SUPPLEMENTARY FIGURES

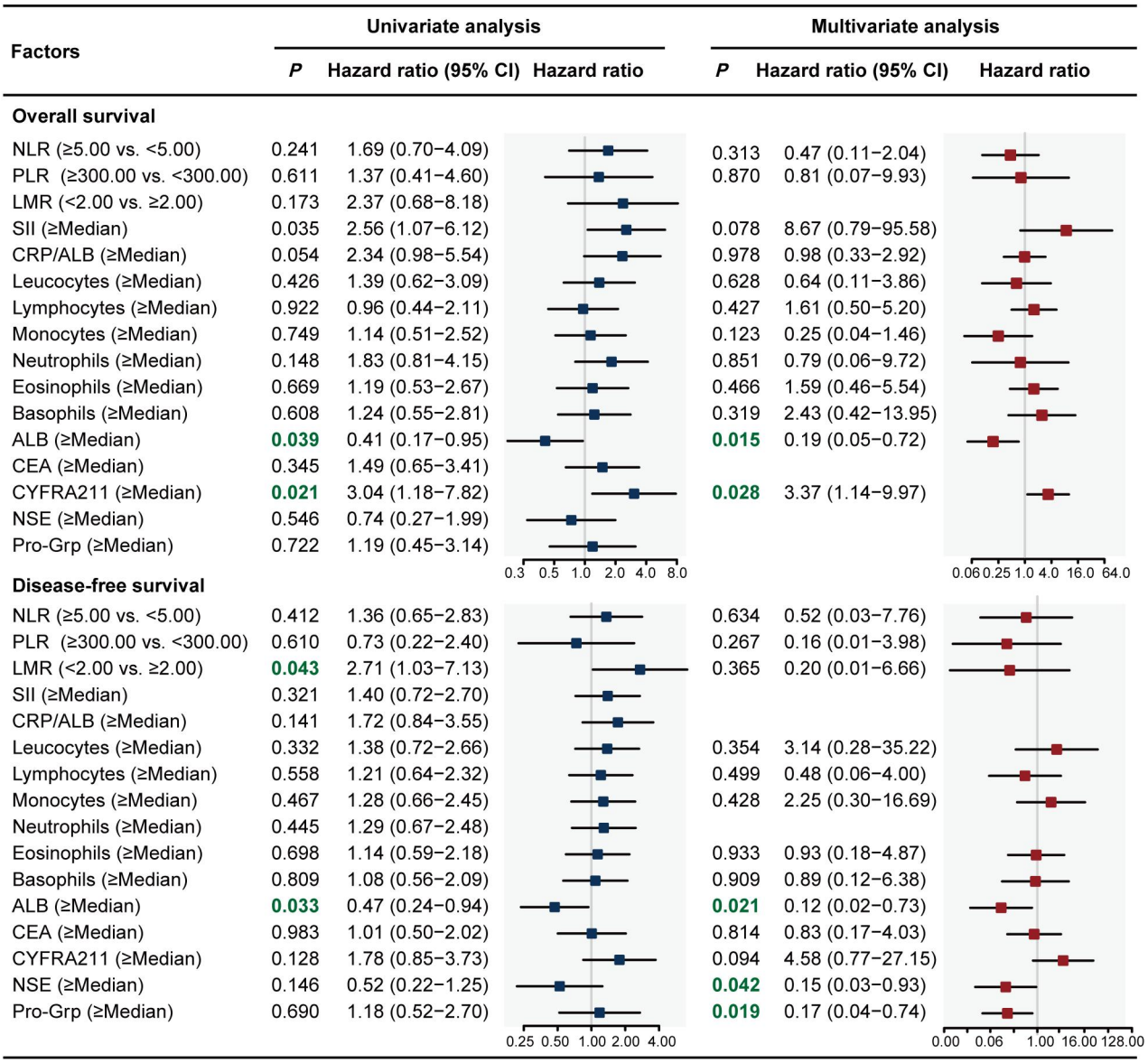


Figure S1. Univariate and multivariate analyses of systemic inflammatory markers on overall survival and disease-free survival. *P*<0.05 are in green text. ALB, albumin; CEA, carcinoembryonic antigen; CRP/ALB, c-reactive protein to albumin ratio; CYFRA211, cytokeratin-19-fragment; LMR, lymphocyte to monocyte ratio; NLR, neutrophil to lymphocyte ratio; NSE, neuron-specific enolase; PLR, platelet to lymphocyte ratio; Pro-Grp, pro-gastrin releasing peptide; SII, systemic immune inflammation index.

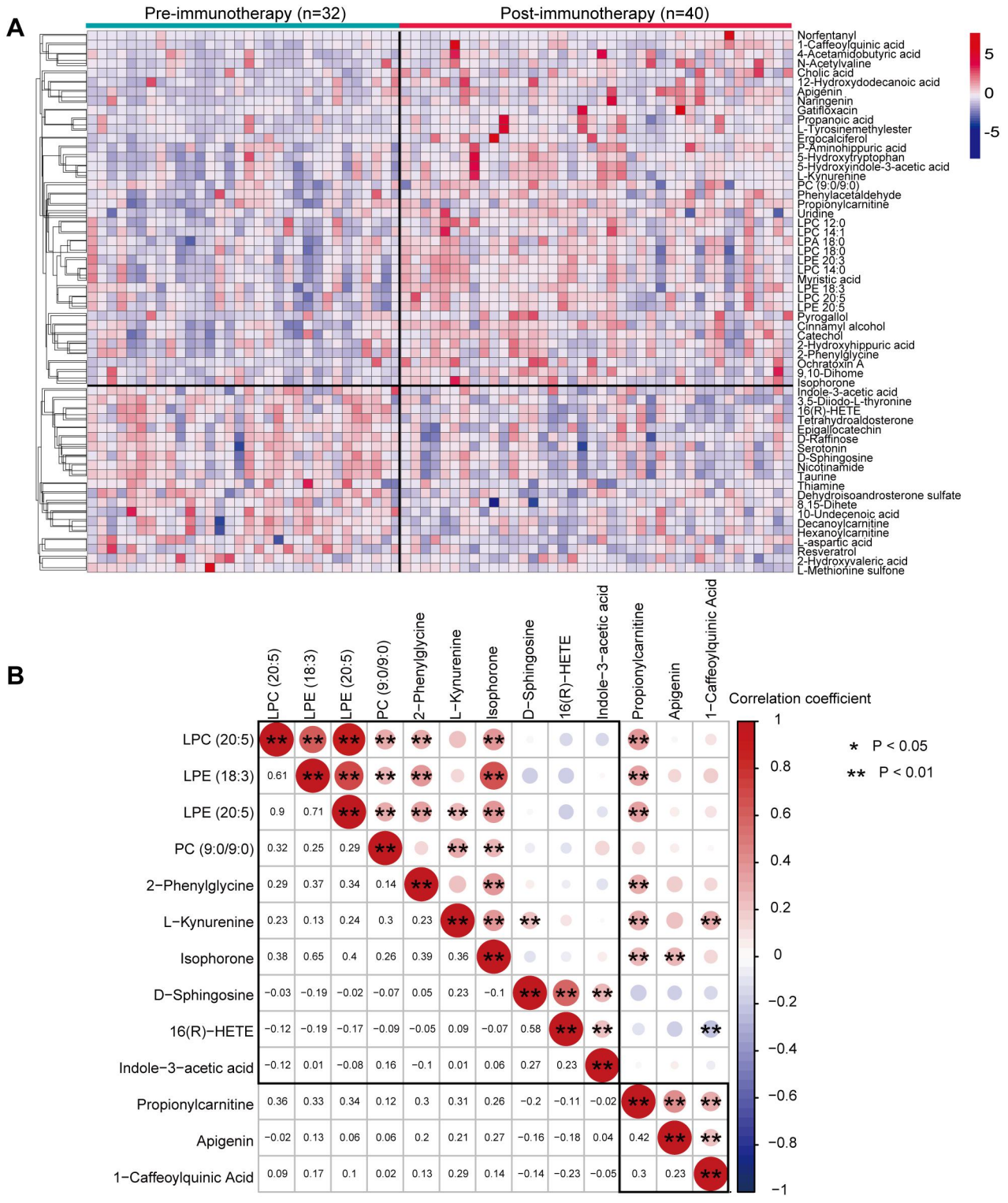


Figure S2. Heatmap of significantly different metabolite clusters before and after immunotherapy and metabolite correlation analysis. (A) Heatmap of cluster analysis of the normalized abundance of metabolites between post- and pre-immunotherapy in LAEC. The color from blue to red indicates an increase in the normalized abundance of metabolites. The data are $\log_2$-transformed and normalized by Z-score. The Z-score is calculated from the mean and standard

deviation of the data set. (B) Spearman's rank correlation analysis between differential metabolites abundance before and after immunotherapy. The size of the circles is based on Spearman's rank correlation coefficient between plasma metabolites. Blue represents negative correlation, red represents positive correlation, *P<0.05, **P<0.01.

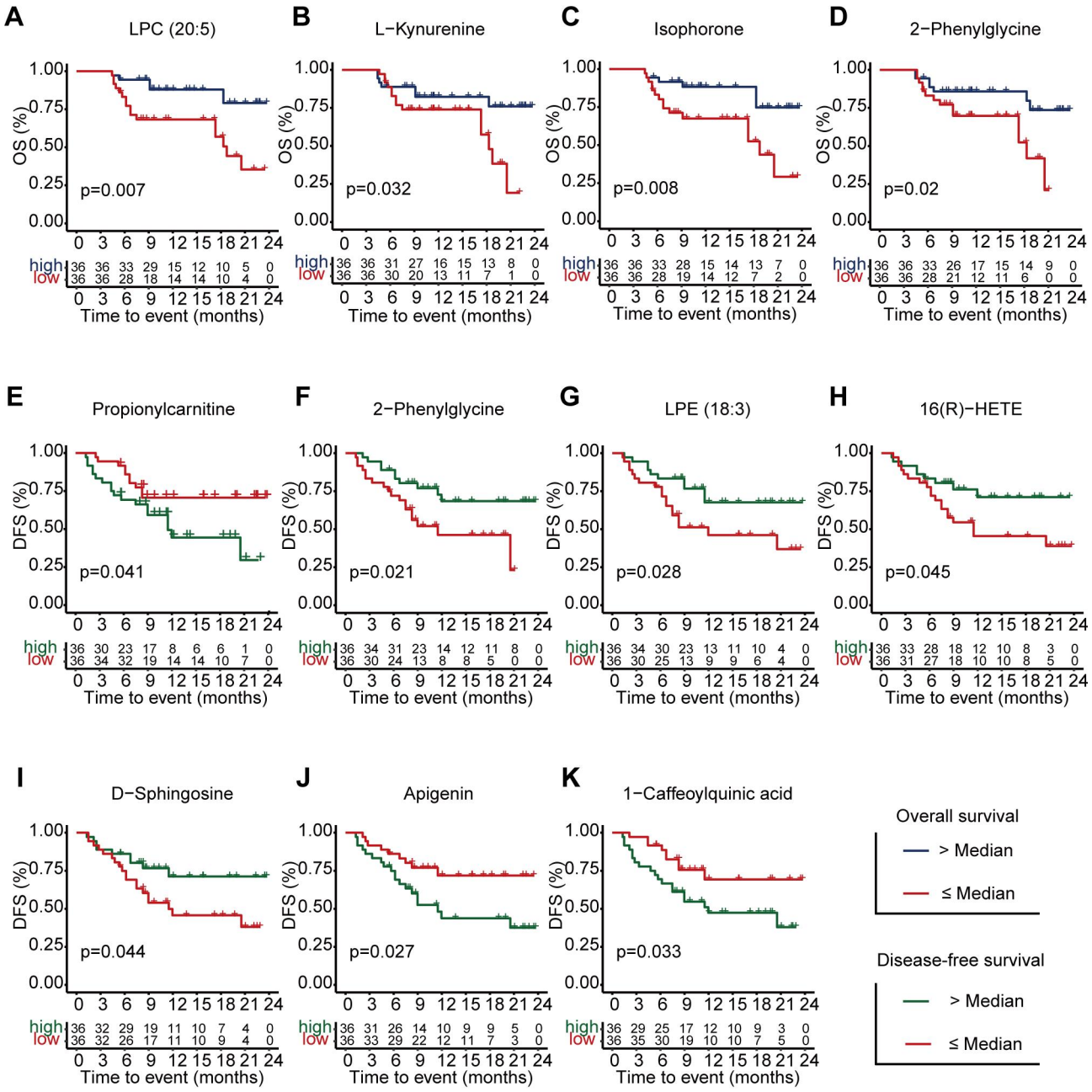


Figure S3. (A-K) Kaplan-Meier estimates of OS and DPS in patients with locally advanced esophageal cancer based on differential metabolite levels before and after neoadjuvant immunotherapy. Log-rank (Mantel-Cox) test for survival comparison. *P*<0.05 was considered statistically significant. DFS, disease-free survival; OS, overall survival.

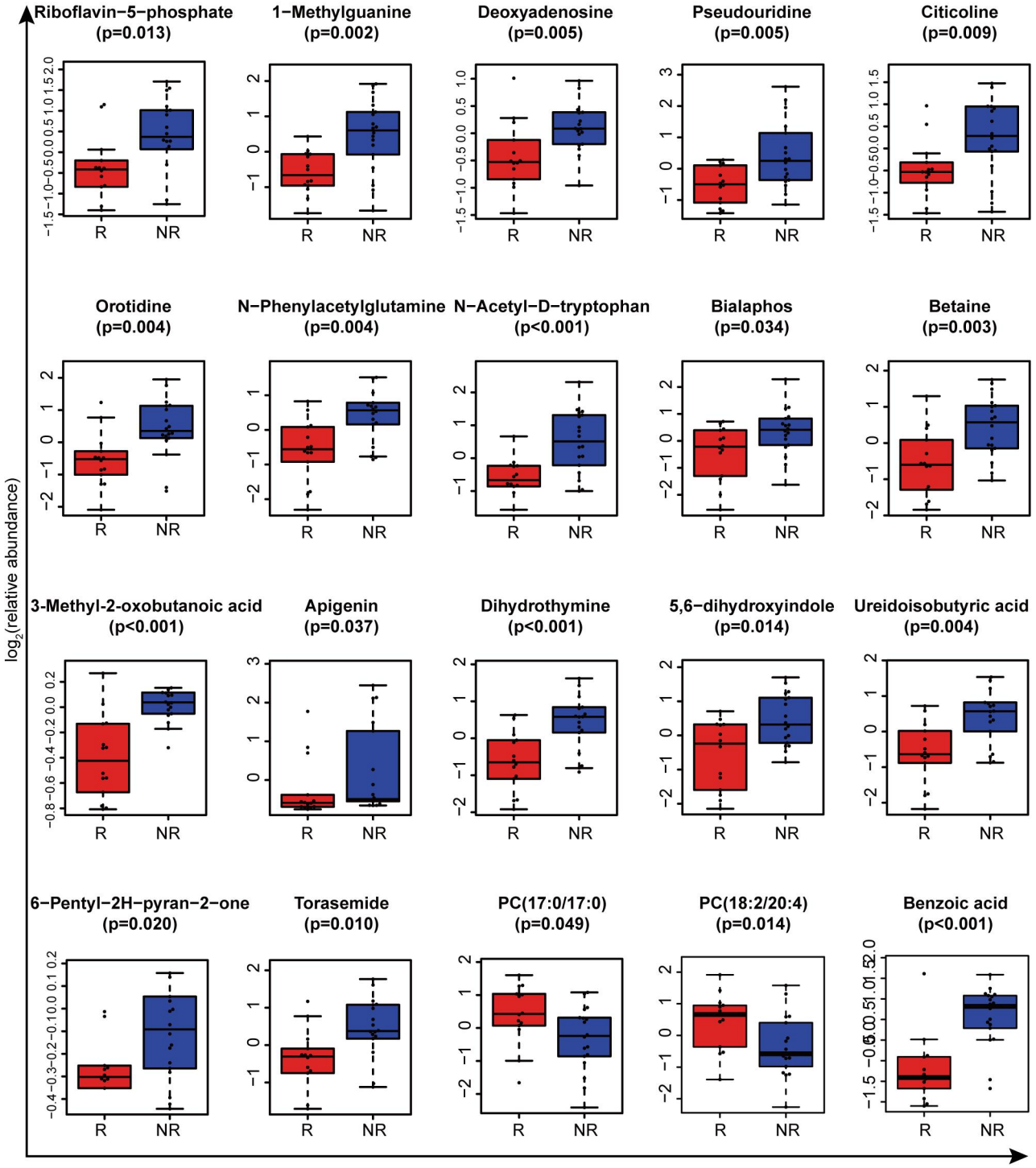
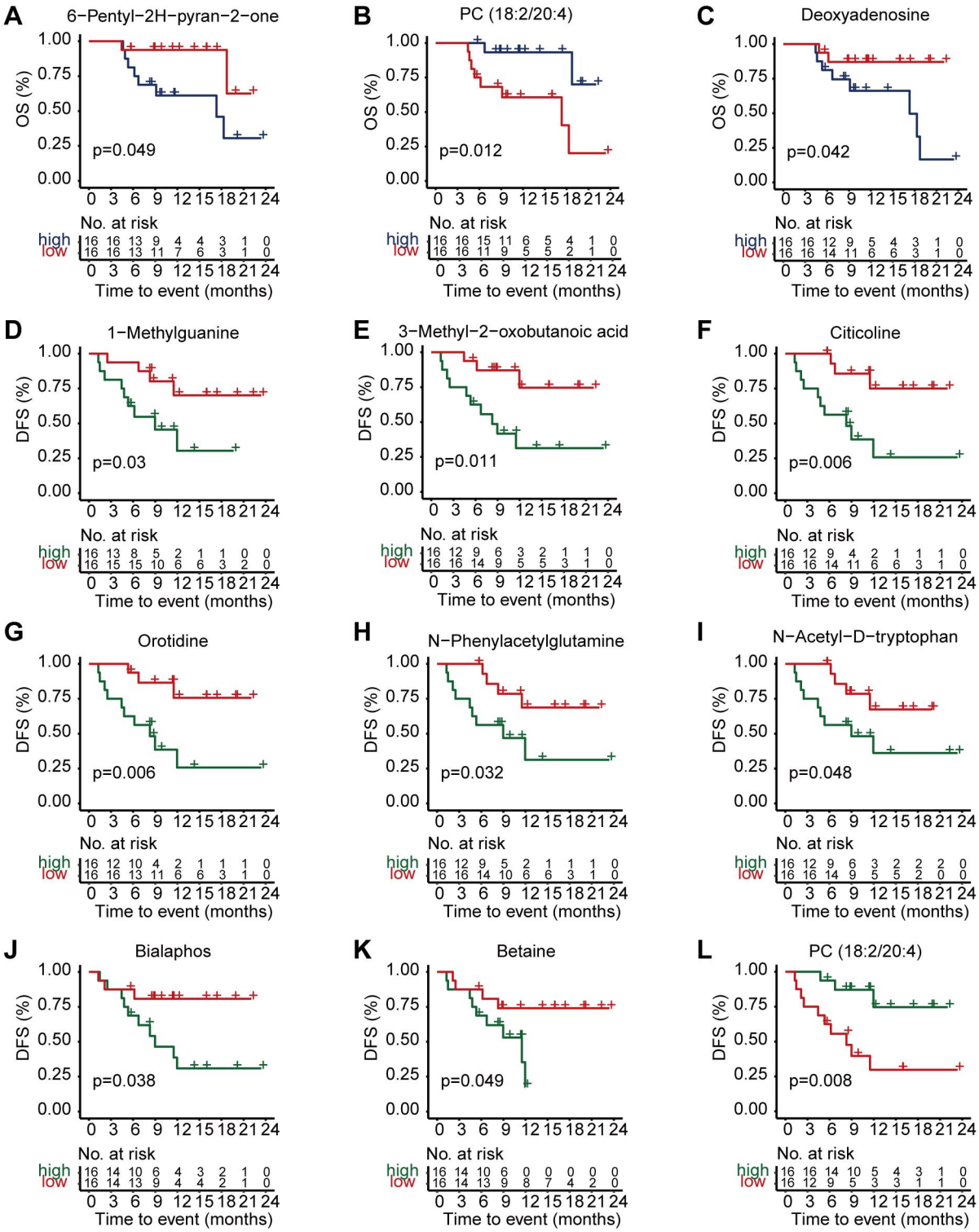


Figure S4. Box plots of the normative abundance distribution of plasma metabolites in responders and non-responders at baseline. Data are presented as means ± SEM. Two-tailed Wilcoxon's test for the relative abundance of metabolites comparison. $P<0.05$ was considered statistically significant. NR, non-responders; R, responders.


A
6−Pentyl−2H−pyran−2−one
OS (%)
p=0.049
No. at risk
high 16 16 13 9 4 4 3 1 0
low 16 16 13 11 7 6 3 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)
B
PC (18:2/20:4)
OS (%)
p=0.012
No. at risk
high 16 16 15 11 6 5 4 1 0
low 16 16 11 9 5 5 2 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)
C
Deoxyadenosine
OS (%)
p=0.042
No. at risk
high 16 16 12 9 5 4 3 1 0
low 16 16 14 11 6 6 3 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)
D
1−Methylguanine
DFS (%)
p=0.03
No. at risk
high 16 13 8 5 2 1 1 0 0
low 16 15 15 10 6 6 3 2 0
0 3 6 9 12 15 18 21 24
Time to event (months)
E
3−Methyl−2−oxobutanoic acid
DFS (%)
p=0.011
No. at risk
high 16 12 9 6 3 2 1 1 0
low 16 16 14 9 5 5 3 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)
F
Citicoline
DFS (%)
p=0.006
No. at risk
high 16 12 9 4 2 1 1 1 0
low 16 16 14 11 6 6 3 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)
G
Orotidine
DFS (%)
p=0.006
No. at risk
high 16 12 10 4 2 1 1 1 0
low 16 16 13 11 6 6 3 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)
H
N−Phenylacetylglutamine
DFS (%)
p=0.032
No. at risk
high 16 12 9 5 2 1 1 1 0
low 16 16 14 10 6 6 3 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)
I
N−Acetyl−D−tryptophan
DFS (%)
p=0.048
No. at risk
high 16 12 9 6 3 2 2 2 0
low 16 16 14 9 5 5 2 0 0
0 3 6 9 12 15 18 21 24
Time to event (months)
J
Bialaphos
DFS (%)
p=0.038
No. at risk
high 16 14 10 6 4 3 2 1 0
low 16 14 13 9 4 4 2 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)
K
Betaine
DFS (%)
p=0.049
No. at risk
high 16 14 10 6 0 0 0 0 0
low 16 14 13 9 8 7 4 2 0
0 3 6 9 12 15 18 21 24
Time to event (months)
L
PC (18:2/20:4)
DFS (%)
p=0.008
No. at risk
high 16 16 14 10 5 4 3 1 0
low 16 12 9 5 3 3 1 1 0
0 3 6 9 12 15 18 21 24
Time to event (months)

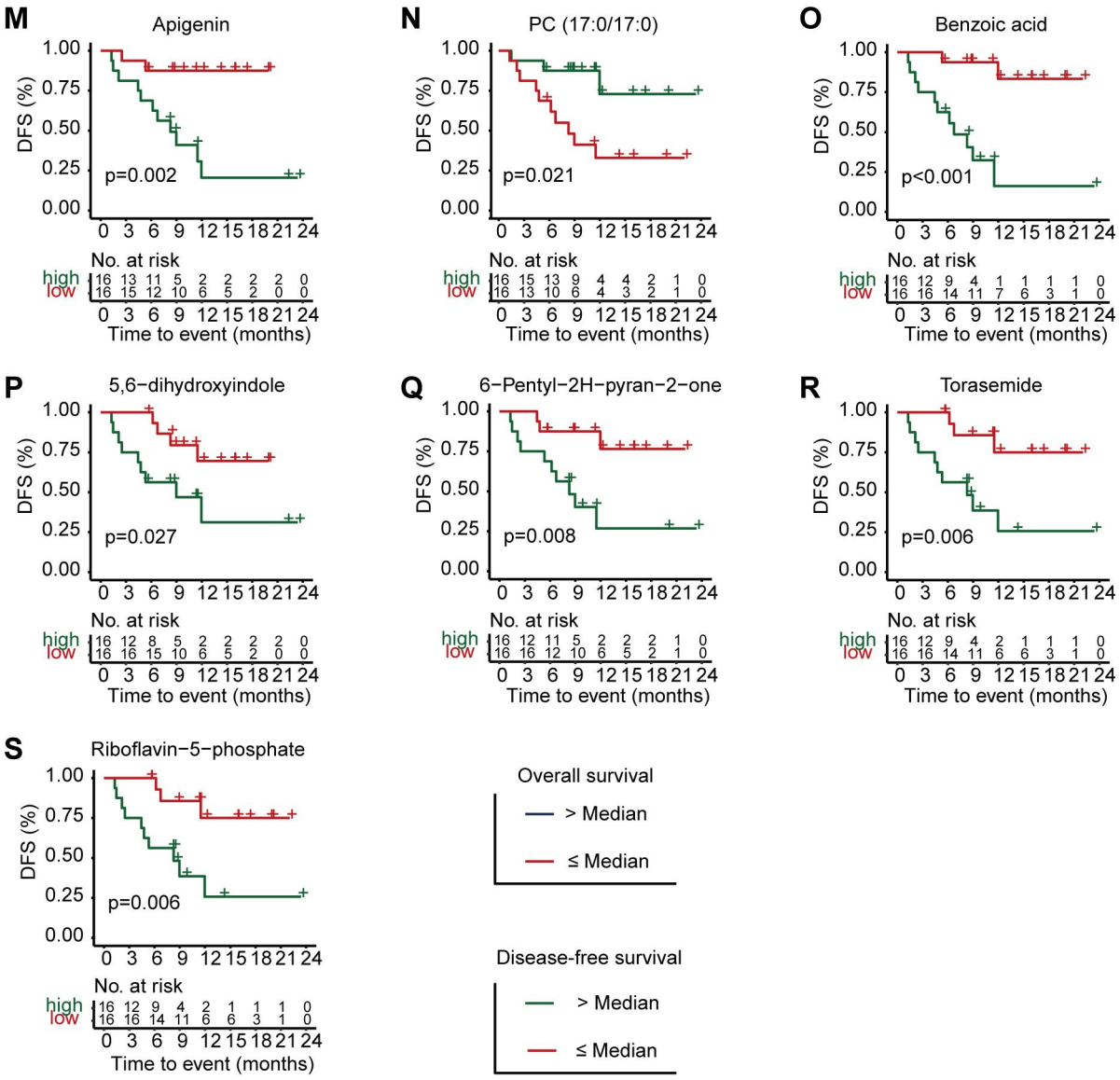


Figure S5. (A-U) Kaplan-Meier estimates of overall survival and disease-free survival based on baseline metabolite levels in patients with locally advanced esophageal cancer receiving neoadjuvant immunotherapy. Log-rank (Mantel-Cox) test for survival comparison. *P*<0.05 was considered statistically significant. DFS, disease-free survival; OS, overall survival.

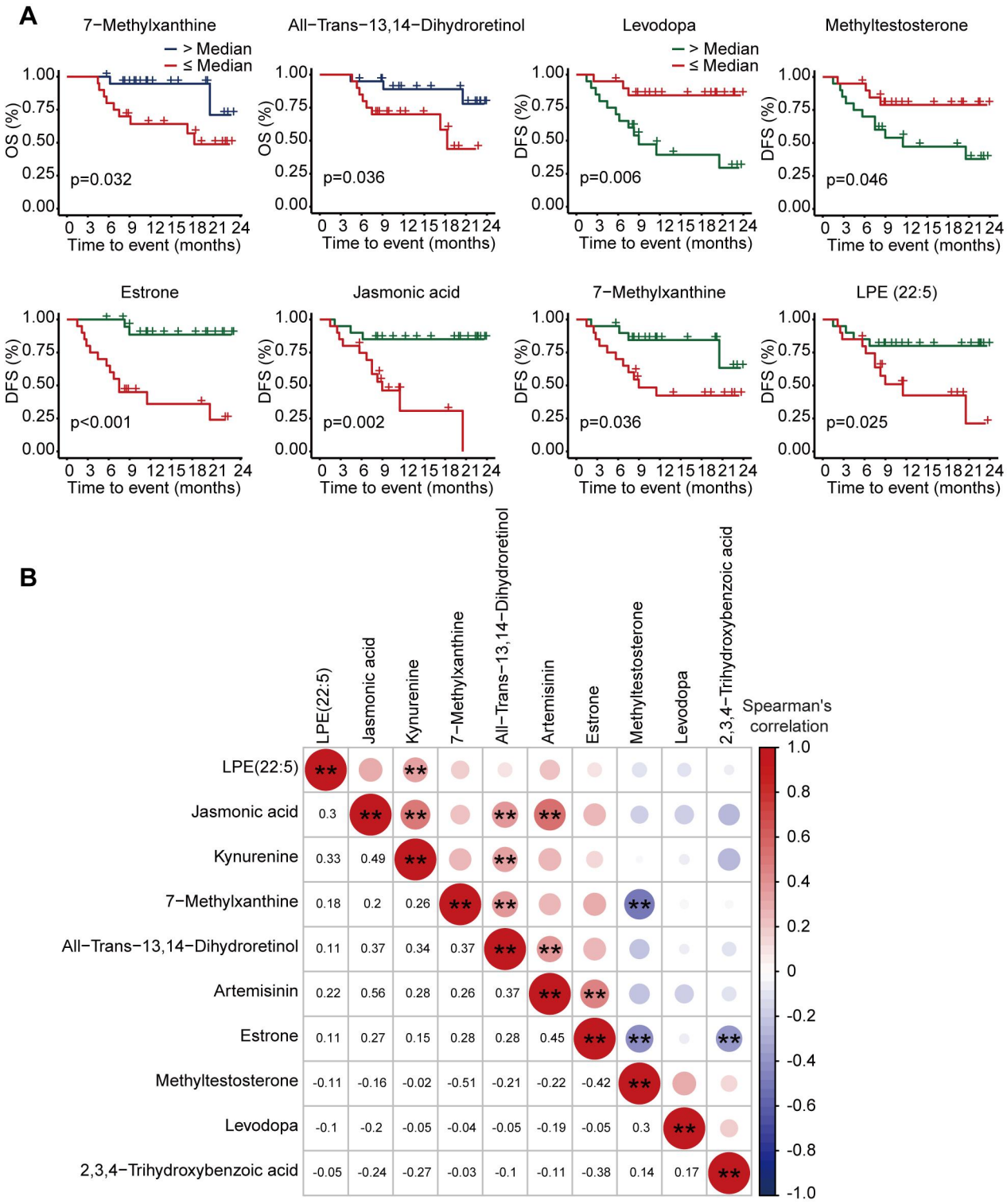


Figure S6. Survival analysis and correlation analysis based on metabolites abundance after immunotherapy. (A) Kaplan-Meier estimates of OS and DFS for patients with locally advanced esophageal cancer receiving neoadjuvant immunotherapy based on post-immunotherapy metabolite levels. The log-rank (Mantel-Cox) test was used to compare the differences in OS and DFS between the high-abundance (>median) and low-abundance (⩽median)metabolite groups. (B) Spearman's

rank correlation analysis of survival-related differential metabolites abundance after immunotherapy. The size and color of the upper half of the circles are based on Spearman's rank correlation coefficient between plasma metabolites. Blue represents negative correlation, red represents positive correlation, and the lower half of the numbers are Spearman's rank correlation coefficient values. *P<0.05, **P<0.01. Log-rank (Mantel-Cox) test for survival comparison. P<0.05 was considered statistically significant. DFS, disease-free survival; OS, overall survival.

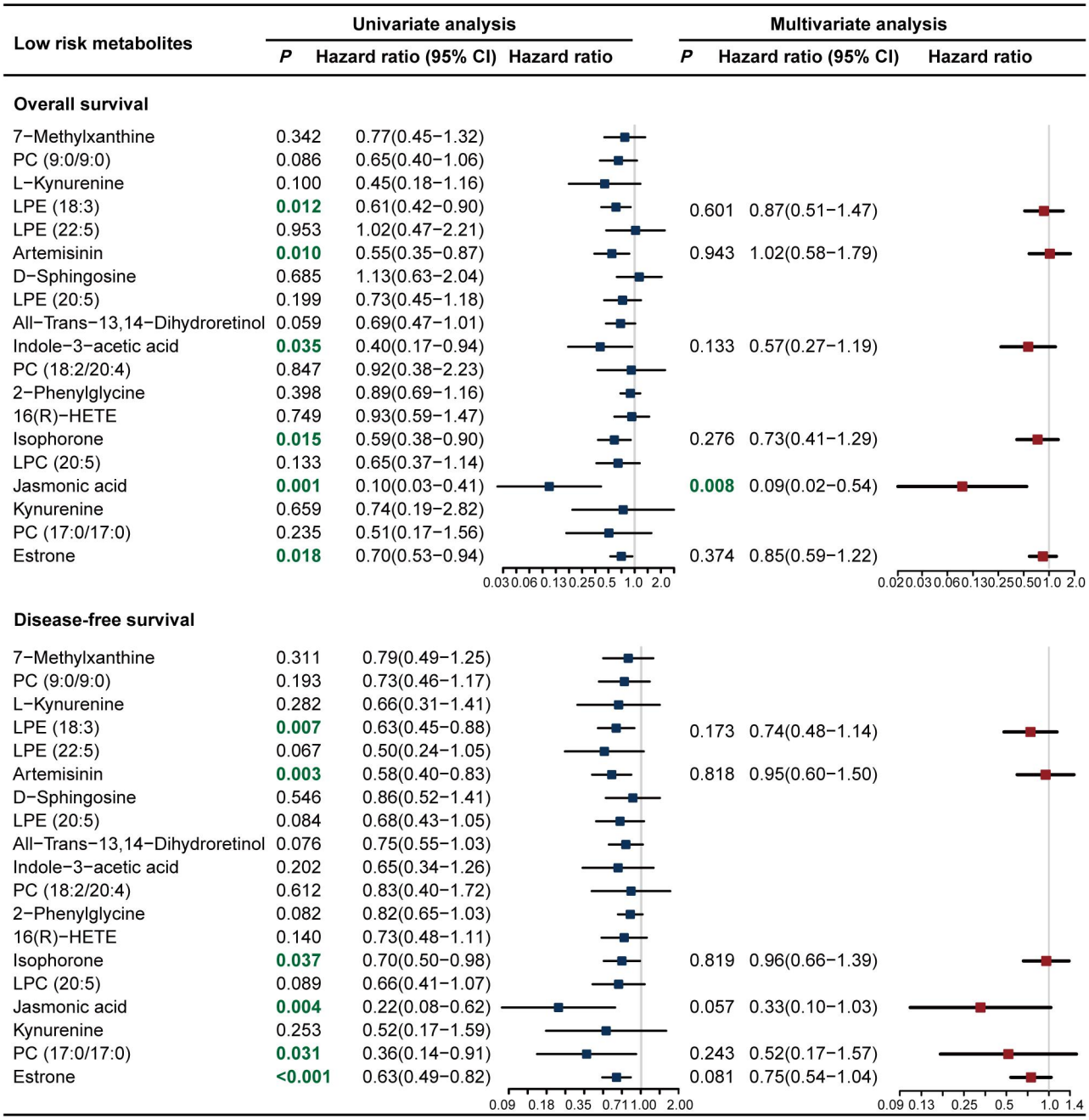


Figure S7. Univariate and multivariate Cox regression analyses of overall survival and disease-free survival of identified metabolic biomarkers associated with favourable prognosis in the total population. $P<0.05$ are in green text. CI, confidence interval.

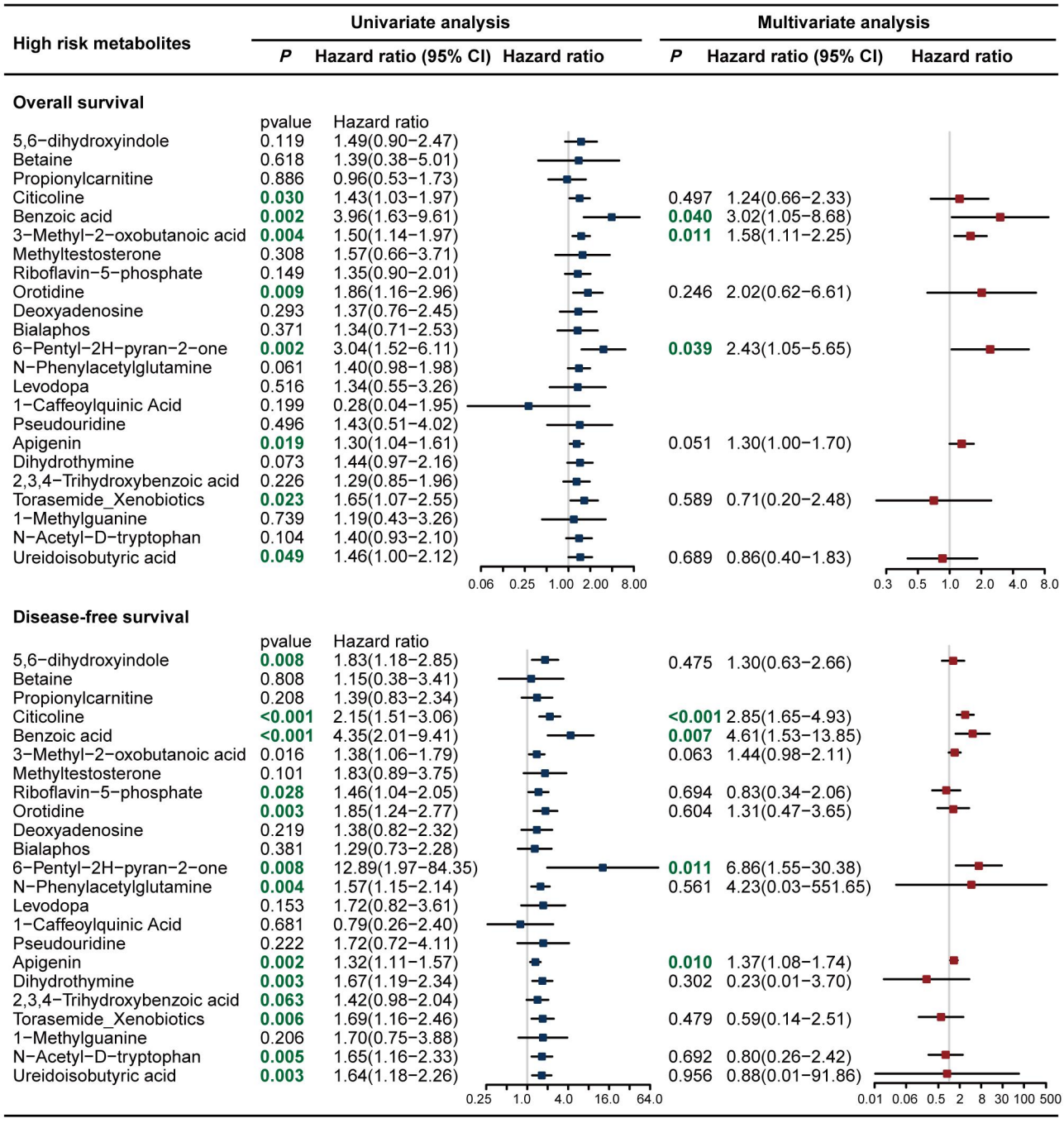


Figure S8. Univariate and multivariate Cox regression analyses of overall survival and disease-free survival of identified metabolic biomarkers associated with poor prognosis in the total population. *P*<0.05 are in green text. CI, confidence interval.

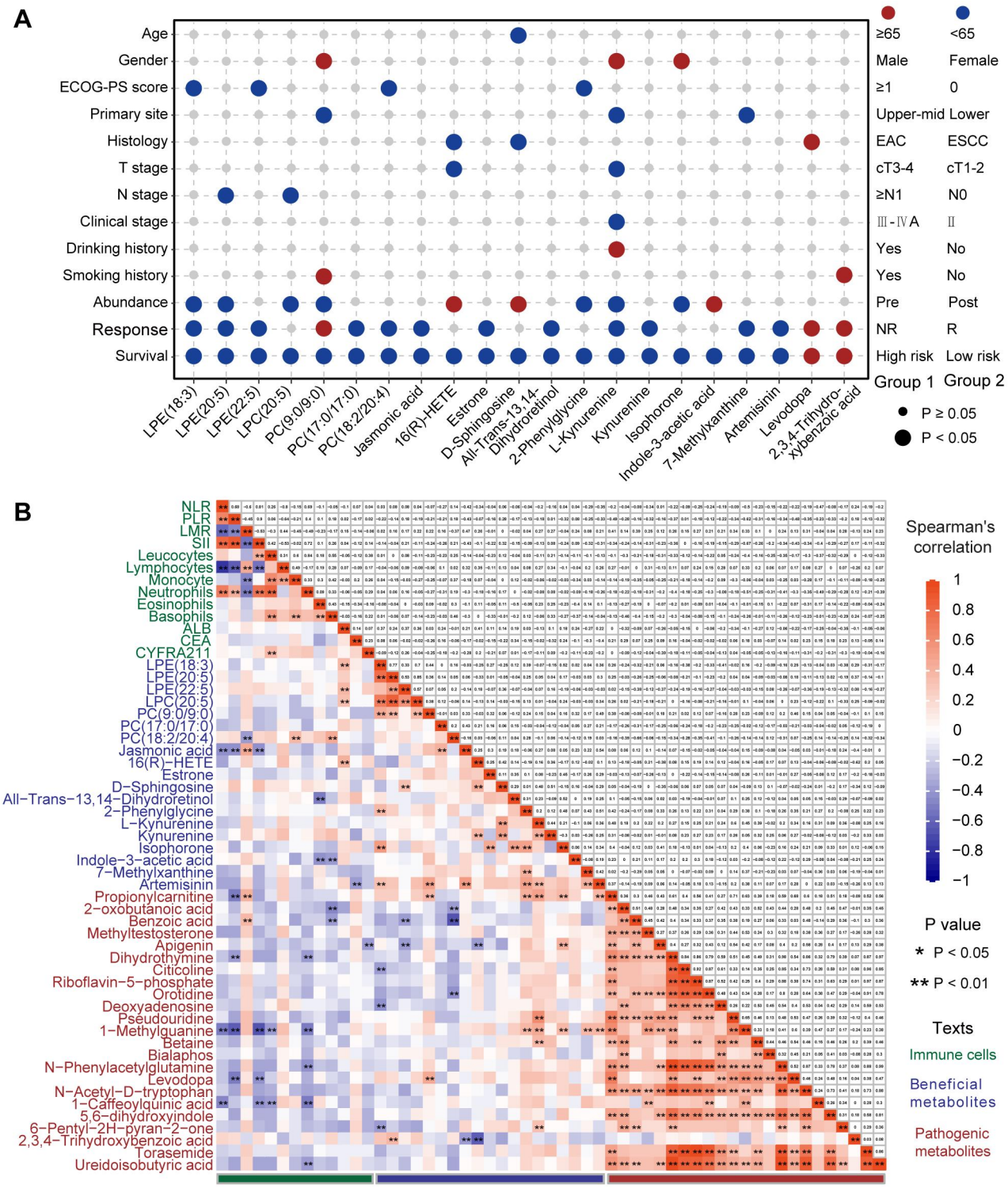


Figure S9. Correlation of candidate biomarkers with clinical parameters and systemic inflammatory markers. (A) Relationship between potential biomarkers abundance and clinical parameters. Red indicates that metabolite abundance is significantly higher in group 1, and blue indicates that metabolite abundance is significantly higher in group 2. The size of the circles represents the P value, with small circles representing $P \geq 0.05$ and large circles representing $P < 0.05$.

P values were calculated using Wilcoxon's test. (B) Correlation analysis between potential biomarker abundance, peripheral blood systemic inflammatory markers content, and clinical tumor marker levels at baseline. The color from blue to red indicates an increased Spearman's rank correlation coefficient, with blue representing negative correlation and red representing positive correlation. The green text indicates peripheral blood immune cells, the blue text indicates metabolites significantly associated with better prognosis, and the red text indicates metabolites significantly associated with poor prognosis. *$P<0.05$, **$P<0.01$. Data are presented as means ± SEM. Two-tailed Wilcoxon's test for the relative abundance of metabolites comparison (A), log-rank (Mantel-Cox) test for survival comparison (A). $P<0.05$ was considered statistically significant. EAC, esophageal adenocarcinoma; ECOG-PS, eastern cooperative oncology group performance status; ESCC, esophageal squamous-cell carcinoma; NR, non-responders; R, responders; SEM, standard error of the mean.

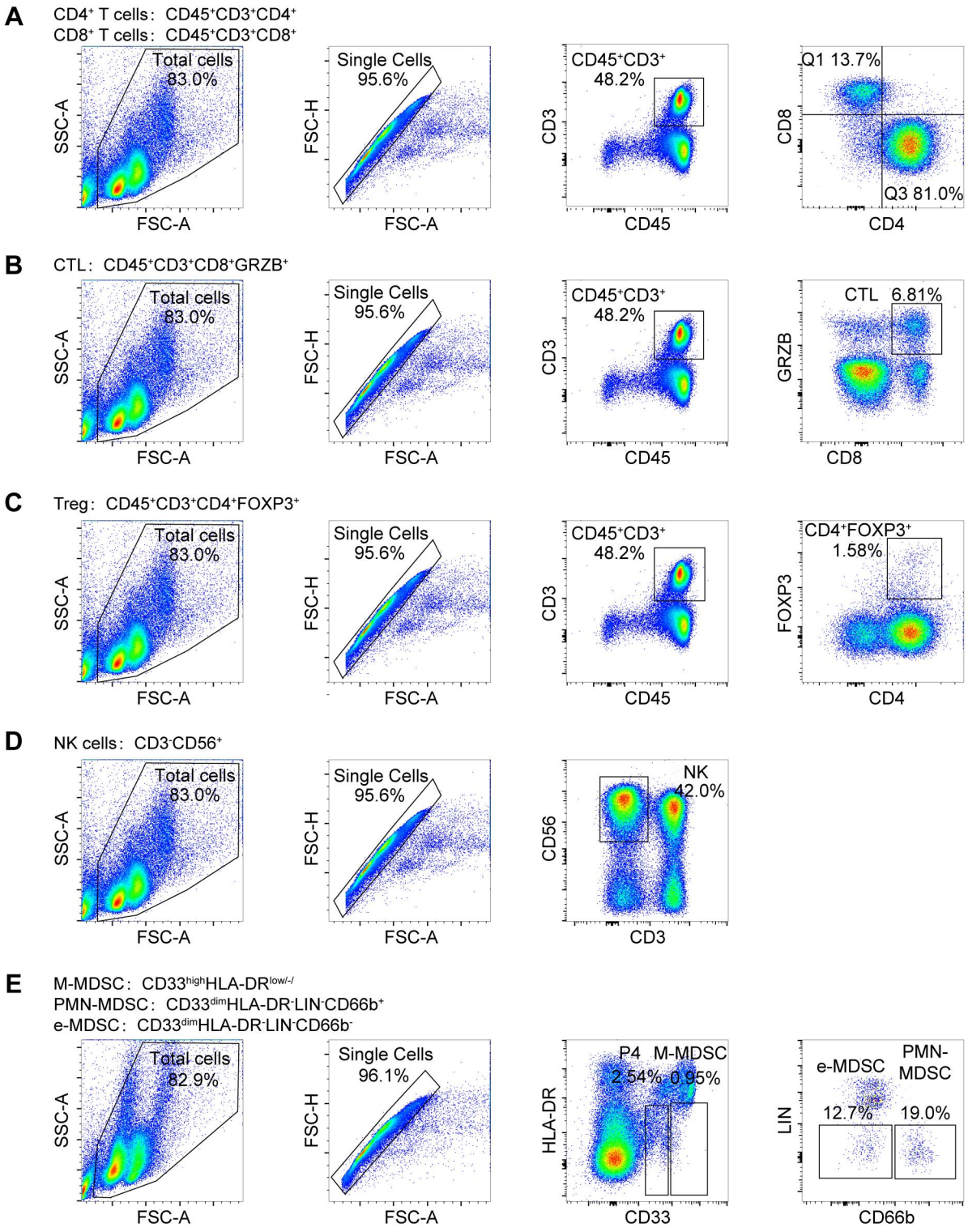


Figure S10. Representative flow chart of a stepwise gating strategy for flow cytometry of T cells, natural killer cells, and myeloid-derived suppressor cells separated from peripheral blood.

Data are presented as means ± SEM. SEM, standard error of the mean.

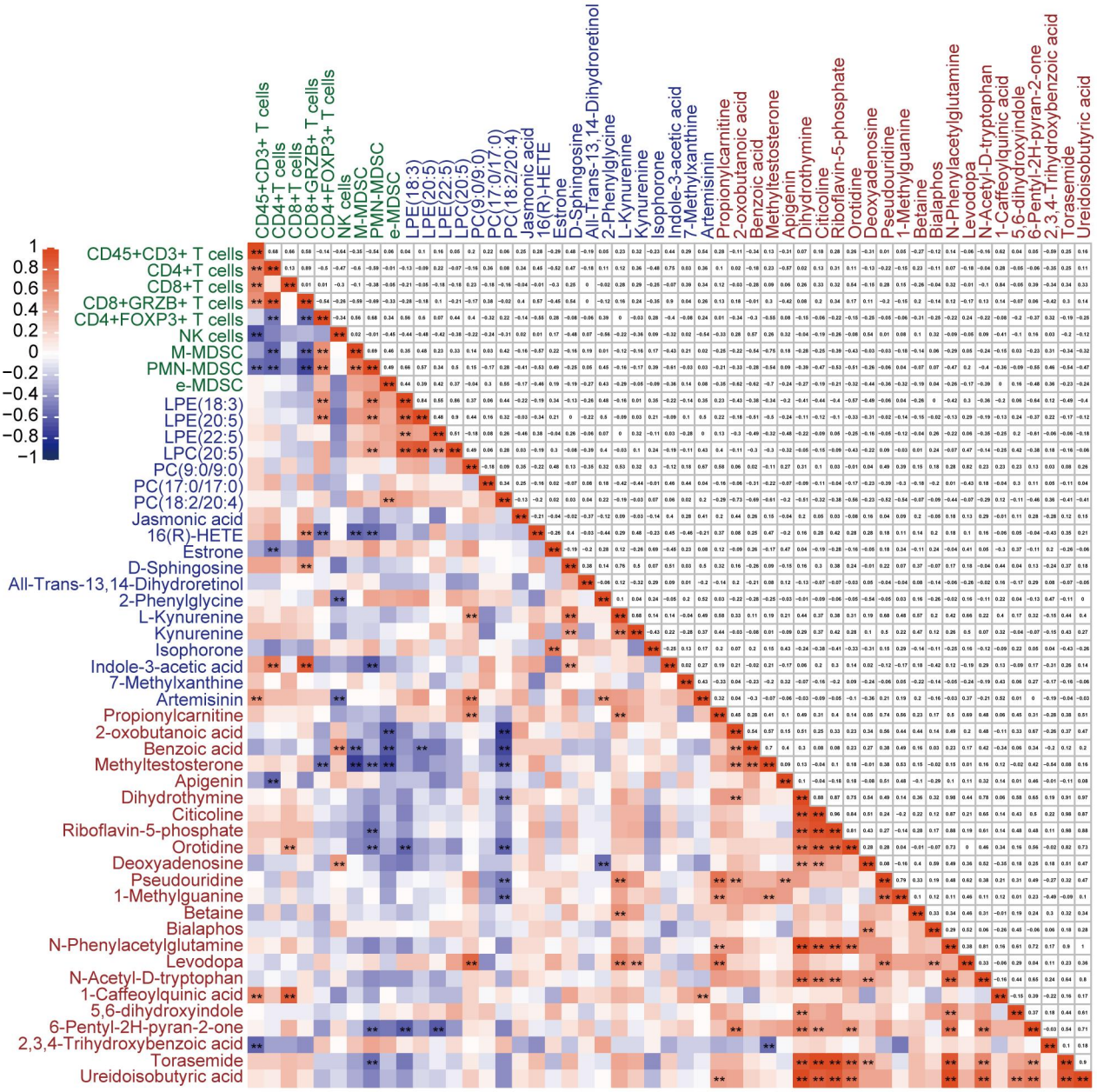


Figure S11. Spearman's rank correlation analysis between potential biomarkers abundances and peripheral blood immune cell levels at baseline. The colors from blue to red in the lower half of the graph indicate Spearman's rank correlation coefficient between plasma metabolites and peripheral blood immune cells, with blue representing a negative correlation and red representing a positive correlation. Numbers in the lower half of the figure represent Rho values. The green text indicates peripheral blood immune cells, the blue text indicates metabolites significantly associated with favorable prognosis, and the red text indicates metabolites significantly associated with poor prognosis. **P*<0.05, ***P*<0.01.

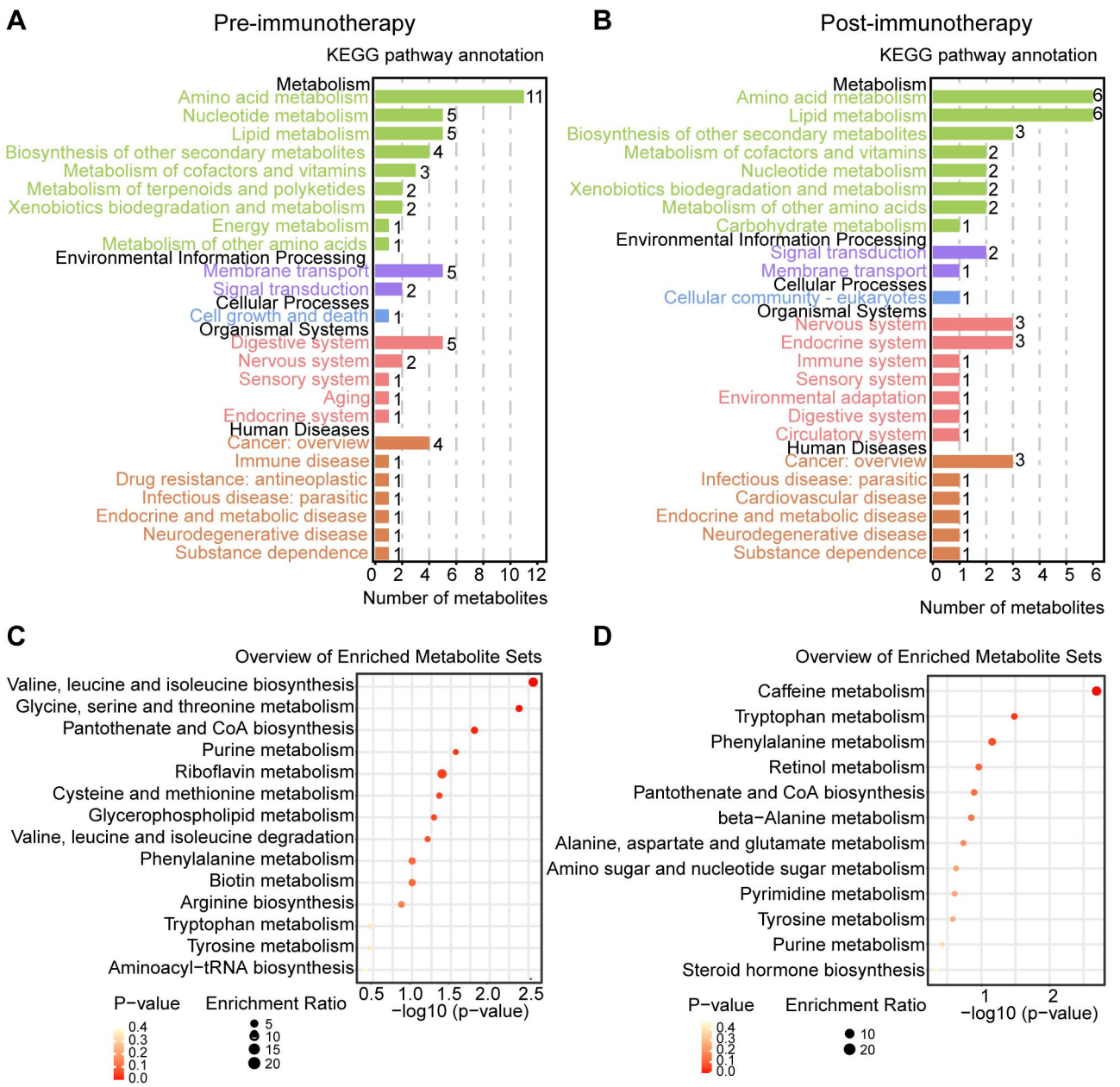


Figure S12. Prognosis-related metabolites pathway annotations and pathway enrichment analysis before and after neoadjuvant immunotherapy. (A-B) Prognosis-related metabolites pathway annotations before (A) and after (B) neoadjuvant immunotherapy. The horizontal coordinate represents the number of metabolites, and the vertical coordinate represents the KEGG pathways annotated; the graph shows the number of metabolites annotated in each secondary subclass under the Pathway primary subclass. (C-D) Prognosis-related metabolites pathway enrichment analysis before (C) and after (D) neoadjuvant immunotherapy. The horizontal coordinate is the number of metabolites in the corresponding metabolic pathway/the total number of metabolites identified in that

pathway, with larger values indicating higher metabolites enrichment. The color of the dot represents the P value. The size of the dots represents the number of metabolites in the pathway.

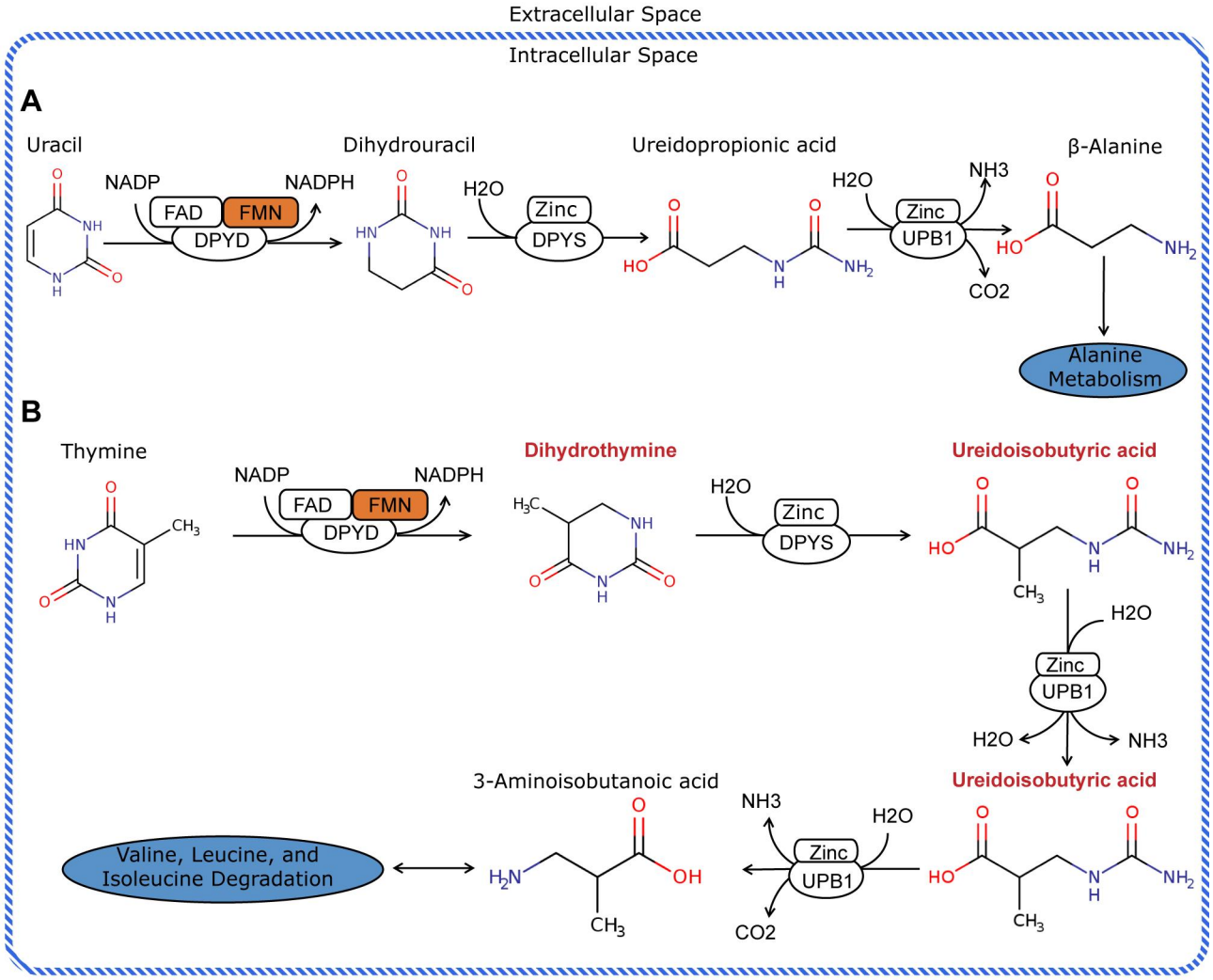


Figure S13. Pyrimidine nucleotide catabolism. (A) Cytosine and uracil catabolism. (B) Thymidine catabolism. DPYD, dihydropyrimidine dehydrogenase; DPYS, dihydropyrimidinase; FAD, flavin adenine dinucletide; FMN, flavin mononucleotide, Riboflavin-5-phosphate; NADP, nicotinamide adenine dinucleotide phosphate; UPB1, Beta-ureidopropionase.

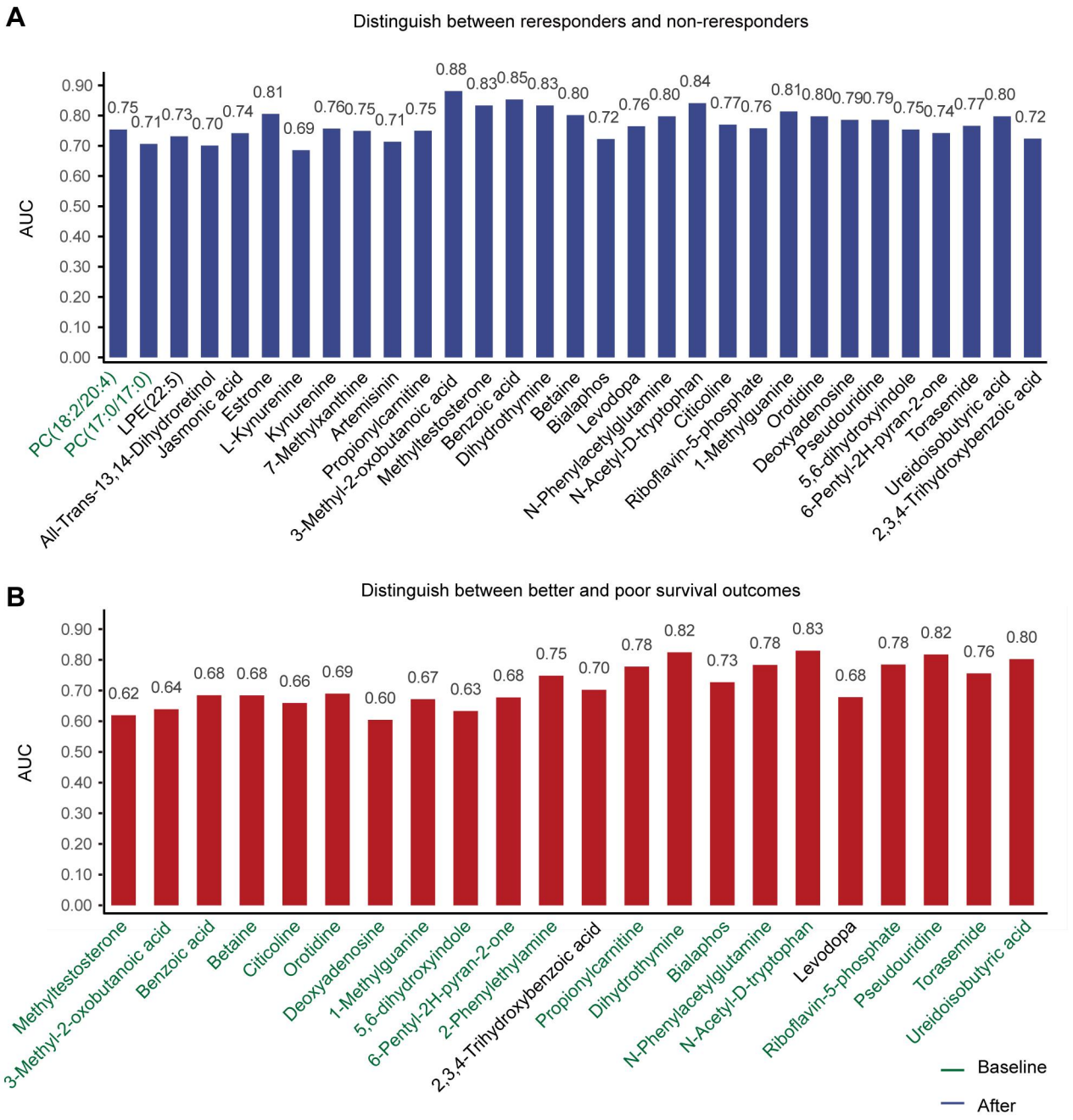


Figure S14. ROC curve analysis of candidate metabolic biomarkers. (A) The ability of individual candidate biomarkers to distinguish immunotherapy responders from non-responders was assessed by the AUC generated from the ROC curves. Analysis was performed using the R package "pROC". (B) The time-dependent AUC assessed the prognostic power of individual candidate metabolic biomarkers under the ROC curve for 12-month survival. The green text indicates the predictive power of biomarkers at baseline, and the black text indicates the predictive power of biomarkers after immunotherapy. The analysis was performed using the R package"survivalROC". AUC, area under the curve; ROC, operating characteristic.