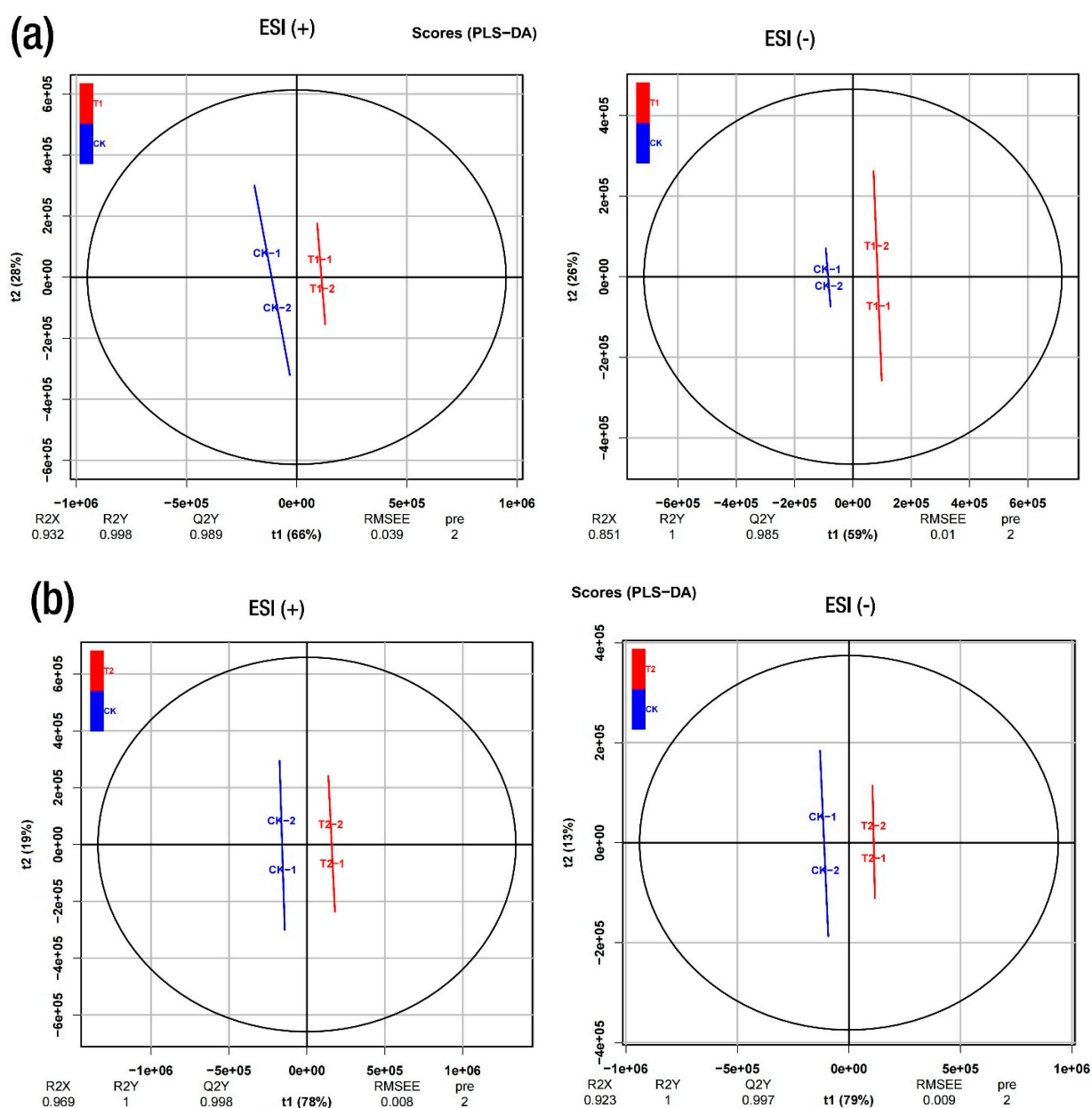
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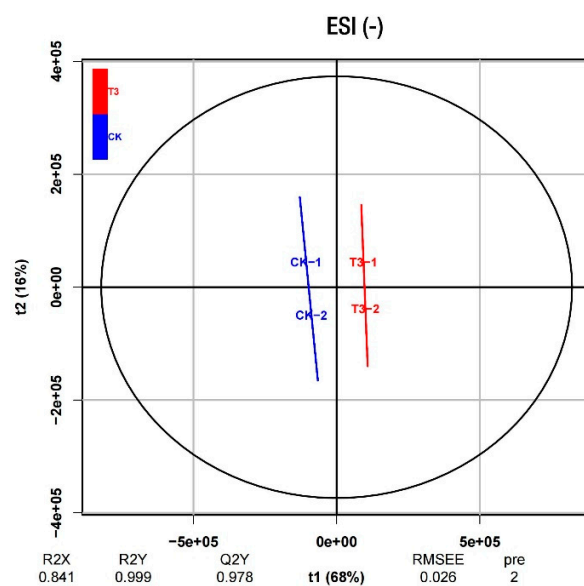
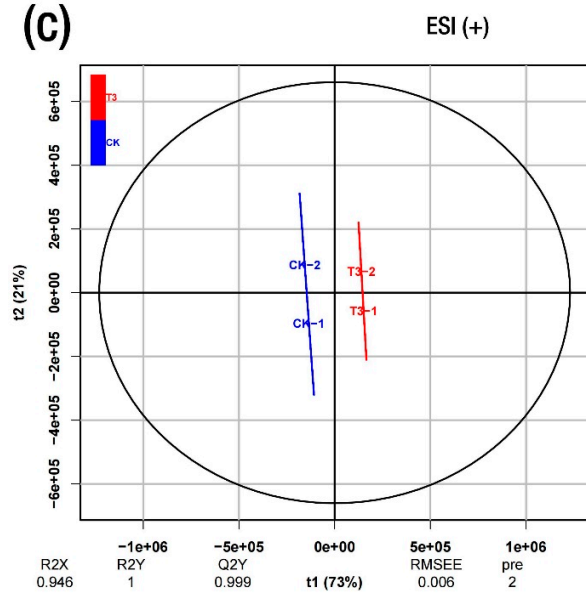
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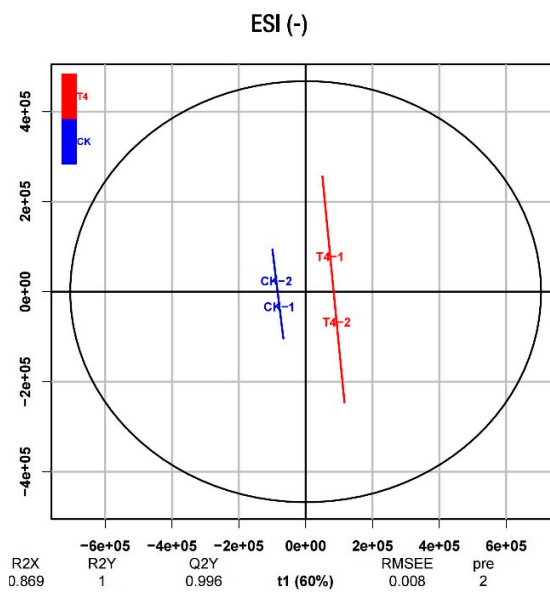
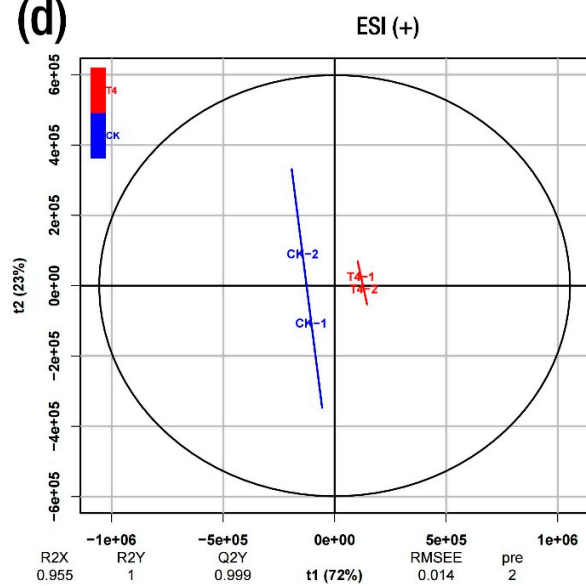
**Figure S1.** Sample PCA analysis chart plot. The PC1 coordinate represents the first principal component, and the percentage in parentheses indicates the contribution value of the first principal component to the sample difference; The PC2 coordinate represents the second principal component, and the percentage in parentheses indicates the contribution of the second principal component to the sample difference. The colored dots in the graph represent each sample, and the closer the same set of samples is, the better the sample repeatability (a) CK-T1. (b) CK-T2. (c) CK-T3. (d) CK-T4. (e) CK-T5. ESI (+); positive ion mode, ESI (-); negative ion mode. CK; sufficiency (3mmol/L), T1; 0mmol/L, T2; 1mmol/L, T3; 2mmol/L, T4; 6mmol/L, T5; 9mmol/L.



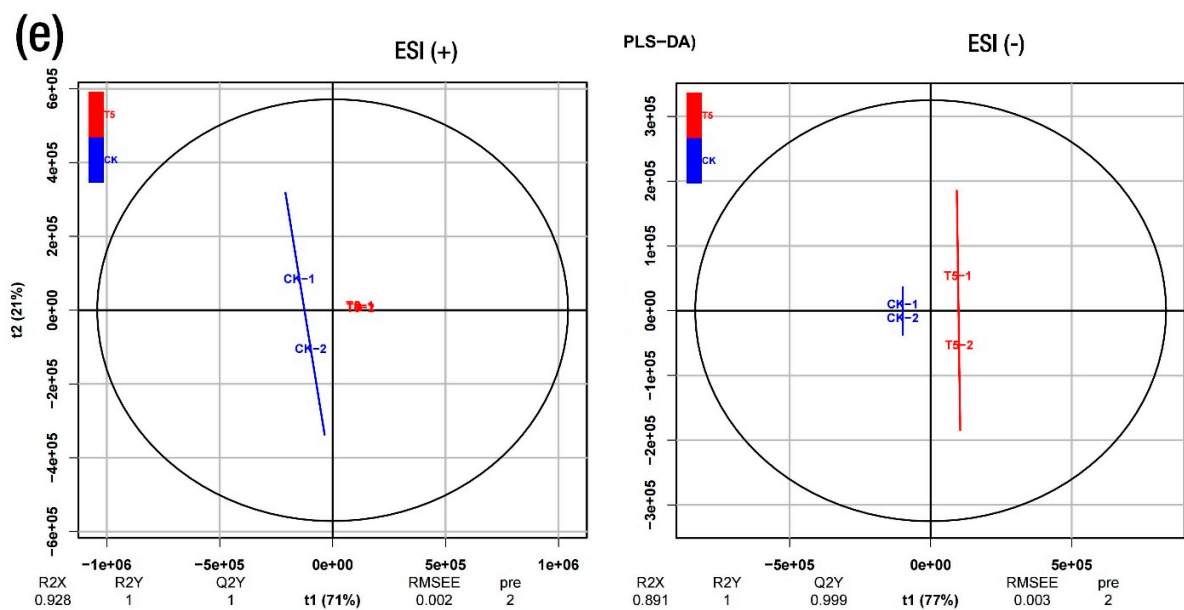
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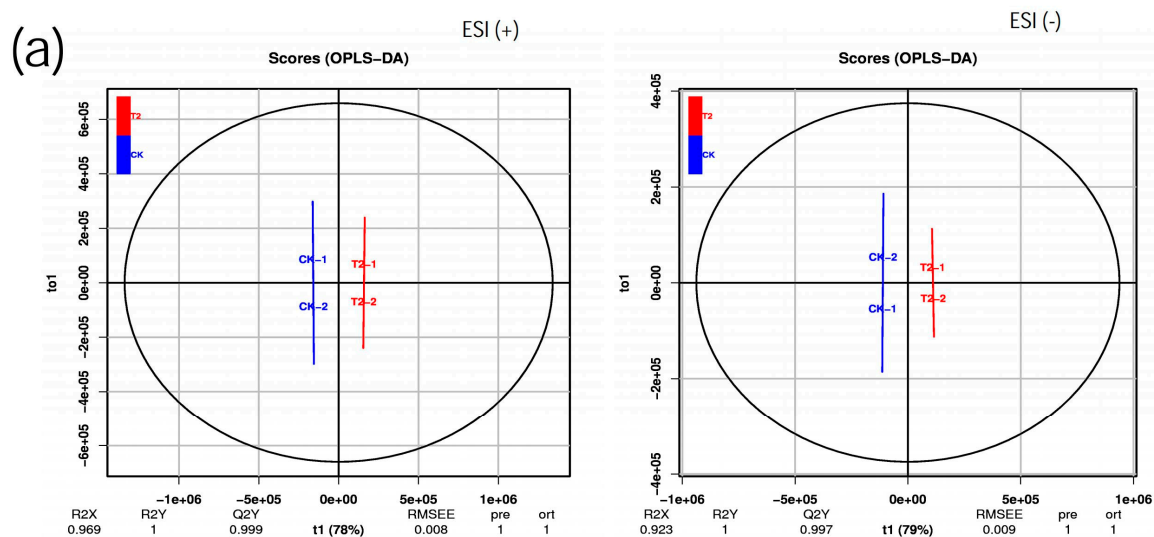
(d)

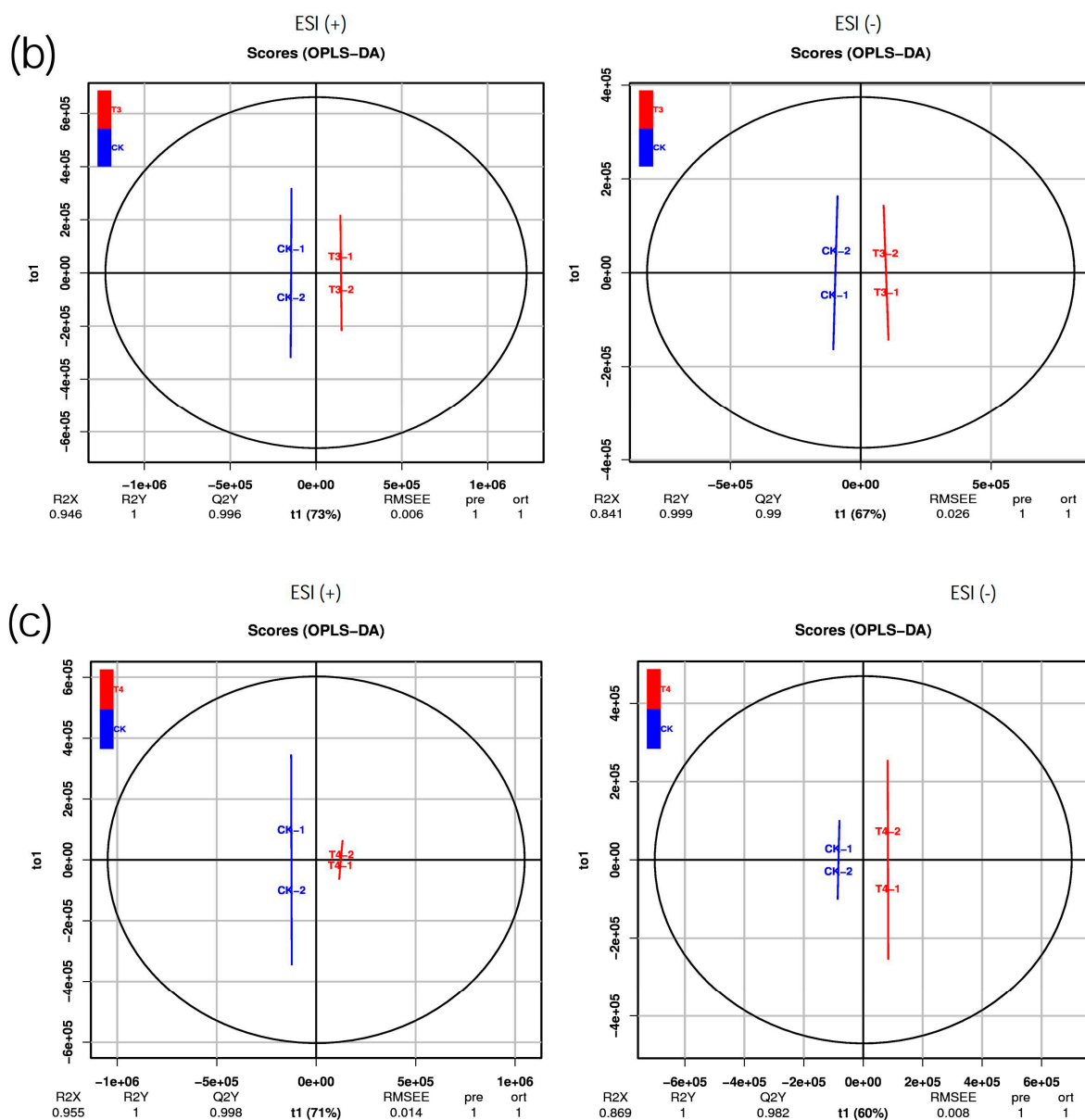




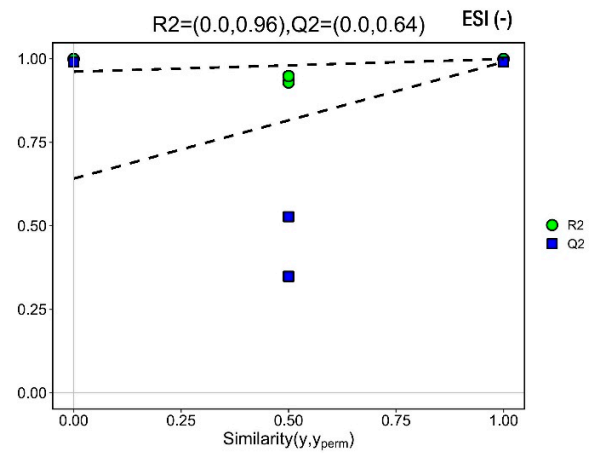
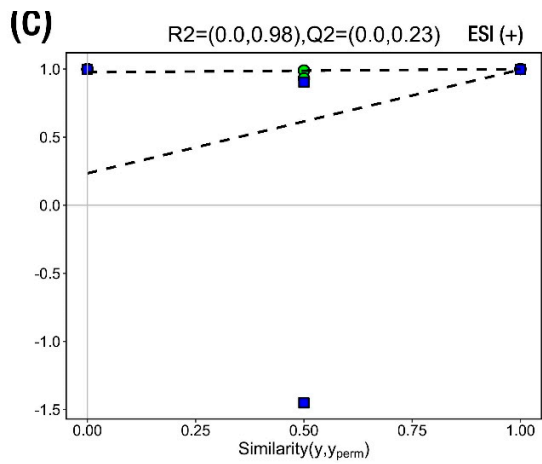
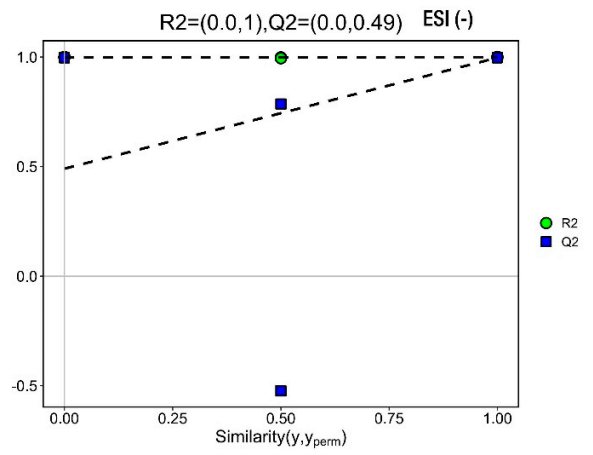
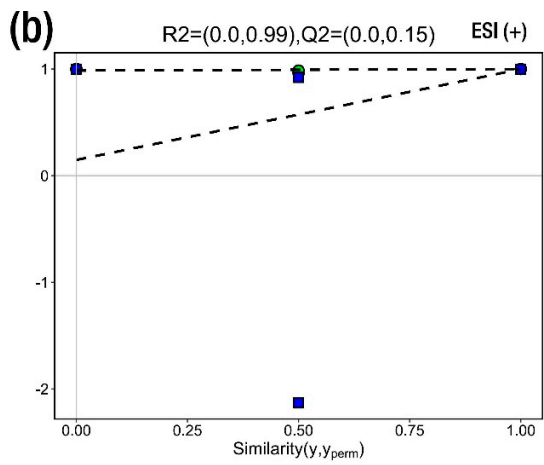
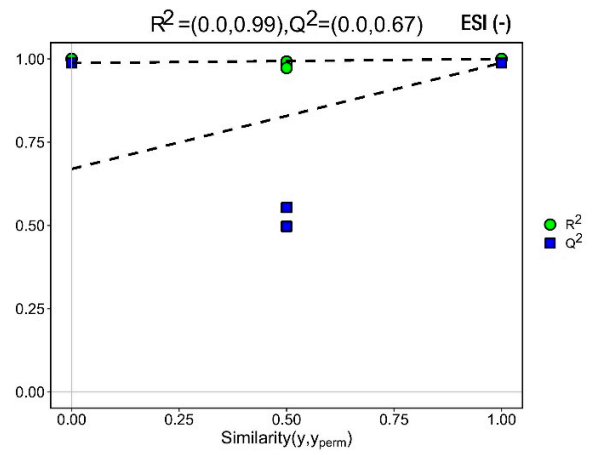
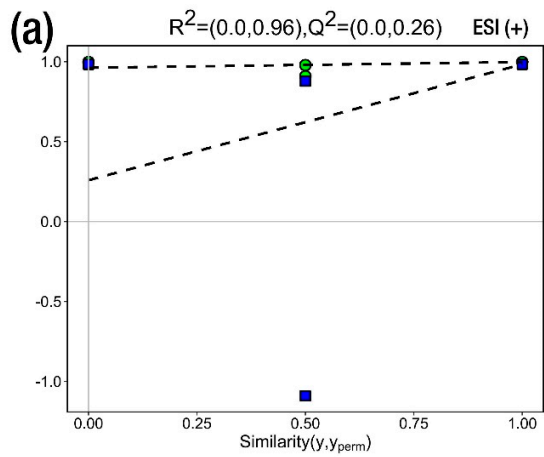


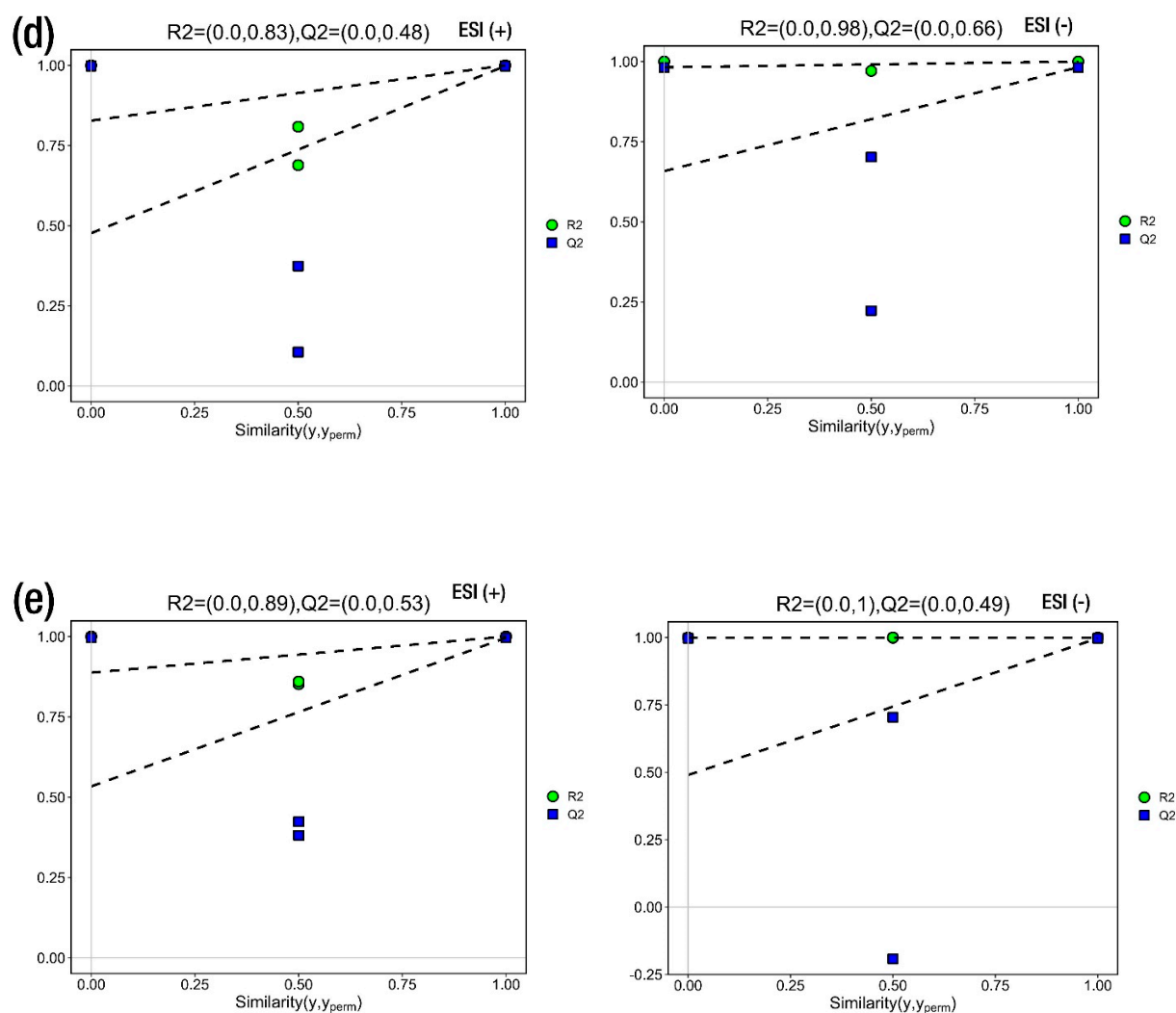
**Figure S2.** Partial least-squares-discriminant analysis (PLS-DA) scores of the metabolites from various Mg treatment in mulberry. (a) CK-T1 (b) CK-T2 (c) CK-T3 (d) CK-T4 (e) CK-T5. The PLS-DA models are R2X, R2Y and Q2. Where R2X and R2Y represent the interpretation rate of the built model to X and Y matrices, respectively, Q2 represents the prediction ability of the model, and the closer these three indicators are to 1, the more stable and reliable the model.  $Q2 > 0.5$  indicates that the model has good prediction ability, and  $Q2 > 0.9$  indicates that the model is excellent. ESI (+); positive ion mode, ESI (-); negative ion mode.



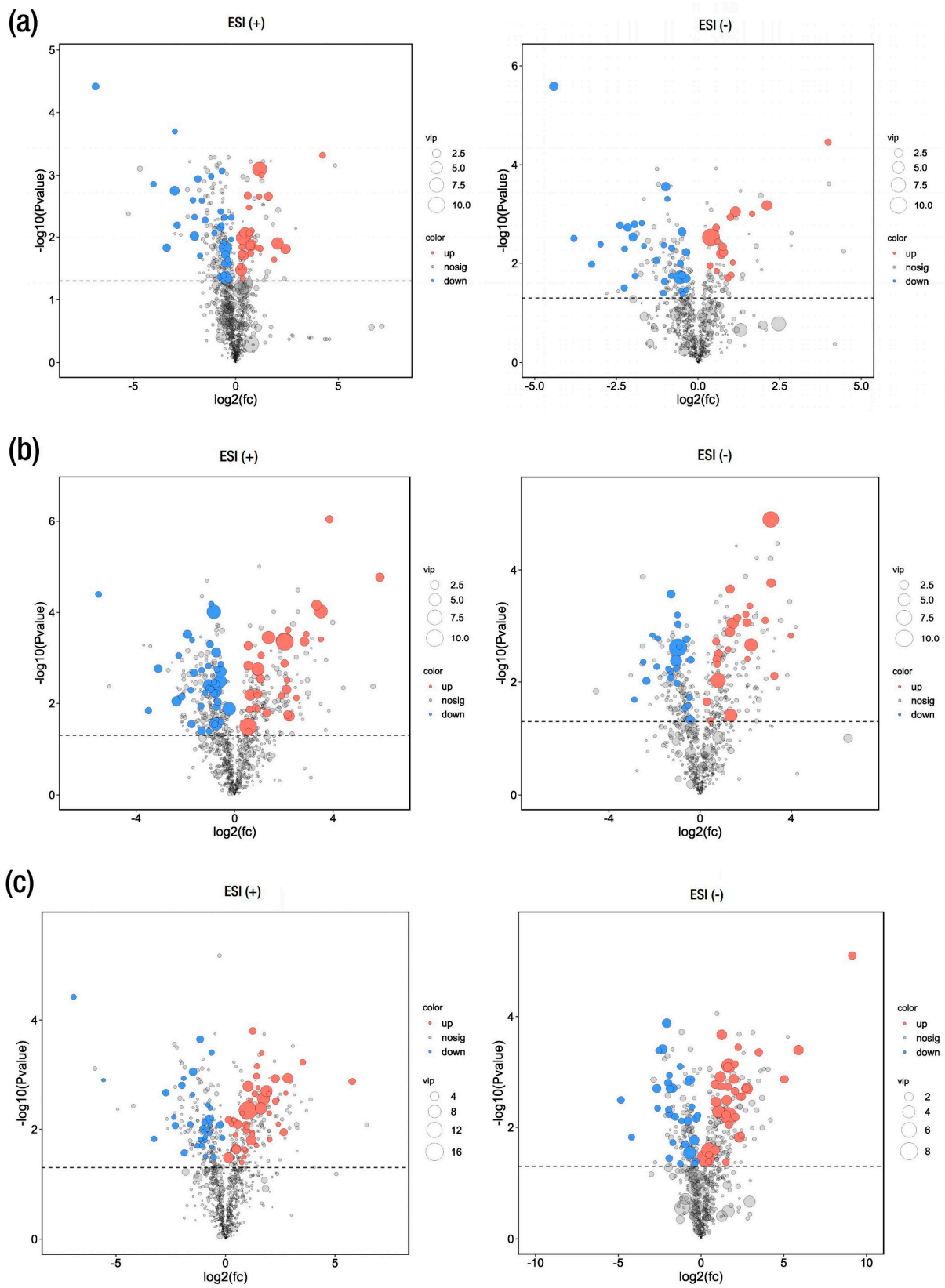


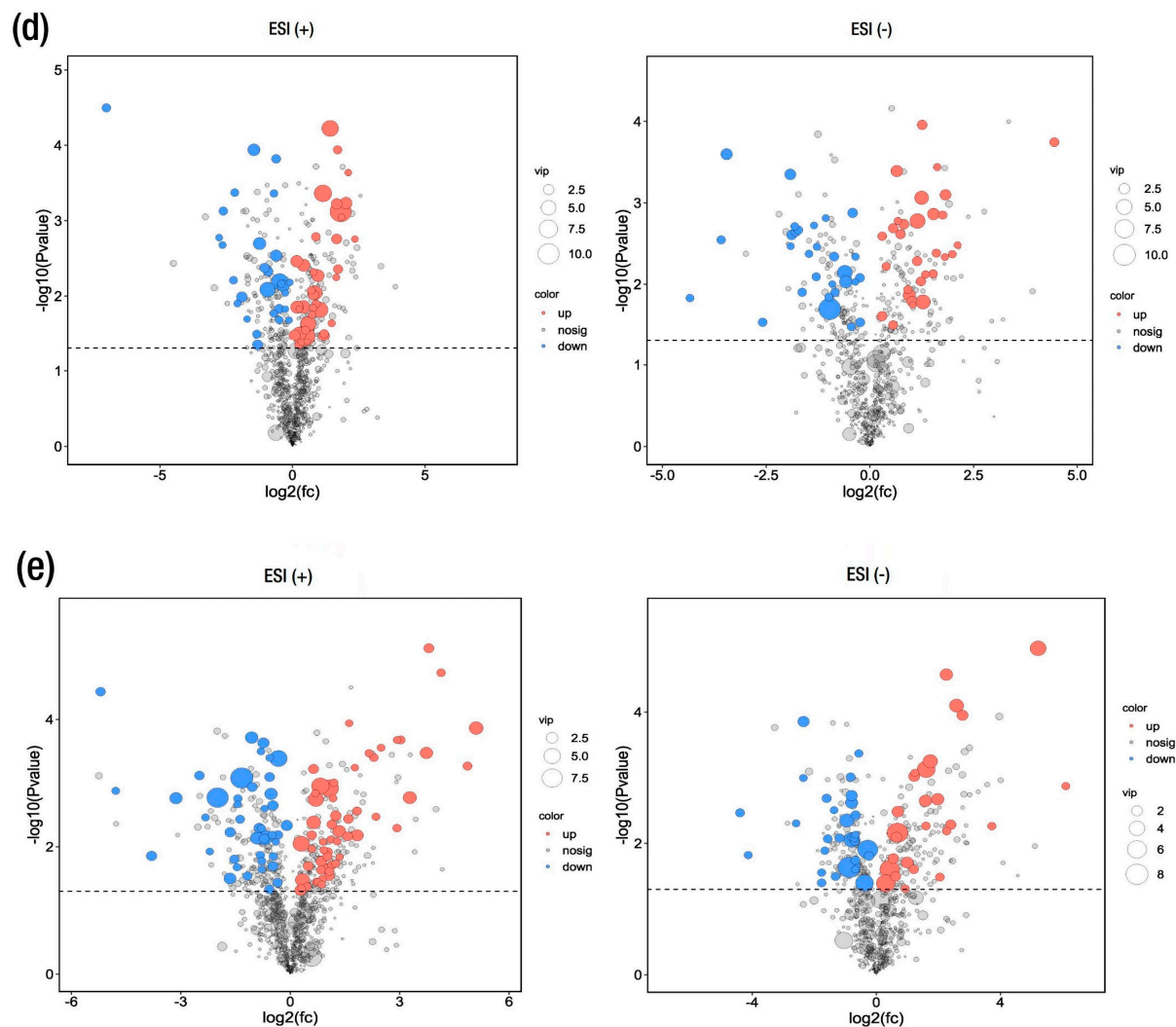
**Figure S3.** Orthogonal partial least-squares-discriminant analysis (OPLS-DA) scores of the metabolites (a) CK-T2 (b) CK-T3 (c) CK-T4. The OPLS-DA models are R2X, R2Y and Q2. Where R2X and R2Y represent the interpretation rate of the built model to X and Y matrices, respectively, Q2 represents the prediction ability of the model, and the closer these three indicators are to 1, the more stable and reliable the model. Q2>0.5 indicates that the model has good prediction ability, and Q2 >0.9 indicates that the model is excellent. ESI (+); positive ion mode, ESI (-); negative ion mode.





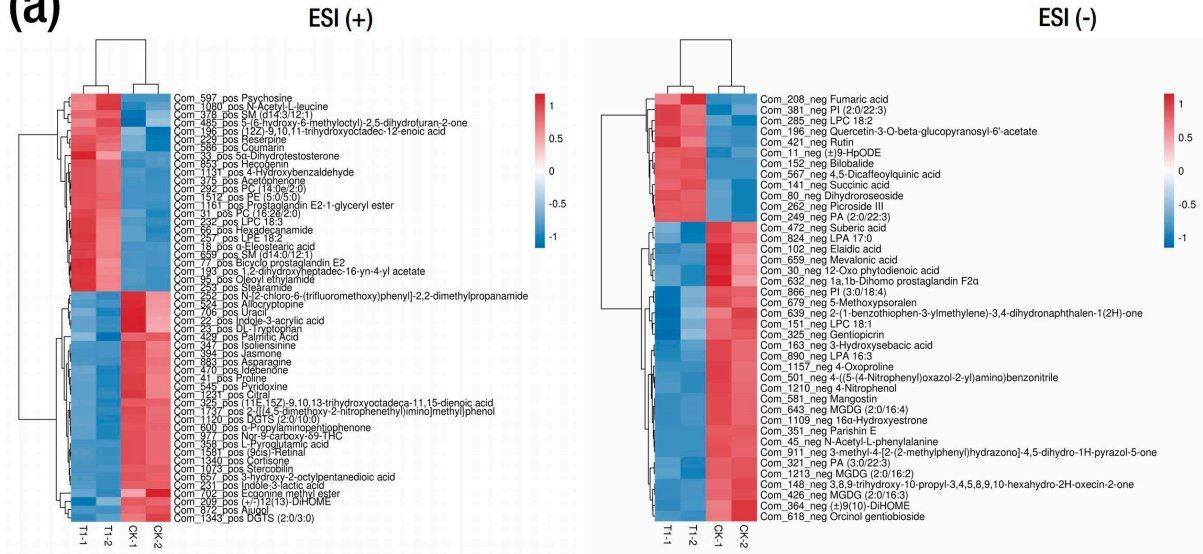
**Figure S4.** Permutation test A random ranking method used to evaluate the accuracy of (O)PLS models. (a) CK-T1 (b) CK-T2 (c) CK-T3 (d) CK-T4 (e) CK-T5. The two rightmost points ( $x=1.0$ ) are  $R^2$  and  $Q^2$  of the original model, and all the points on the left are the  $R^2'$  and  $Q^2'$  of the model after Y displacement, if all the blue  $Q^2$  points from left to left are lower than the original blue  $Q^2$  points on the far right, or the regression line of  $Q^2$  points is less than or equal to 0 at the intersection of ordinates, it means that the model prediction results are reliable.



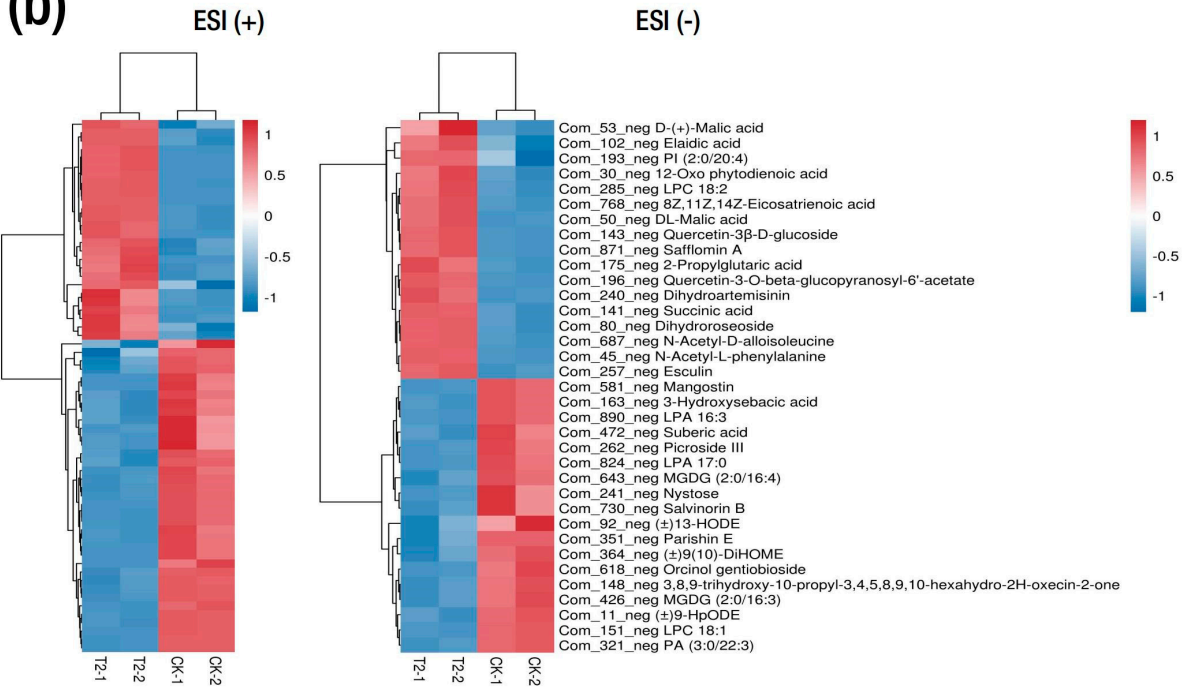


**Figure S5.** Volcano plot of the differential metabolites from various Mg treatment in mulberry. (a) CK-T1 (b) CK-T2 (c) CK-T3 (d) CK-T4 (e) CK-T5. ESI (+); positive ion mode, ESI (-); negative ion mode. The abscissa is the value after  $\log_2$  for the different multiple of the metabolite abundance in each comparison group, the ordinate is the p-value after the t-test, and the dashed line perpendicular to the y-axis is the p-value threshold for differential metabolite screening. The red dots represent  $\text{VIP} \geq 1$  and  $p < 0.05$ , differential metabolites that are up-regulated ( $\text{FC} > 1$ ); The blue dots represent  $\text{VIP} \geq 1$  and  $p < 0.05$ , differentiating metabolites that are downregulated ( $\text{FC} < -1$ ). The larger the point, the greater the metabolite VIP value.

(a)



(b)

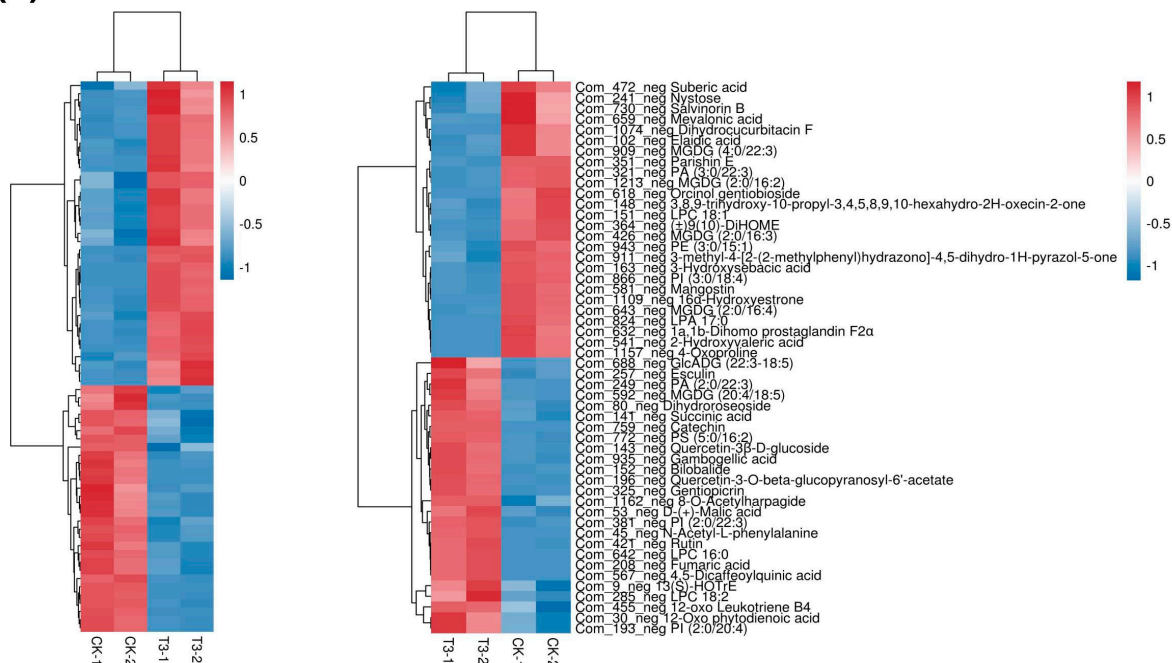




**(c)**

ESI (+)

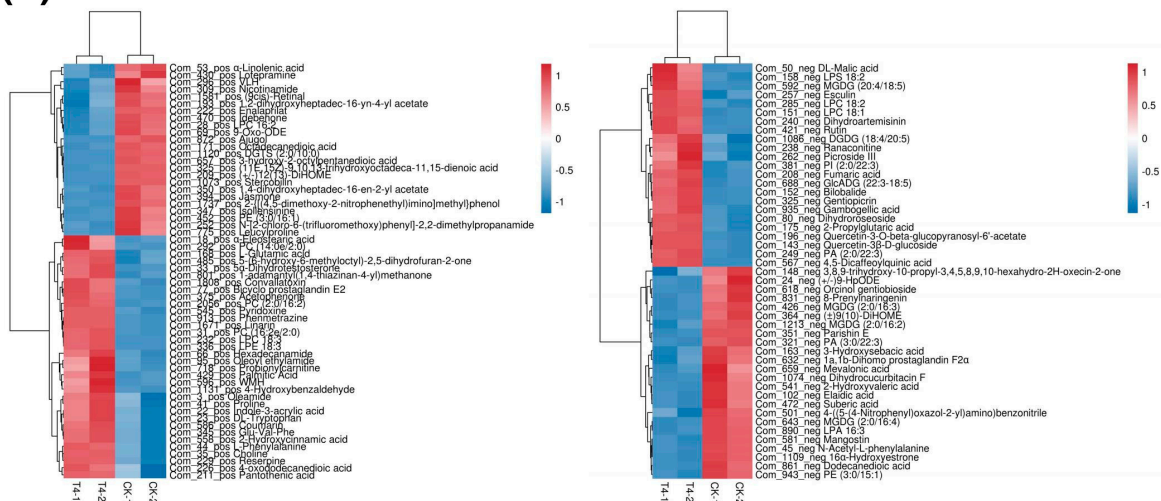
ESI (-)



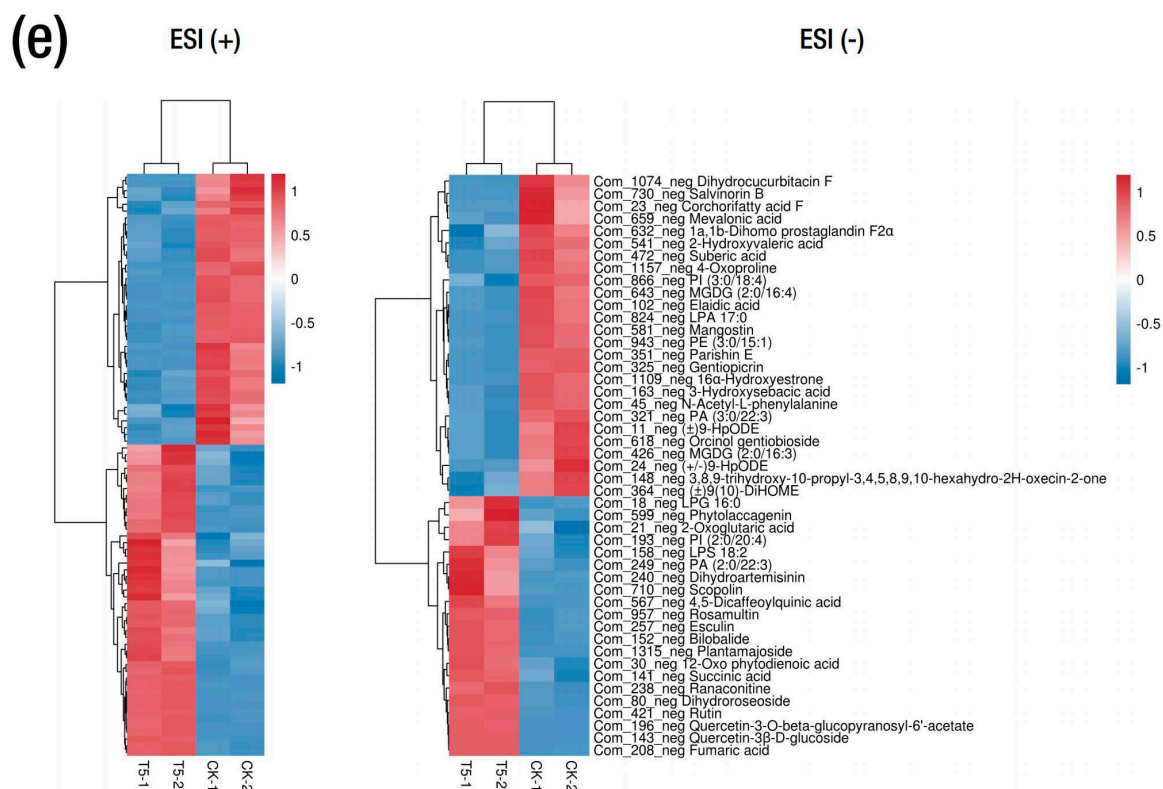
(d)

ESI (+)

ESI (-)

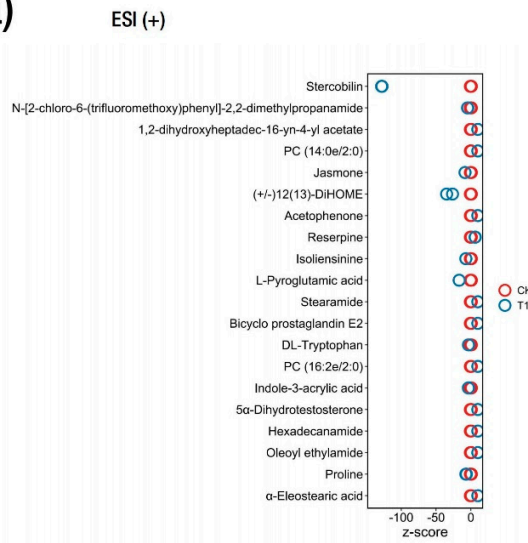




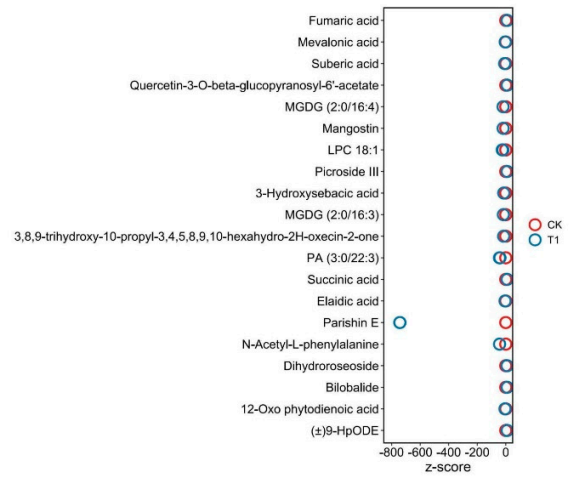


**Figure S6.** Differential metabolite clustering heatmap from various Mg treatment in mulberry. (a) CK-T1 (b) CK-T2 (c) CK-T3 (d) CK-T4 (e) CK-T5. ESI (+); positive ion mode, ESI (-); negative ion mode. Each row in the figure represents a metabolite, each column represents a sample, the different metabolites in each comparison group are normalized by rows, the red color, represent higher metabolite abundance, and the blue represents lower metabolite abundance. Metabolites and samples clustered separately according to their metabolite abundance, and the expression patterns of samples or metabolites under the same branch represent more similarity.

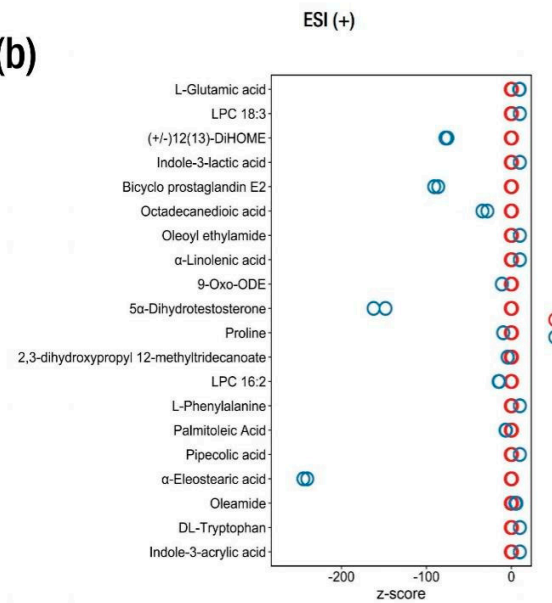
(a)



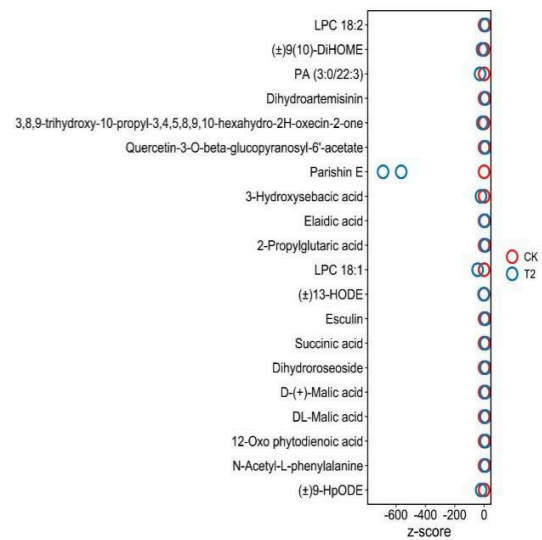
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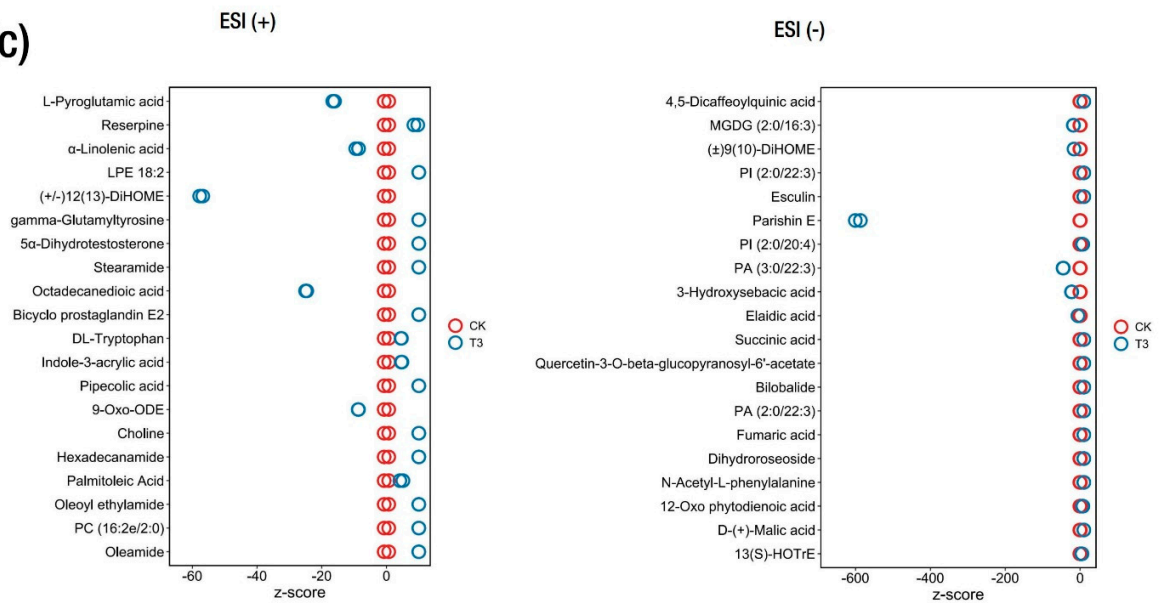
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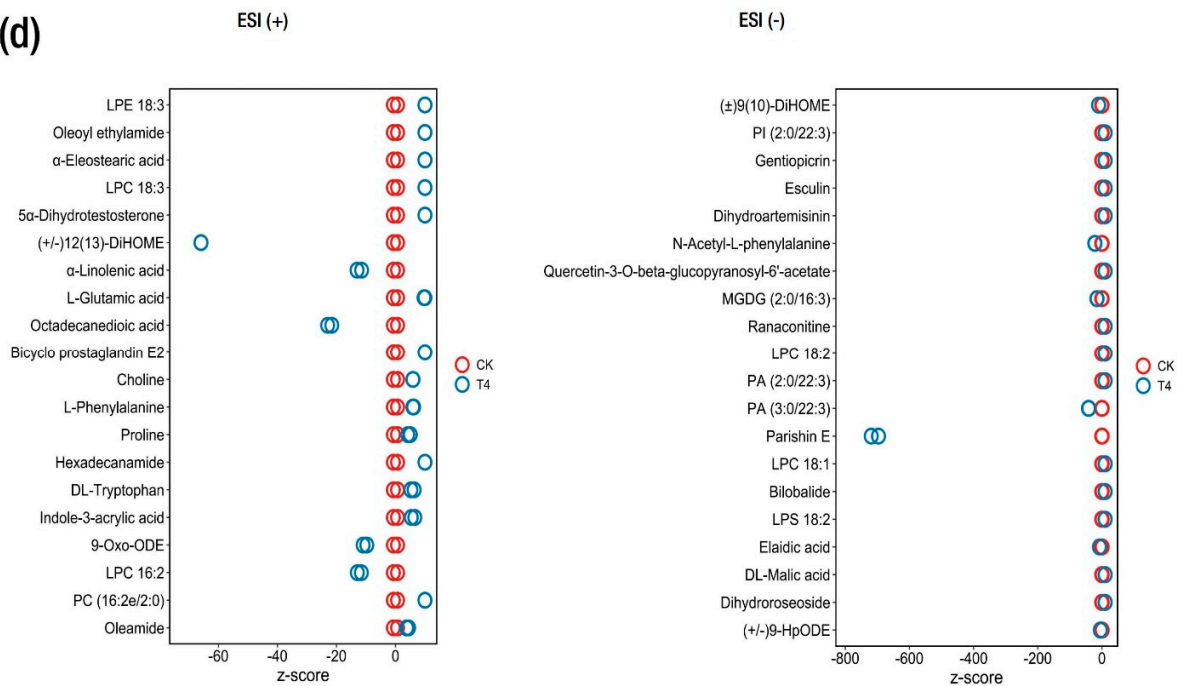
ESI (-)

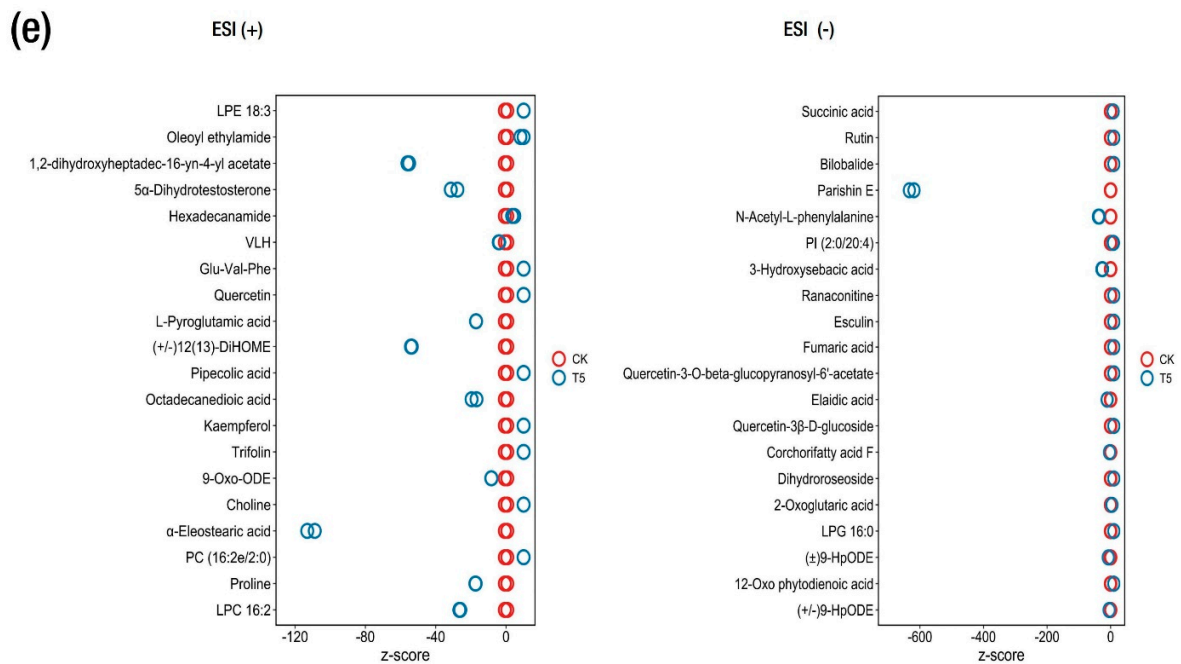


(c)

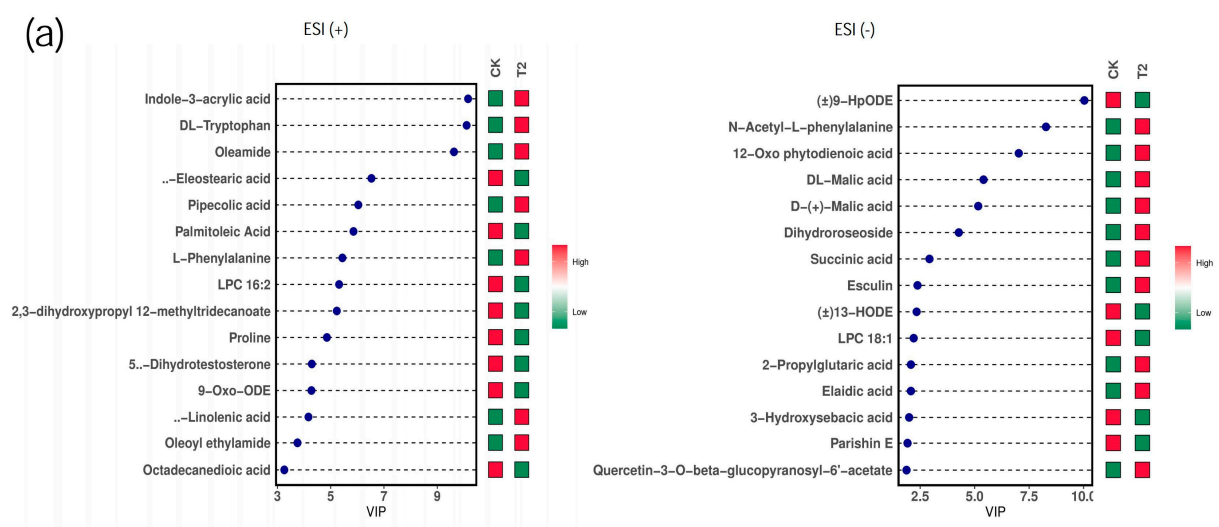


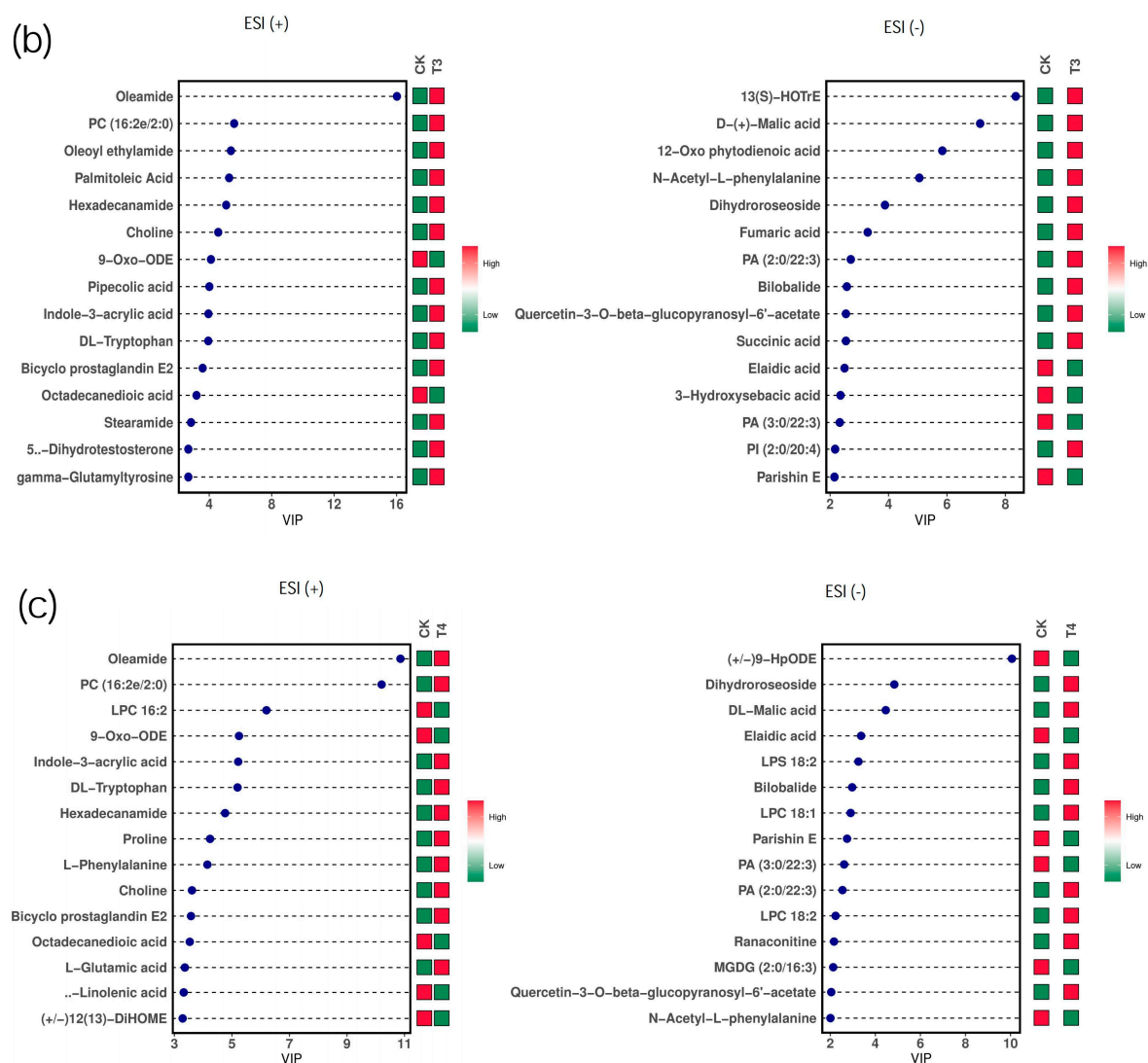
(d)





**Figure S7.** Differential metabolite Z-score plot from various Mg treatment in mulberry. (a) CK-T1 (b) CK-T2 (c) CK-T3 (d) CK-T4 (e) CK-T5. ESI (+); positive ion mode, ESI (-); negative ion mode. The abundance of metabolites after the abscissa is z-score, each ordinate is a metabolite, red represents the control group sample in the comparison group, blue represents the sample of the treatment group, each circle represents a metabolite, each sample  $z\text{-score} < 0$  represents the downward regulation of metabolites, and  $z\text{-score} > 0$  represents the upward regulation of metabolite abundance.

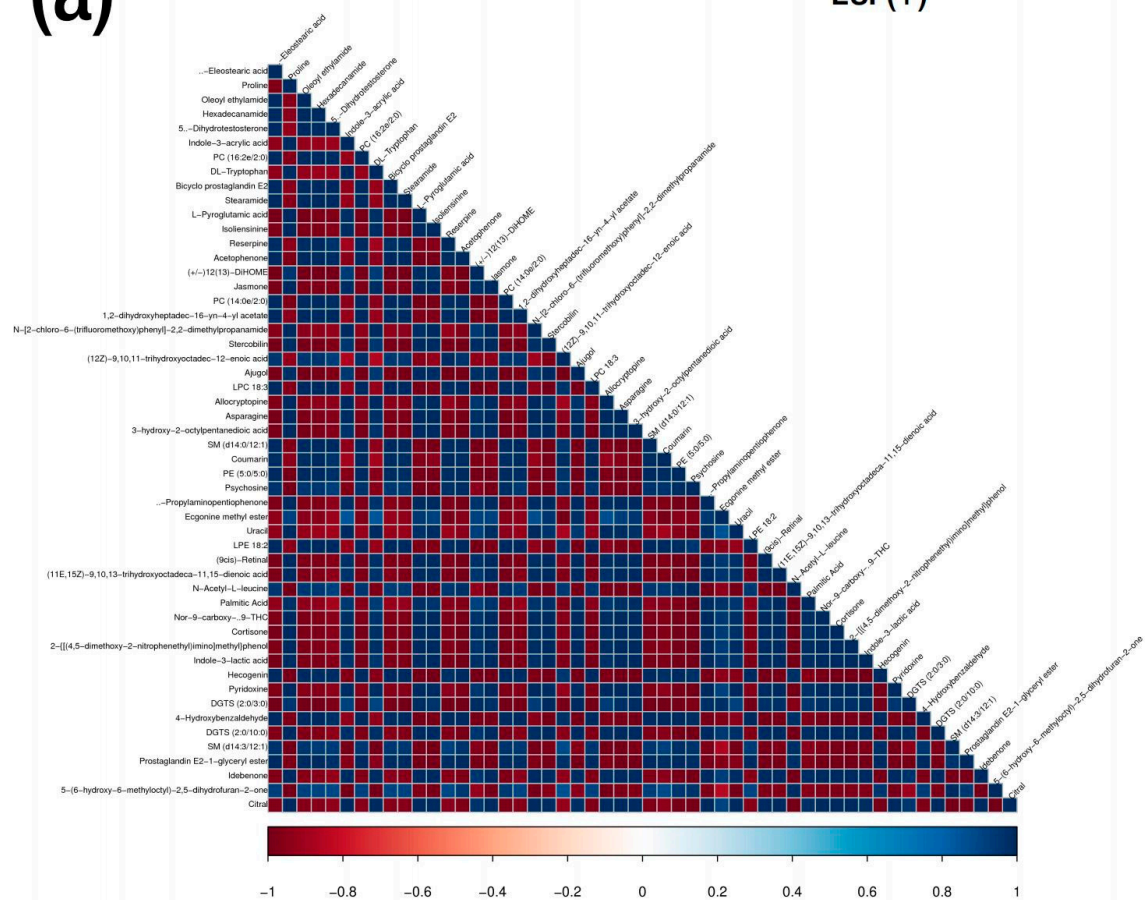




**Figure S8.** Orthogonal partial least-squares-discriminant analysis (OPLS-DA) scores of the metabolites (a) CK-T2 (b) CK-T3 (c) CK-T4. The OPLS-DA models are R2X, R2Y and Q2. Where R2X and R2Y represent the interpretation rate of the built model to X and Y matrices, respectively, Q2 represents the prediction ability of the model, and the closer these three indicators are to 1, the more stable and reliable the model. Q2>0.5 indicates that the model has good prediction ability, and Q2 >0.9 indicates that the model is excellent.

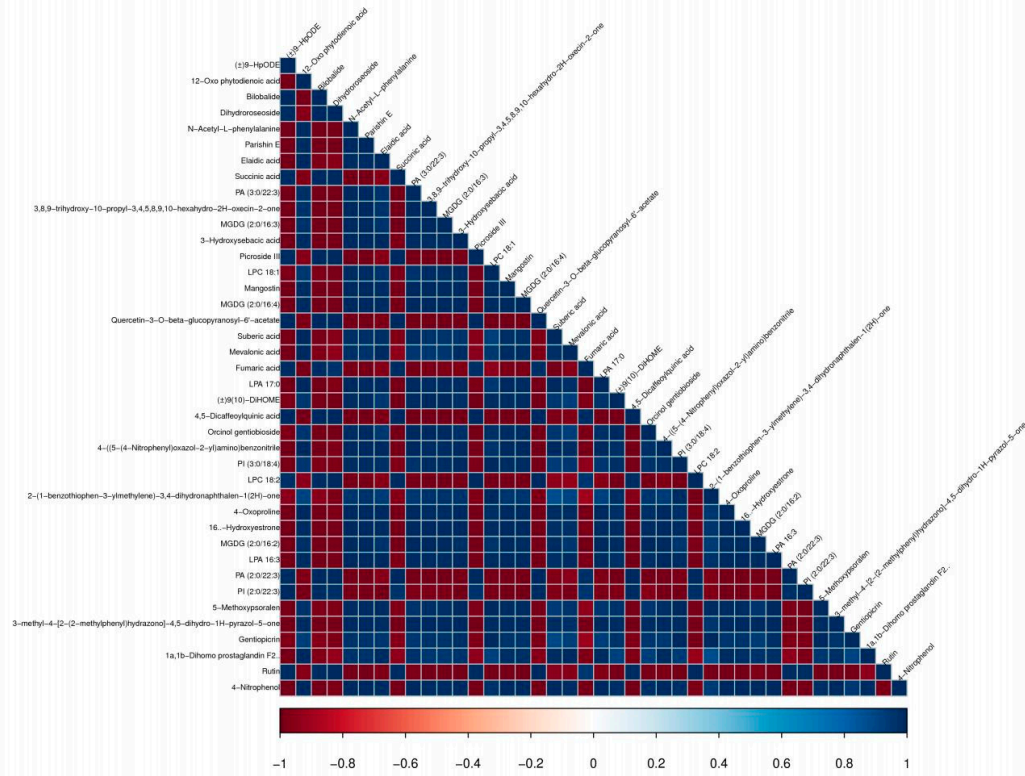
(a)

ESI (+)



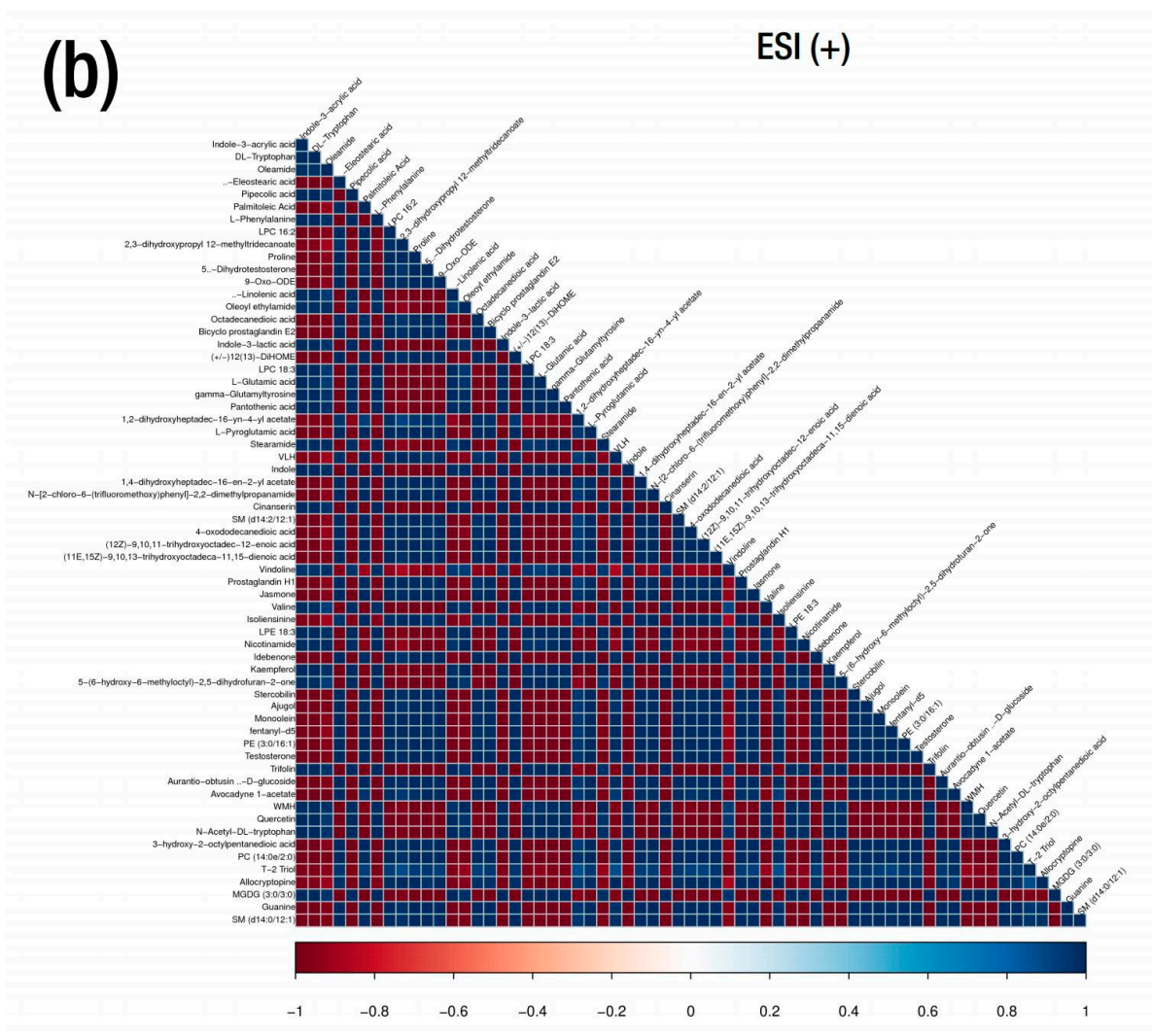


ESI (-)



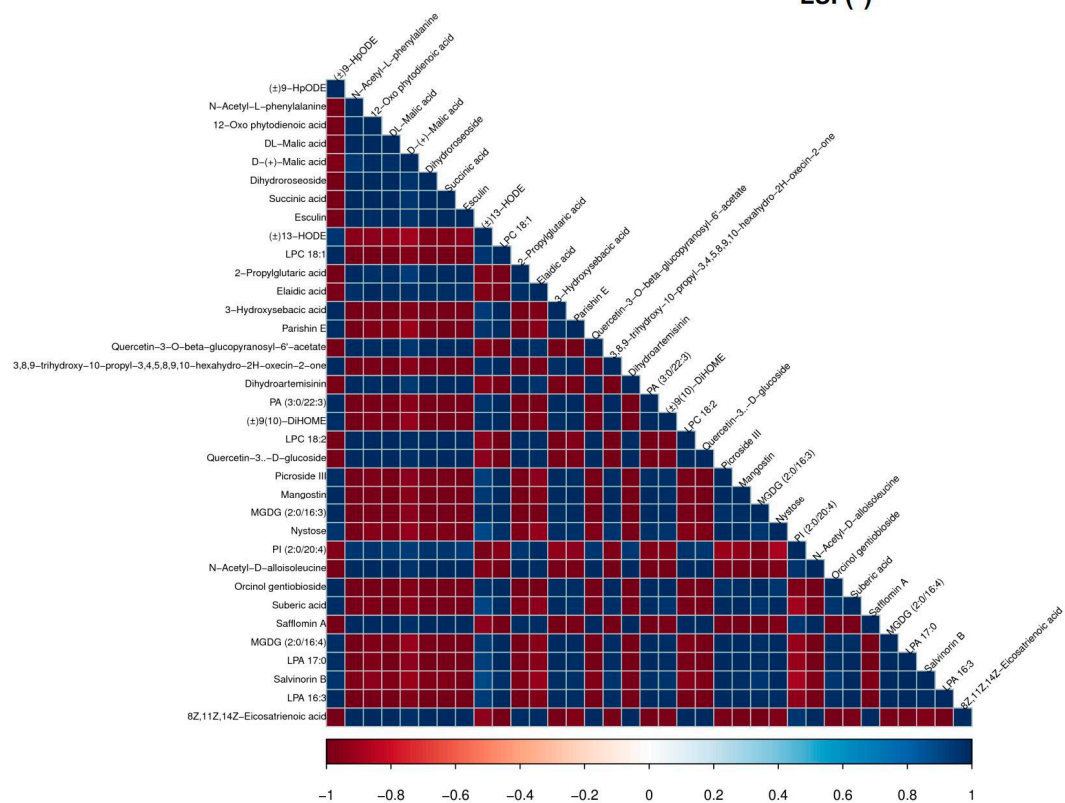
(b)

ESI (+)

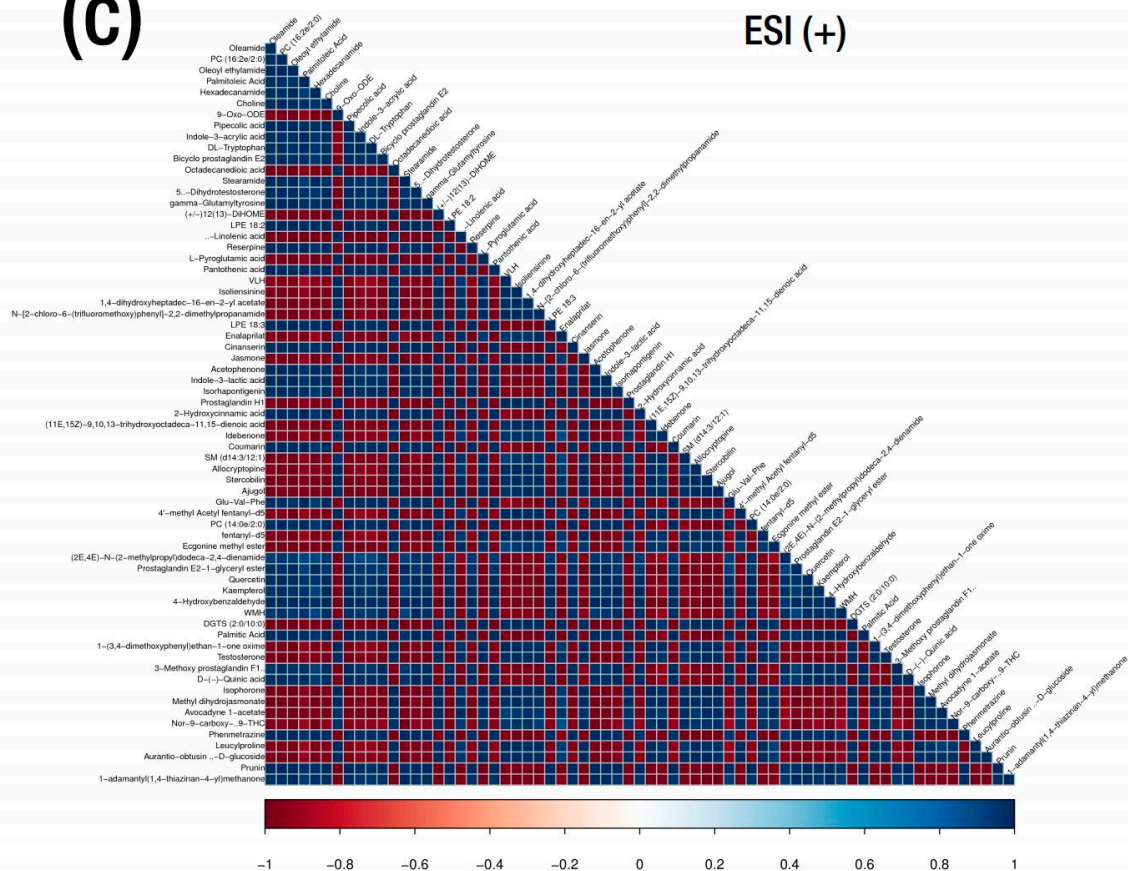




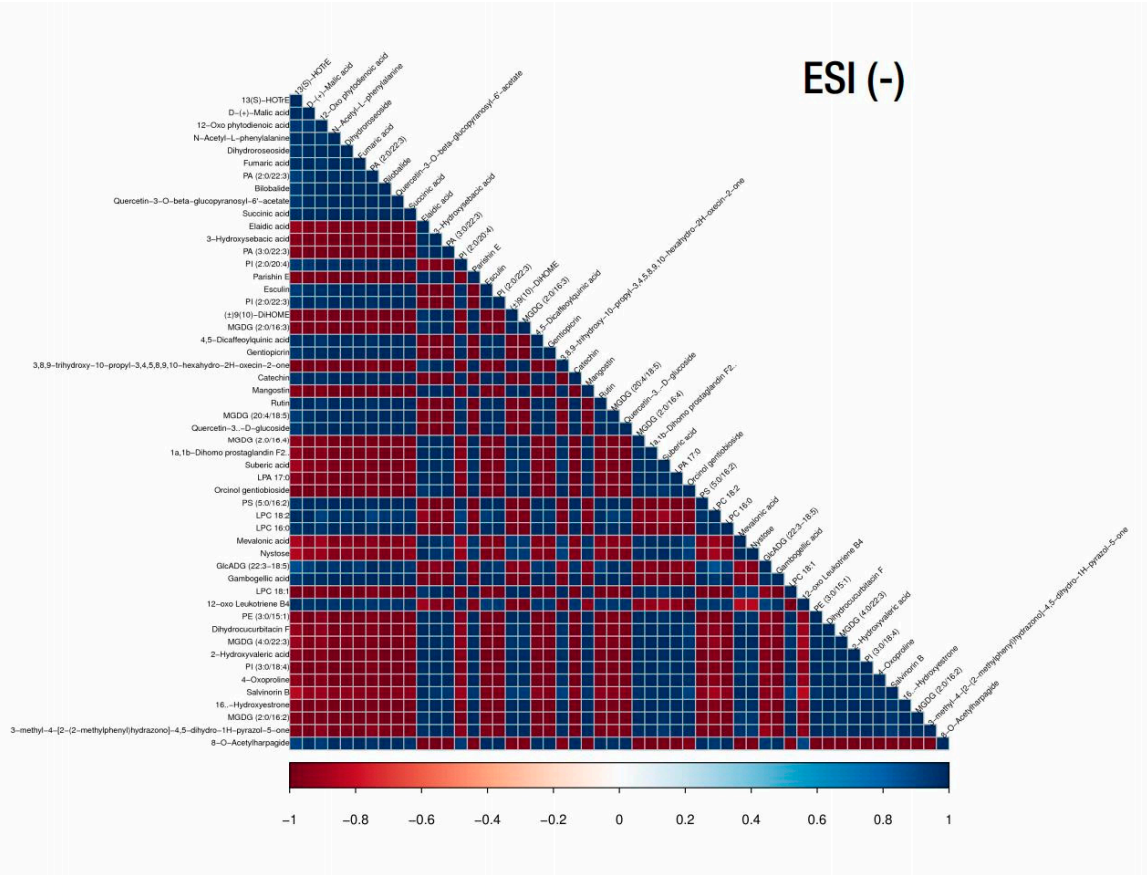
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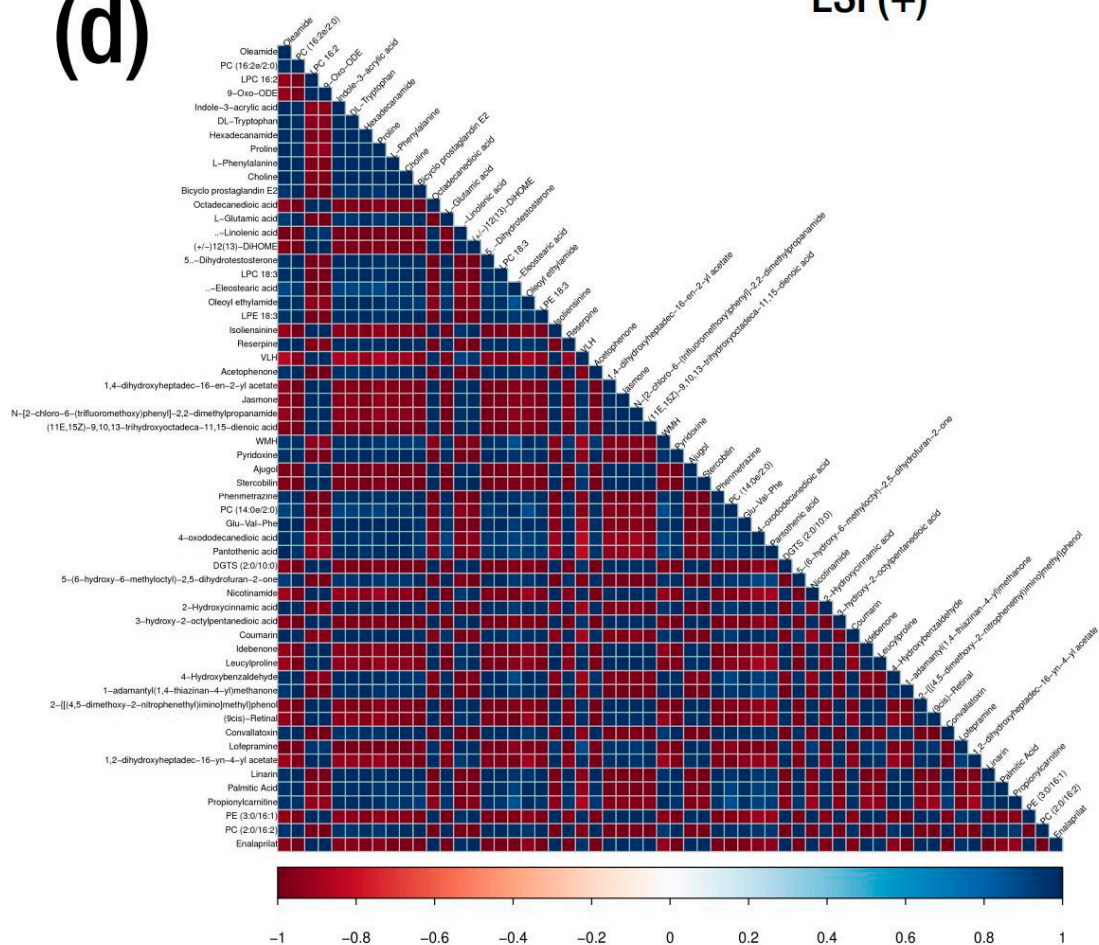
**ESI (+)**



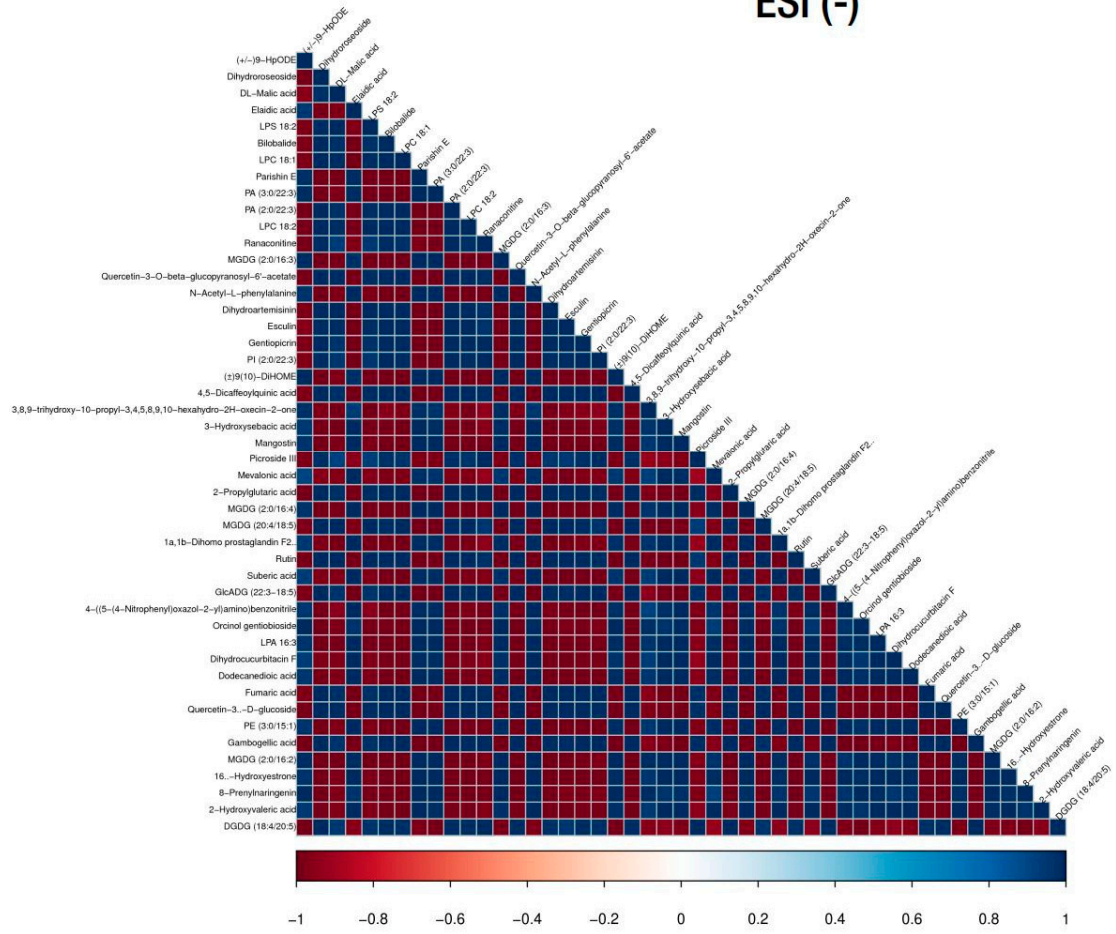
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## ESI (+)

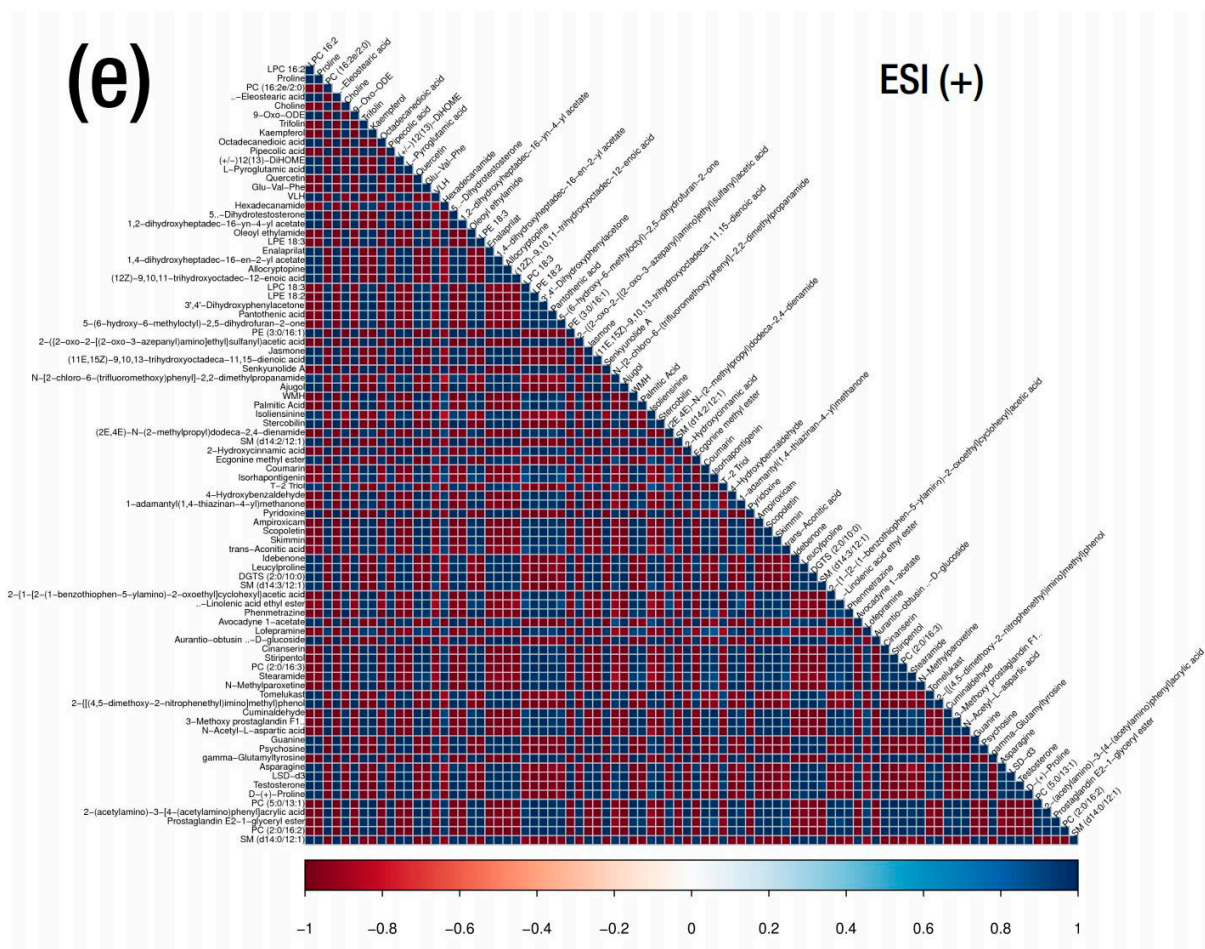


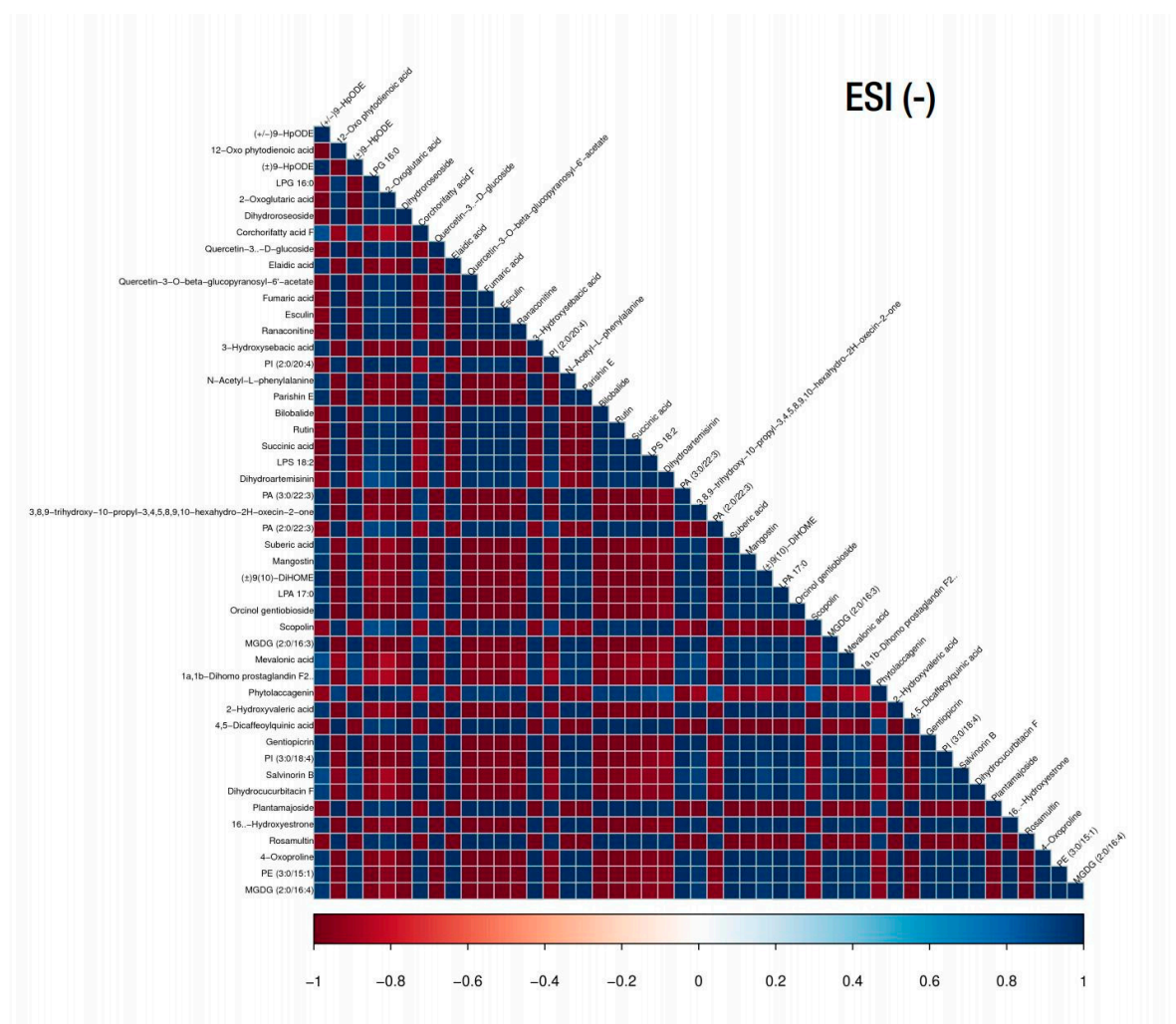
ESI (-)





ESI (+)

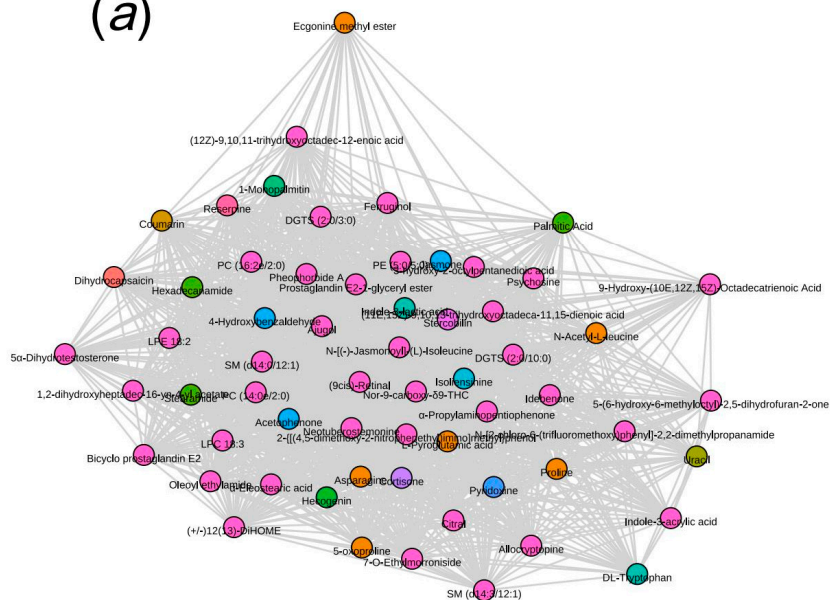




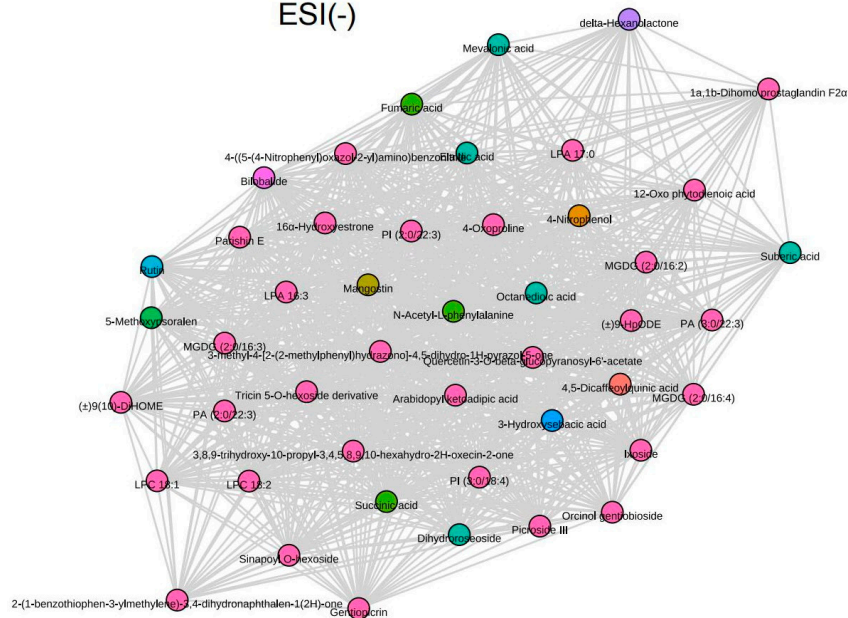
**Figure S9** Sample correlation heatmap chart description. (a) CK-T1 (b) CK-T2 (c) CK-T3 (d) CK-T4 (e) CK-T5. Red color represents negative correlation and blue color represent positive correlation Each row and column in the graph represent metabolite, and the value in each cell represents the Pearson correlation coefficient of the corresponding two metabolites, the higher the value, the darker the color, indicating that the two samples are more correlated.

ESI(+)  

(a)



ESI(-)



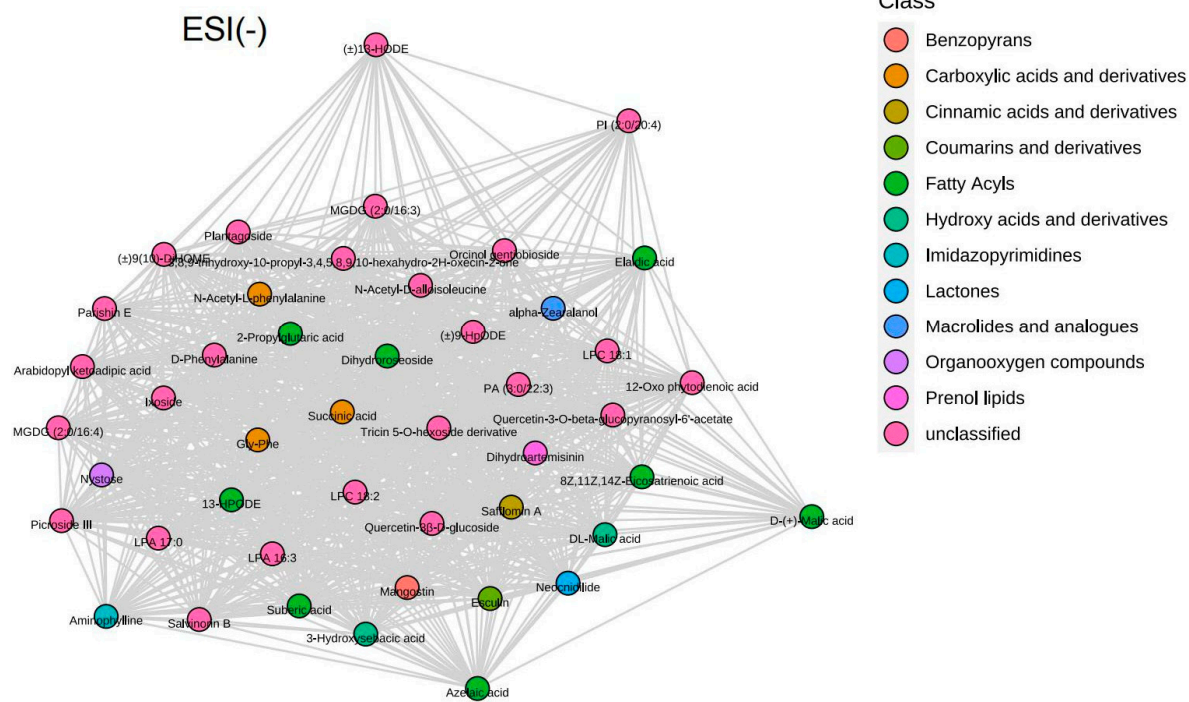
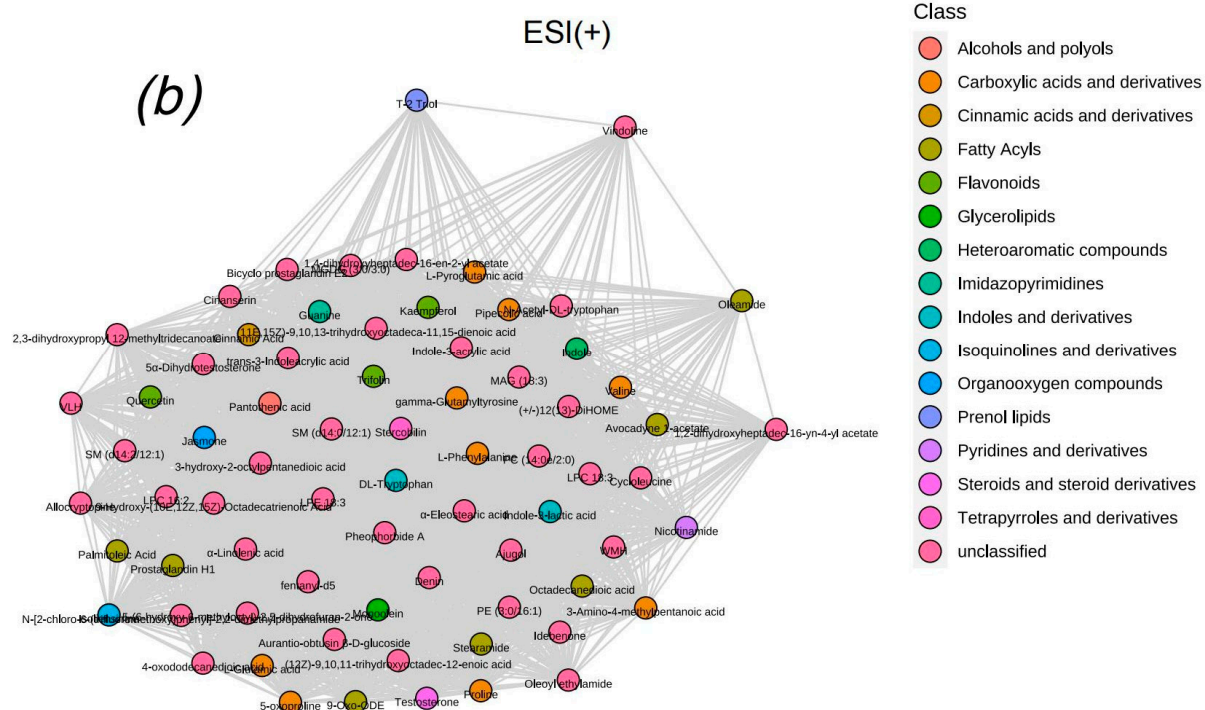
## Class

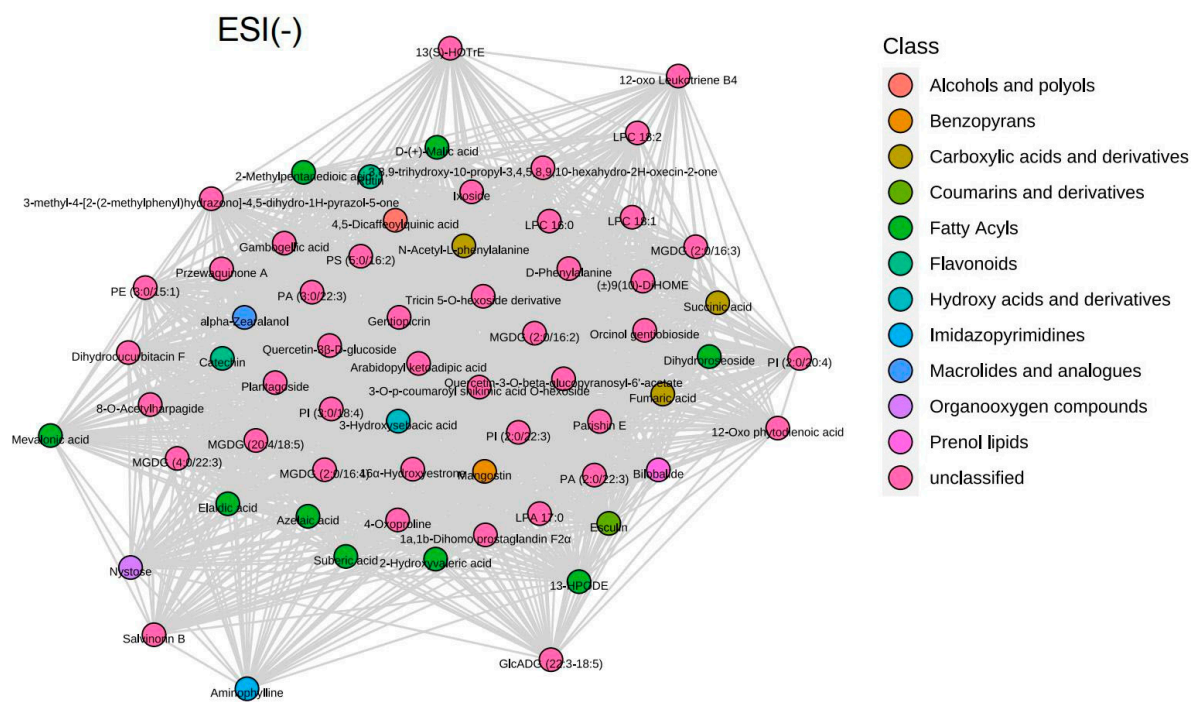
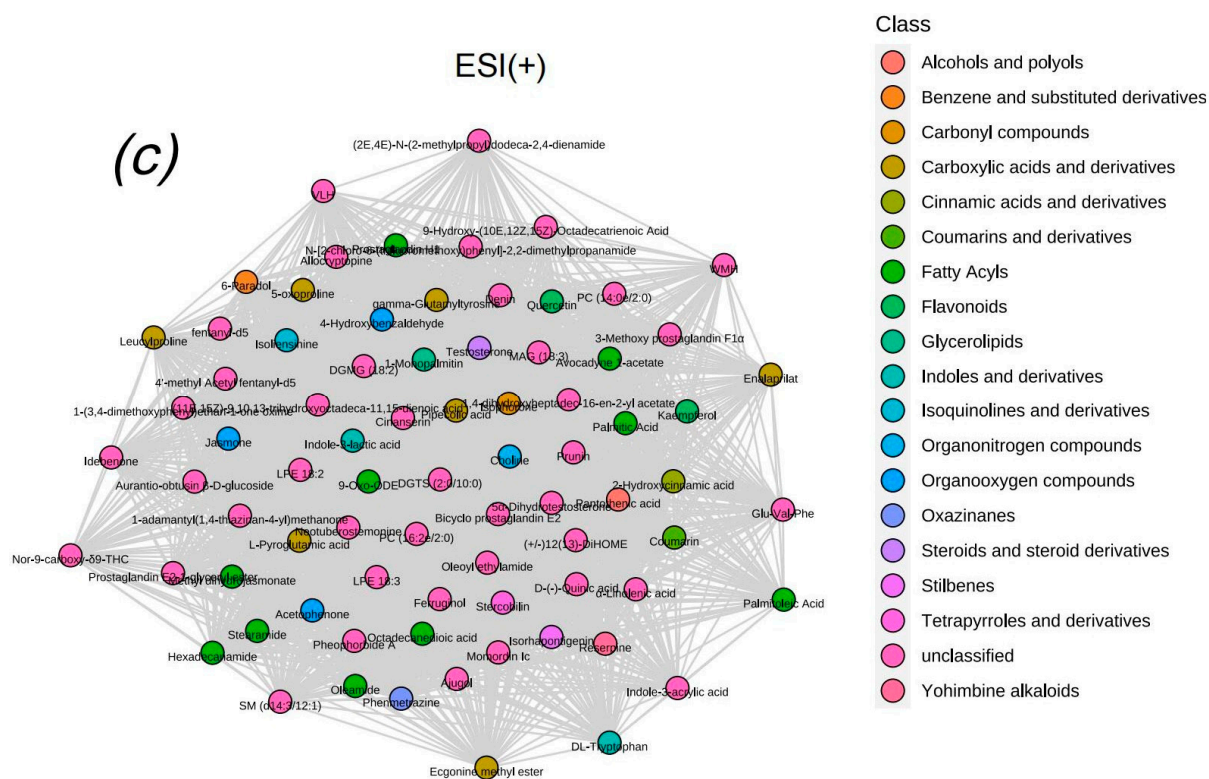
-  Benzene and substituted derivatives
-  Carboxylic acids and derivatives
-  Coumarins and derivatives
-  Diazines
-  Fatty Acyls
-  Flavonoids
-  Glycerolipids
-  Indoles and derivatives
-  Isoquinolines and derivatives
-  Organoxygen compounds
-  Pyridines and derivatives
-  Steroids and steroid derivatives
-  Tetrapyrroles and derivatives
-  unclassified
-  Yohimbine alkaloids

## Class

- Alcohols and polyols
- Benzene and substituted derivatives
- Benzopyrans
- Carboxylic acids and derivatives
- Coumarins and derivatives
- Fatty Acyls
- Flavonoids
- Hydroxy acids and derivatives
- Lactones
- Prenol lipids
- unclassified







(d)

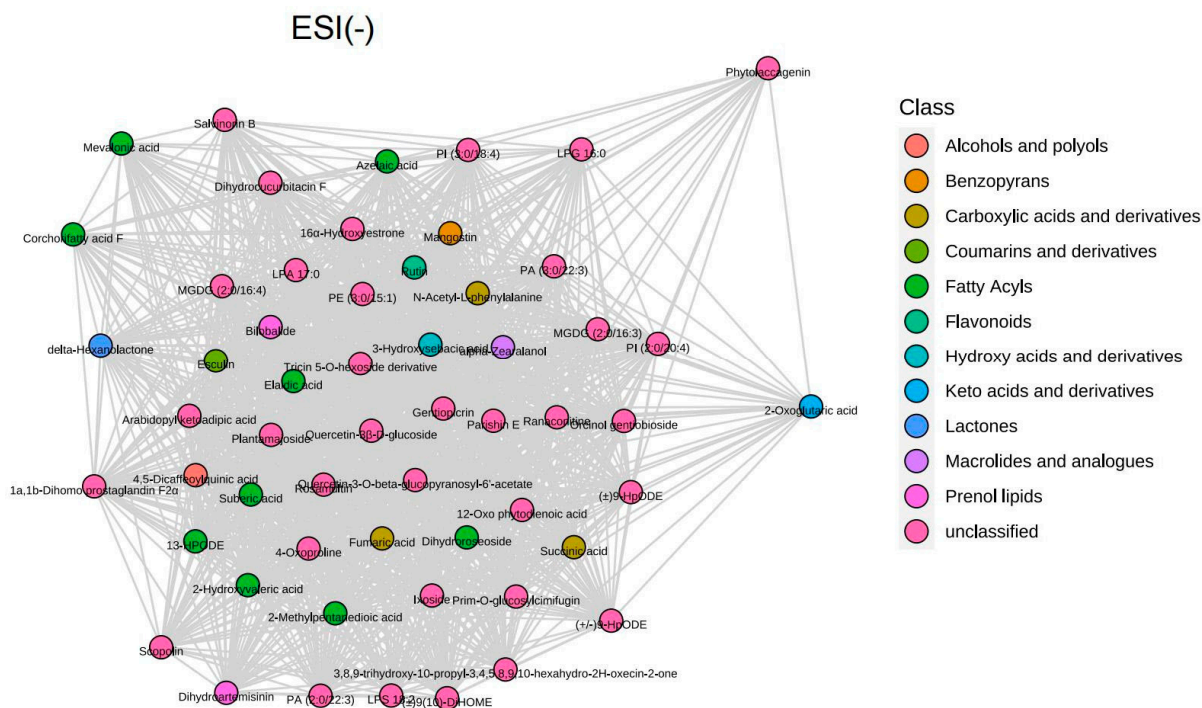
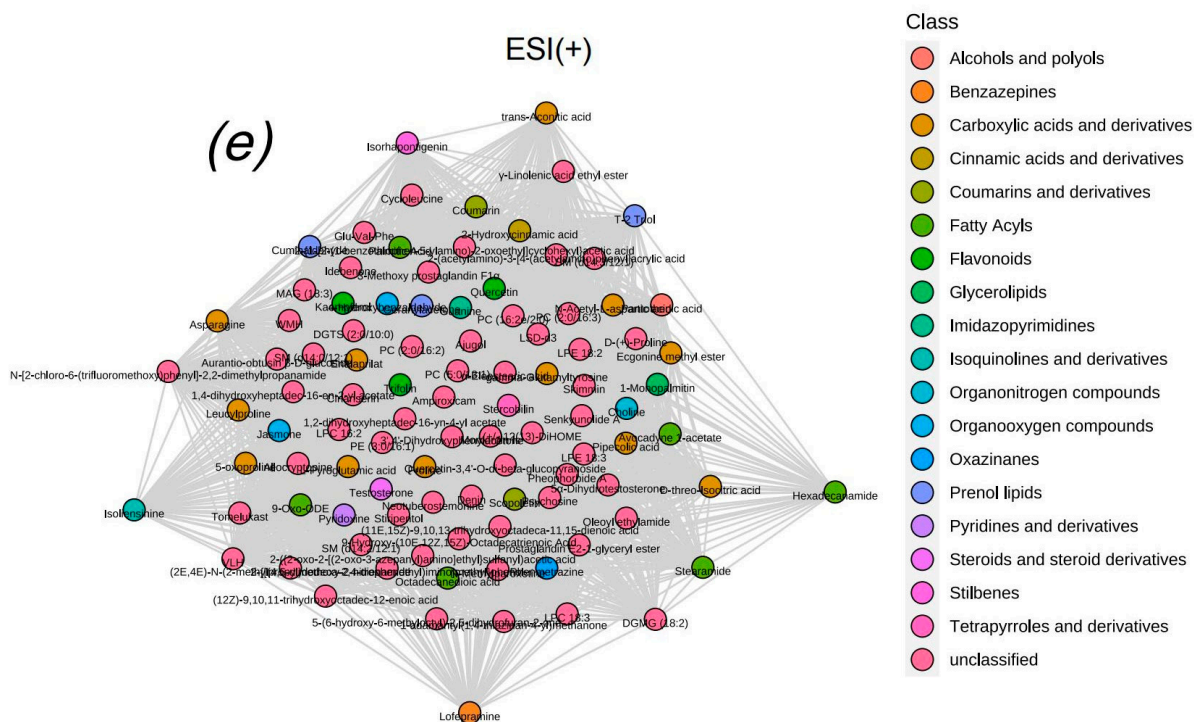


- ESI(-)

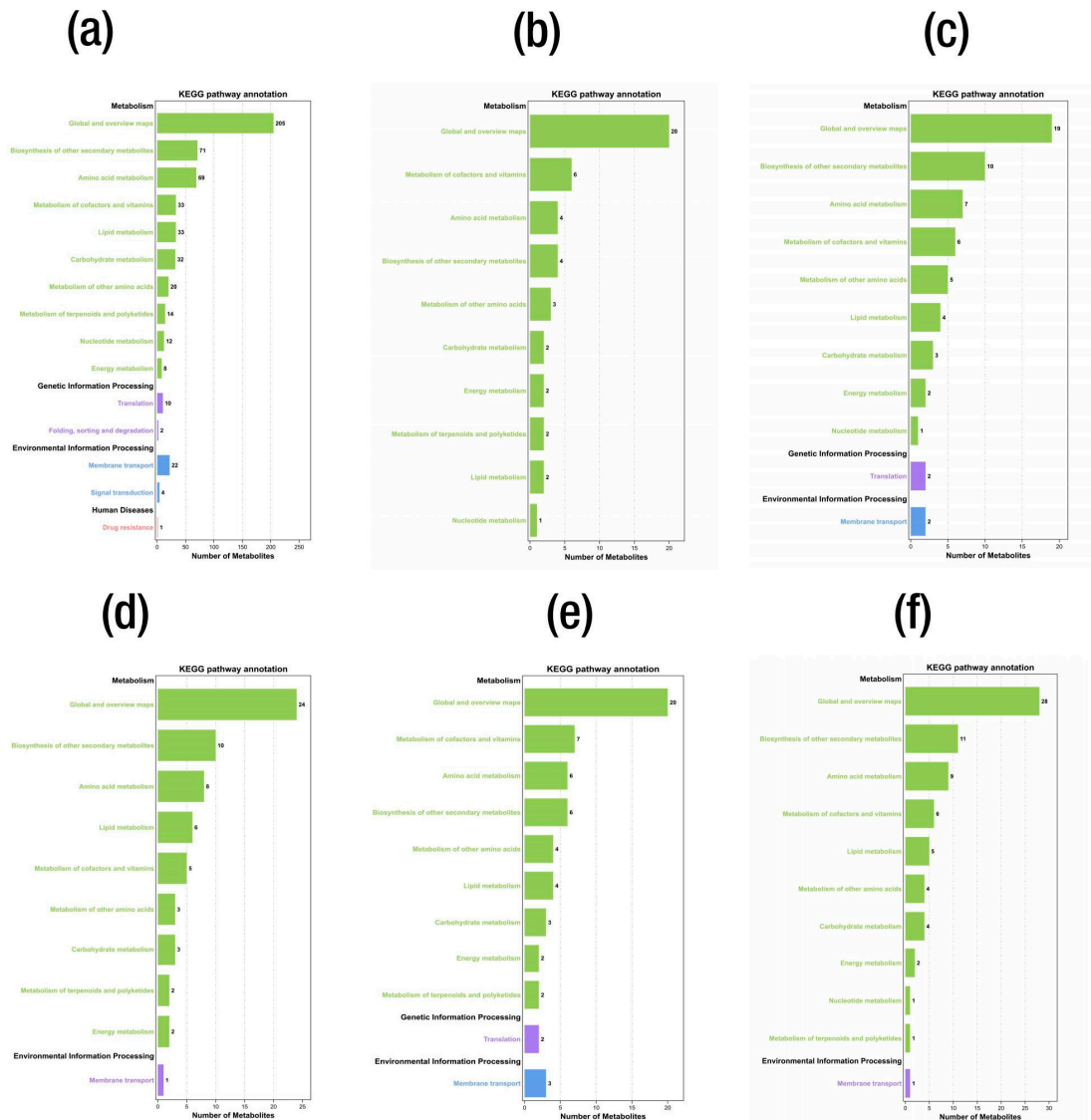


- Alcohols and polyols
- Benzopyrans
- Carboxylic acids and derivatives
- Coumarins and derivatives
- Fatty Acyls
- Flavonoids
- Hydroxy acids and derivatives
- Imidazopyrimidines
- Macrolides and analogues
- Prenol lipids
- unclassified



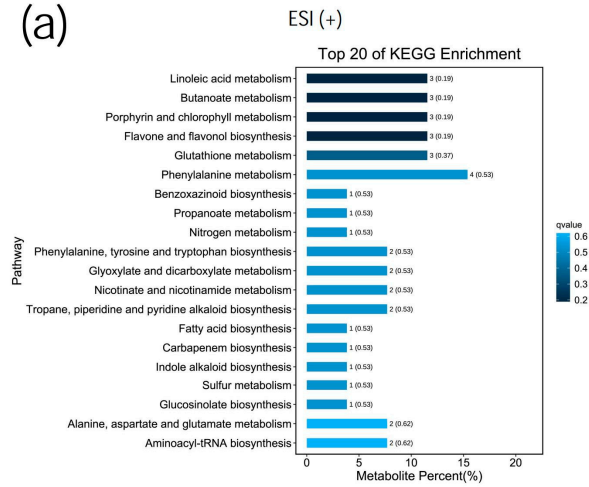


**Figure S10.** Differential metabolites network analysis from various Mg supply in mulberry. (a) CK-T1 (b) CK-T2 (c) CK-T3 (d) CK-T4 (e) CK-T5. The network was built using the differential metabolites classes.

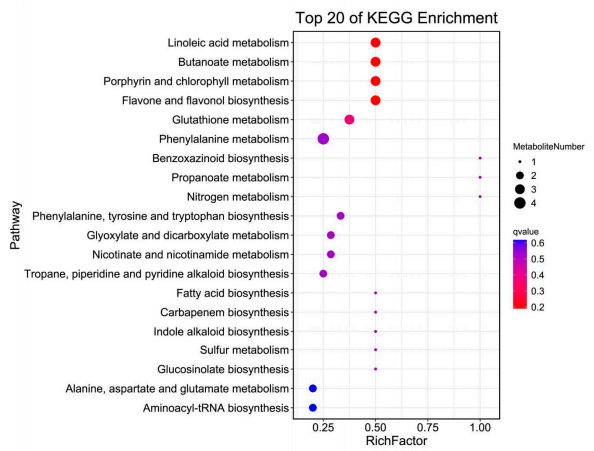


**Figure S11.** Differential metabolites KEGG annotation from various Mg application in mulberry. (a) general KEGG annotation of all the extracted metabolites (b) differential in CK-T1 (c) differential in CK-T2 (d) differential in CK-T3 (e) differential in CK-T4 (f) differential in CK-T5. KEGG pathways were retrieved from [www.kegg.jp/kegg/kegg1.html](http://www.kegg.jp/kegg/kegg1.html).

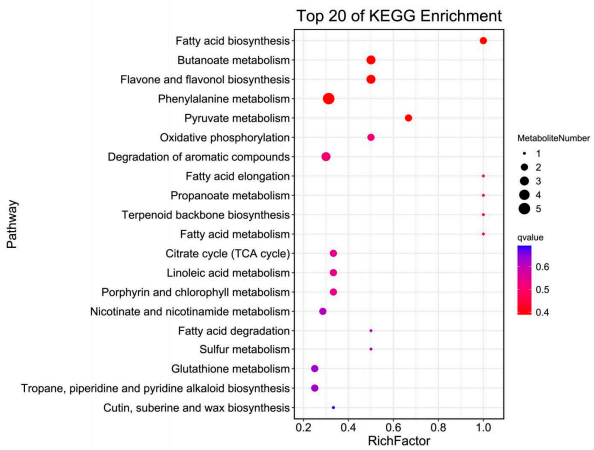
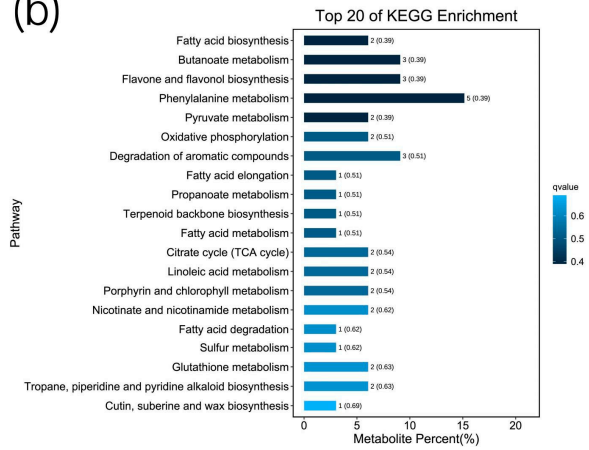
(a)



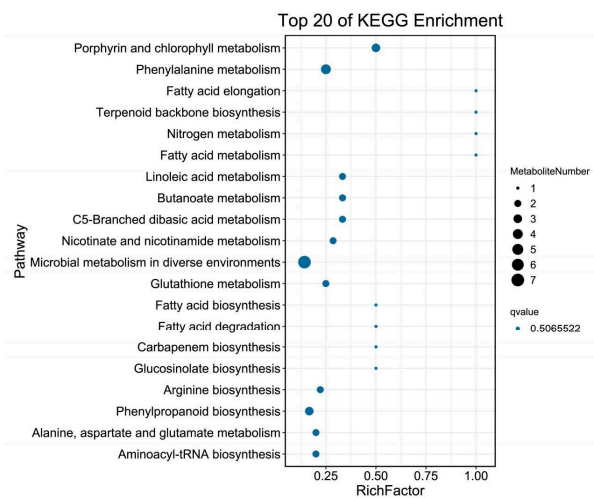
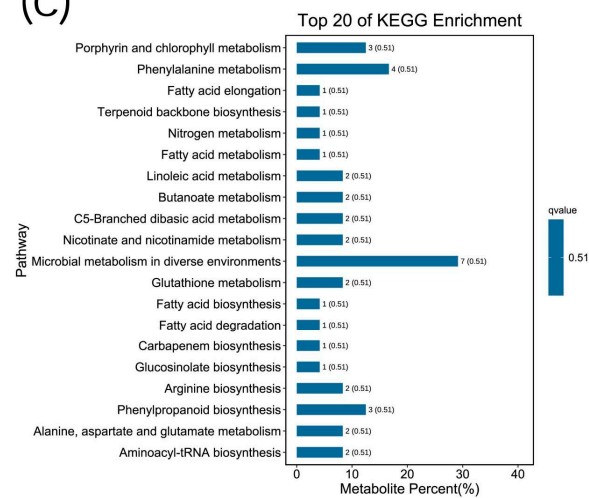
ESI (-)



(b)



(c)



**Figure S12.** Differential metabolites KEGG enrichment analysis of the top 20 pathways from Mg treatments in mulberry. (a) CK-T2 (b) CK-T3, (c) CK-T4. The bar graph is the metabolites percentages in the pathways and the bubble plot on the right side represents enrichment factor of the metabolites in the pathways using the smallest q-value.