

# Tophi removal surgery improves the metabolic landscape of synovial tissue and serum by regulating lipid metabolism pathways

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## Research Article

**Keywords:** gout, synovial tissue, serum, metabolomics

**Posted Date:** October 14th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-11063463/v1>

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**Additional Declarations:** No competing interests reported.

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# Abstract

## Background

To explore the effects of surgical tophi removal on local synovial tissue and systemic serum metabolic networks in patients with advanced gout, and to clarify the related metabolic changes and inflammatory mechanisms.

## Methods

Liquid chromatography-mass spectrometry (LC-MS) was used to detect and compare metabolic alterations in synovial tissue and serum from gout patients before and after surgical tophi resection. Gene expression levels of *ALOX15*, *PLA2G3*, *TNF $\alpha$* , and *NF- $\kappa$ B1* were determined by using RT-qPCR and their protein expression levels were detected by Western blotting (WB).

## Results

Compared with healthy controls, gout patients showed significant metabolic dysregulation in synovial tissue. Furthermore, we also found that significant metabolic dysregulation in serum preoperatively compared with the control group, while the serum exhibited a shift toward healthier metabolic pathways in postoperative. Notably, we identified several key differential metabolites and pathways, with linoleic acid metabolism and glycerophospholipid metabolism showing the most pronounced improvements. The levels of various lipid metabolites associated with inflammation and cell membrane stability were restored to near-normal in the serum. Mechanistic investigation demonstrated that surgery significantly downregulated the expression of pro-inflammatory genes such as *PLA2G3*, *TNF $\alpha$* , and *NF- $\kappa$ B1* in the serum.

## Conclusions

The synovial tissue and serum metabolism of patients with gout stones are severely disordered. However, Surgical removal of tophi systemically improves serum metabolic dysregulation in gout patients, which may be mediated by restoring abnormal lipid metabolism and inhibiting serum inflammatory responses.

## 1. Introduction

Gouty arthritis is a metabolic rheumatic disorder characterized by purine metabolism dysregulation, leading to hyperuricemia and deposition of monosodium urate (MSU) crystals in synovial and periarticular tissues. These crystals trigger innate immune responses and acute inflammation [1]. Clinical manifestations include severe peripheral joint synovitis and intense pain. Although inflammatory

episodes are usually self-limiting, recurrent attacks can cause joint damage, deformity, and subcutaneous tophi [2–4]. Global gout prevalence ranges from 0.1% to 10%, higher in developed countries [5]. Recently, increased high-purine diet consumption and altered physical activity have driven epidemiological shifts, including earlier onset and geographic expansion from coastal to inland regions [6]. In China, the age-standardized prevalence reached 8.1% in 2019 (12.3% in males, 3.9% in females), with a consistent upward trend from 1990 to 2019, approaching Western levels [7]. In traditional medicine, this shift is linked to spleen dysfunction and dampness accumulation [8].

Tophi, a severe complication of advanced gout, develop in 12–35% of patients (some reports up to 52.56%) [9, 10]. They infiltrate tendons, ligaments, and nerves, causing bone erosion, tendon rupture, and structural destruction [11, 12]. Enlarging tophi, especially in extremities, can lead to skin ulceration and impaired joint mobility, reducing quality of life [13]. Gout patients also exhibit systemic metabolic dysregulation (lipid, amino acid, energy metabolism) [14, 15], which drives uric acid overproduction and chronic inflammation, contributing to tissue damage and tophus formation. For tophaceous disease, conventional pharmacotherapy often fails to eliminate deposited urate crystals or reverse metabolic disturbances [16, 17]. Thus, surgical intervention is a superior strategy for tophi removal, offering shorter treatment duration and significant symptom relief.

Surgical indications for tophaceous gout address mechanical complications: joint impairment, nerve/soft tissue compression, and ulcerated or non-healing lesions. Established criteria include: 1) functional deficits from joint deformity or restricted mobility; 2) local complications (skin ulceration, sinus tracts, neurovascular compression); and 3) persistent disease burden (massive tophi or refractory hyperuricemia despite optimal therapy). Beyond mechanical obstacles, tophi act as dynamic urate reservoirs perpetuating systemic metabolic disturbance. Early surgical excision upon tophus formation may reduce total body urate pool, relieve renal burden, and prevent joint destruction [18]. Yen et al. [19] reported that pre-complication surgery significantly shortens hospital stay. Wang et al. [20] successfully treated refractory ulcers using vacuum sealing drainage combined with autologous platelet-rich gel, promoting wound healing. However, the systemic and local metabolic consequences of tophi removal, and the molecular mechanisms underlying its therapeutic effects, remain largely unexplored.

In this study, we used untargeted metabolomics to investigate metabolic changes in synovial tissue and serum from gout patients before and after tophi removal surgery. Our findings show that surgery significantly reversed gout-associated metabolic disorders, particularly improving linoleic acid and glycerophospholipid metabolism in serum. These results indicate that tophi excision not only relieves mechanical compression but also systemically corrects metabolic imbalances via key lipid pathways, revealing a metabolic therapeutic value beyond mechanical debridement and providing a novel molecular rationale for managing tophaceous gout.

## 2. Materials and Methods

### Sample collection and preparation

This study was approved by the Ethics Committee of The People's Hospital of Suzhou New District (Approval No. 2023 – 123), and all participants gave written informed consent. Gout patients met the 2015 ACR/EULAR classification criteria (score  $\geq 8$ ) [21]. Surgical candidates underwent preoperative dual-energy CT and physical examination for target localization. Operative specimens were divided into: (1) Tophus group (OG, n = 15), with characteristic trilaminar histopathology; (2) Control group (CG, n = 9), consisting of synovial specimens from patients with chronic ligament or meniscus injuries during arthroscopic repair. Tophi and synovium were dissected intraoperatively and stored in MACS Tissue Storage Solution (Miltenyi Biotec). Additionally, fasting morning venous blood was collected from healthy controls (n = 12) and tophus patients (n = 17) before and after surgery.

### **Liquid sample extraction**

Frozen samples ( $-80^{\circ}\text{C}$ ) were thawed on ice and vortexed for 10 s. A 50- $\mu\text{L}$  aliquot was mixed with 300  $\mu\text{L}$  extraction solution (acetonitrile:methanol = 1:4, v/v) containing internal standards, vortexed for 3 min, and centrifuged at 12,000 rpm for 10 min ( $4^{\circ}\text{C}$ ). A 200- $\mu\text{L}$  supernatant was kept at  $-20^{\circ}\text{C}$  for 30 min, then centrifuged again at 12,000 rpm for 3 min ( $4^{\circ}\text{C}$ ). Finally, 180  $\mu\text{L}$  of supernatant was used for LC-MS analysis.

### **HPLC conditions**

Two Liquid Chromatography–Mass Spectrometry (LC/MS) methods were used. For positive ion mode, samples were eluted from a T3 column (Waters ACQUITY Premier HSS T3, 1.8  $\mu\text{m}$ , 2.1 $\times$ 100 mm) with mobile phase A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). Gradient: 5–20% B in 1 min, to 99% in 2 min, hold 1.5 min, back to 5% in 0.1 min, hold 1.4 min. Column temperature  $40^{\circ}\text{C}$ , flow rate 0.4 mL/min, injection volume 4  $\mu\text{L}$ . Negative ion mode used the same gradient.

### **HPLC-MS conditions (QE)**

MS analysis used electrospray ionization in positive and negative modes, full scan ( $m/z$  75–1000) at 35,000 resolutions. Settings: ion spray voltage 3.5 kV (positive) or 3.2 kV (negative); sheath gas 30 Arb, aux gas 5; ion transfer tube  $320^{\circ}\text{C}$ , vaporizer  $300^{\circ}\text{C}$ ; collision energy 30, 40, 50 V; intensity threshold  $1.00\text{E} + 06$  cps; Top N = 10; exclusion duration 3 s.

### **Data processing and metabolomics analysis**

Unsupervised principal component analysis (PCA) was performed in R (prcomp function) with unit variance scaling. Hierarchical cluster analysis (HCA) and Pearson correlation coefficients (PCC) were computed using R package Complex Heatmap. For two-group comparison, differential metabolites were defined by VIP > 1 (from Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) and Student's t-test  $p < 0.05$ . OPLS-DA, including score and permutation plots (200 permutations), was performed using R package MetaboAnalystR, with log2 transformation and mean centering. Metabolites were annotated using Kyoto Encyclopedia of Genes and Genomes (KEGG) compound and pathway databases.

## **Total protein extraction**

Synovial tissue was ground to powder and lysed in 1 mL ice-cold RIPA buffer containing 1 mM PMSF and protease inhibitor cocktail. After incubation on ice for 30–60 min with occasional vortexing, the lysate was centrifuged at 12,000×g for 10 min at 4°C. The supernatant (total protein) was collected, and protein concentration was determined using a BCA assay kit (Solarbio, China) with BSA as standard. Extracts were stored at – 80°C.

## **Western blot assay**

Protein samples were mixed with loading buffer (10:1), heated at 95°C for 10 min, separated by SDS-PAGE, and transferred to PVDF membranes. Membranes were blocked and incubated with primary antibody in blocking buffer (5% skim milk in TBST) for 3 h at 25°C with gentle shaking. After three TBST washes (10 min each), membranes were incubated with HRP-conjugated secondary antibody (1:5000 in TBST) for 2 h at 25°C, then washed three times. Proteins were visualized using an ECL detection kit (Cwbio, China) and imaged with an ultrasensitive multifunctional imager (UVItec, UK).

## **RNA extraction and cDNA synthesis**

Total RNA was extracted from synovial tissue (≈ 30 mg) using a Total RNA Extraction Kit (Omega Bio-Tek) with TRIzol. After homogenization in 1 mL TRIzol and incubation at – 20°C for 5 min, 200 µL chloroform was added, shaken vigorously for 30 s, and centrifuged at 12,000×g for 10 min at 4°C. The aqueous phase was transferred to a new tube, and RNA was precipitated with ice-cold isopropanol, washed twice with 75% ethanol, air-dried, and dissolved in 30 µL RNase-free water. First-strand cDNA was synthesized using the TaKaRa PrimeScript™ RT reagent Kit with gDNA Eraser, following the manufacturer's protocol (37°C for 15 min, 85°C for 5 s). cDNA was stored at – 20°C.

## **Reverse Transcription quantitative real-time Polymerase Chain Reaction (RT-qPCR) analysis**

RT-qPCR was performed on an Applied Biosystems instrument using a 10-µL reaction mixture containing 5 µL PerfectStart® Green qPCR SuperMix, 1 µL diluted cDNA, 0.1 µL each of forward and reverse primer (10 mM), and 3.6 µL ddH<sub>2</sub>O. Relative gene expression was calculated using the  $2^{-\Delta\Delta C_t}$  method [22] and normalized to GAPDH. Primer sequences are listed in Supplemental Table 1.

## **Statistical analysis**

Statistical analyses were carried out using SPSS 26.0 and GraphPad Prism 9. Measurement data conforming to normal distribution were expressed as mean ± standard deviation (SD). Comparisons between two groups with normal distribution adopted independent samples t-test. One-way ANOVA with Tukey post-hoc test or Kruskal-Wallis H test was used for multi-group comparisons. Categorical variables were compared by Chi-square test or Fisher's exact test.  $P < 0.05$  indicated statistically significant difference.

### 3. Results

#### 3.1. Patient characteristics

The clinical characteristics of recruited gout patients were documented and analyzed. These characteristics included age, body mass index (BMI), uric acid and creatinine levels, cholesterol levels, triglyceride levels, blood glucose levels. Other comorbidities were also documented. A summary of patient characteristics is shown in Table 1. In total, the study recruited 17 tophi patients and 12 healthy controls. Participants were age- and sex-matched. It's important to note that the creatinine levels are higher in patients with gout than in normal subjects, reflecting the fact that gout can impair kidney function. What's more, UA, were all significantly higher in OG patients than in HC patients. It is demonstrated that poor uric acid control is a crucial factor in tophus formation. Besides, there were differences in the hypertension rates, and diabetes rates in the OG patients vs. the NG patients (Table 1).

Table 1  
Patient characteristics

| Variable              | OG             | NG             | p      |
|-----------------------|----------------|----------------|--------|
| Age, y                | 52.65 ± 16.61  | 54.5 ± 15.68   |        |
| BMI, kg/m2            | 27.69 ± 3.79   | 25.78 ± 2.85   |        |
| UA, umol/L            | 469.12 ± 91.57 | 325.17 ± 62.92 | < 0.01 |
| Cr, umol/L            | 114.61 ± 34.95 | 77.05 ± 9.95   | = 0.01 |
| Cholesterol, mmol/L   | 4.70 ± 0.99    | 4.22 ± 0.68    |        |
| Triglycericle, mmol/L | 2.60 ± 1.41    | 1.85 ± 0.84    |        |
| Blood glucose, mmol/L | 5.88 ± 1.92    | 5.71 ± 0.06    |        |
| Comorbidities         |                |                |        |
| Hypertension          | 10(58.82%)     | 3(25%)         |        |
| Diabates              | 2(11.76%)      | 0              |        |

#### 3.2. Gout patients effectively reshape the metabolic landscape of the synovial tissue

To investigate local metabolic changes in gout, we performed untargeted metabolomics on synovial tissue from gout patients after surgery (OG) and from patients with chronic ligament/meniscus injuries (CG). High data reliability was confirmed by QC sample correlation (Fig. 1A). Identified metabolites fell into 12 major categories, including heterocyclic compounds, benzene derivatives, and organic acids (Fig. 1B). Principal component analysis (PCA) showed clear separation between OG and CG groups within the 95% confidence interval (Fig. 1C), indicating a significant alteration of the synovial metabolic

profile in gout. Unsupervised hierarchical clustering confirmed distinct segregation between groups (Fig. 1D). Volcano plot analysis revealed 134 significantly differential metabolites ( $P < 0.05$ , fold change  $> 2.0$ ), with 45 downregulated and 89 upregulated (Fig. 1E). The top 10 altered metabolites are shown in Fig. 1F. Collectively, these results demonstrate that gout substantially reshapes the synovial tissue metabolic landscape.

A: Correlation analysis on QC samples showing credibility of metabolomics data. B: Category analysis of all metabolites identified in metabolomics profiles of synovial tissue. C: Principal component analysis (PCA) plots of metabolic compositions of synovial tissue of gout patients after (OG) surgery and the chronic ligament injuries or meniscus injuries patients during arthroscopic repair surgeries (CG). Ellipses show 95% confidence intervals. The samples with different treatments were shown in different colour. The PCA plots are constructed using top 2 principal components (PC1 and PC2). D: Unsupervised hierarchical clustering displays the separation in metabolic composition CG and OG samples. The correlation value between each sample is calculated using Pearson algorithm. The higher correlation relationship between samples is shown in red. E: Volcano plots of differential metabolites in OG vs. CG comparison. Each plot represented a metabolite, and the up-regulated metabolites ( $\text{Log}_2 0 \text{ Foldchange} > 1.0$ ;  $P < 0.05$ ) were labelled red, whereas the down-regulated metabolites were shown in green. The number of differential metabolites of each comparison are shown in the figure panel. F: Top 10 differential metabolites in OG vs. CG comparison. The top 10 metabolites are identified based on foldchange.

### 3.3. Differential metabolites and related metabolic pathways in synovial tissue of gout patients

To further validate identified metabolites and explore their biological relevance, we constructed an Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) model based on metabolites from the CG and OG groups. The score plot showed clear separation between groups (Fig. 2A), and permutation testing confirmed model reliability (Fig. 2B). Differential metabolites (DEMs) were identified using a VIP threshold  $> 1.0$ . A scatter plot of the top 30 DEMs highlighted key metabolites, including valeric acid, uric acid, dioleoyl phosphatidylcholine, and linoleic acid (Fig. 2C), implicating fatty acid and purine metabolism in tophaceous gout. KEGG pathway enrichment analysis revealed significant enrichment of several metabolic pathways (Fig. 2D), notably those linked to inflammation and membrane remodeling, such as linoleic acid, arachidonic acid, and glycerophospholipid metabolism.

To unravel interrelationships among DEMs, we visualized them by category, expression level, and correlation pattern (Fig. 2E). Organic acids, fatty acids (FAs), and amino acid derivatives were the most abundant metabolite classes, revealing complex synergistic and antagonistic interactions. Heatmap analysis showed that lipid-related metabolites (e.g., fatty acids and glycerophospholipids) and organic acids underwent the most pronounced changes after tophi removal (Fig. 2F). In the CG vs. OG comparison, most FAs were upregulated, whereas glycerophospholipids and amino acids were significantly down-regulated. Notably, levels of 1-3-phenyllactic acid, dioleoylphosphatidylcholine, 1-

stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine, triamterene, and 1,4-dimethylphenanthrene were markedly elevated in the OG group (Fig. 2G). Together, these findings provide strong evidence that gout induces extensive metabolic reprogramming in synovial tissue.

A: OPLS-DA scatter plots derived from metabolic profiles of CG vs. OG comparison. The OPLS-DA model was validated using a permutation test that was randomly permitted 1000 times with the first component extracts. B: Permutation test of the OPLS-DA model for CG vs. OG comparison. C: Scatter plot displays the top 30 differential metabolites from CG vs. OG comparison. The differential metabolites were identified using  $VIP > 1.0$  from OPLS-DA model. D: KEGG pathway enrichment analysis on differential metabolites from CG vs. OG comparison. Each plot in the diagram represents a metabolic pathway, and the abscissa coordinate and the plot size represent the metabolite number of related pathway. The vertical coordinate and plot colour represent the P-value [ $-\ln(P\text{-value})$ ] of enrichment analysis. E: Correlation analysis on all differential metabolites from CG vs. OG comparison. All differential metabolites involved in a complex correlation network. F: Heat maps display the relative changes in contents of differential metabolites in CG vs. OG comparison. The up- and down-regulated metabolites were shown in red and green, respectively. The scale represents the normalized peak area of metabolite in each biological replicates. G: Box plots display the contents of hub metabolites in CG and OG samples.

### 3.4. Surgery effectively reshapes the serum metabolic landscape

To assess the systemic metabolic impact of surgery, we analyzed serum metabolites from postoperative (Postop), preoperative (Preop), and control groups. PCA showed that principal component 1 (PC1) explained 36.3% of total variance, primarily separating Postop from Preop/Control; PC2 explained 16.8%, distinguishing Postop from Control (Fig. 3A). This pattern suggests that surgery reduces the metabolic gap between gout patients and controls. Identified serum metabolites fell into nine categories, with benzene derivatives (17.14%), heterocyclic compounds (15.11%), organic acids (12.96%), and amino acids (10.66%) being the most abundant; notably, glycerophospholipids (GP) accounted for 7.15% (Fig. 3B).

OPLS-DA with  $VIP > 1.0$  identified DEMs. A scatter plot of the top 30 DEMs highlighted metabolites including hydroxyminaline, fluorobenzoylpropionic acid, and artemisyl propionste, implicating fatty acid metabolism in postoperative recovery (Fig. 3C). Volcano plot analysis ( $P < 0.05$ , fold change  $> 2.0$ ) showed 215 DEMs in Preop vs. Control (61 down, 154 up), confirming significant gout-related metabolic alterations (Fig. 3D). Strikingly, Postop vs. Preop revealed 315 DEMs (239 down, 76 up), indicating that surgery markedly reversed these alterations. Furthermore, Postop vs. Control showed only 108 DEMs (63 down, 45 up), demonstrating that surgery substantially normalized the serum metabolic profile toward control levels (Fig. 3D). Together, these findings indicate that tophi removal effectively restores systemic metabolism in gout patients.

A: Principal component analysis (PCA) plots of metabolic compositions of serum from normal (Control), postoperation (Postop) and preoperation (Preop) groups. Ellipses show 95% confidence intervals. The samples with different treatments were shown in different color. The PCA plots are constructed using top 2 principal components (PC1 and PC2). B: Category analysis of all metabolites identified in metabolomics profiles of serum from all groups. C: Scatter plot displays the top 30 differential metabolites among Control, Postop and Preop groups. The differential metabolites were identified using VIP > 1.0 from PLS-DA model. D: Volcano plots of differential metabolites in Preop vs. Control (left), Postop vs. Preop (medium) and Postop vs. Control (right) comparisons. Each plot represented a metabolite, and the up-regulated metabolites ( $\text{Log}_2.0$  Foldchange > 1.0;  $P < 0.05$ ) were labeled by red, whereas the down-regulated metabolites were shown in green. The number of differential metabolites of each comparison are shown in the figure panel.

### 3.5. Surgery improves gout associated metabolic pathways in serum

To further evaluate systemic metabolic changes after surgery, we performed OPLS-DA on serum from three pairwise comparisons: Preop vs. Control, Postop vs. Preop, and Postop vs. Control. Score plots showed clear separation along the first predictive component for each comparison (Fig. 4A). The explained variance was 39.1% for Preop vs. Control and 36.5% for Postop vs. Preop, indicating substantial differences. Notably, it dropped to 26.3% for Postop vs. Control, suggesting a marked reduction in metabolic disparity following surgery (Fig. 4A).

KEGG enrichment analysis revealed distinct pathway alterations (Fig. 4B). Preop vs. Control was enriched in drug metabolism-cytochrome P450, branched-chain amino acid biosynthesis, and aminoacyl-tRNA biosynthesis, highlighting disturbed amino acid metabolism in gout serum. Postop vs. Preop showed enrichment in glycerophospholipid metabolism, glycerolipid metabolism, and purine metabolism, indicating that tophi removal reshapes serum metabolic profiles. Postop vs. Control was enriched in vitamin B6 metabolism, taurine/hypotaurine metabolism, and drug metabolism pathways.

Heatmaps displayed relative changes in metabolite contents (Fig. 4C). In Preop vs. Control, benzenes, alcohols/amines, organic acids, and heterocyclic compounds were upregulated, reflecting metabolic disturbances in gout. In Postop vs. Preop, the most pronounced changes involved aldehydes/ketones, carbohydrates, glycerophospholipids (GP), and benzenes, confirming that surgery reshapes the serum profile. In Postop vs. Control, the most significant differences were observed for GA, FA, aldehydes/ketones, and heterocyclic compounds (Fig. 4C). Collectively, these findings demonstrate that surgical removal of tophi restores key metabolic pathways in gout patients.

A: OPLS-DA scatter plots derived from metabolic profiles about Preop vs. Control (left), Postop vs. Preop (medium) and Postop vs. Control (right) comparisons. B: KEGG pathway enrichment analysis on differential metabolites from Preop vs. Control (left), Postop vs. Preop (medium) and Postop vs. Control (right) comparisons. Each plot in the diagram represents a metabolic pathway, and the abscissa coordinate and the plot size represent the metabolite number of related pathway. The vertical coordinate

and plot color represent the P-value [ $-\ln(\text{P-value})$ ] of enrichment analysis. C: Heat maps display the relative changes in contents of differential metabolites in Preop vs. Control (left), Postop vs. Preop (medium) and Postop vs. Control (right) comparisons. The up- and down-regulated metabolites were shown in red and green, respectively. The scale represents the normalized peak area of metabolite in each biological replicates.

### **3.6. Improvement of linoleic acid and glycerophospholipid metabolisms for improving gout**

To delineate the serum metabolites changes by surgical intervention in tophi patients, we conducted an Upset plots analysis of the differential metabolites from two key comparisons: Preop vs. Control and Postop vs. Preop. The 154 up- and 61 down-regulated metabolites from the Preop vs. Control comparison were combined as Set A (Fig. 5A), while the 76 up- and 239 down-regulated metabolites from the Postop vs. Preop were designated as Set B (Fig. 5A). This identified 37 hub DEMs from both the Postop vs. Preop and Preop vs. Control comparisons, highlighted in red (Fig. 5A). KEGG analysis of these hub metabolites identified linoleic acid metabolism and glycerophospholipid metabolism as the primary pathways concurrently associated with gout pathogenesis and surgical intervention (Fig. 5B). Venn diagram analysis revealed 47 overlapping metabolites between synovial tissue and serum. Enrichment analysis confirmed tophi resection significantly influenced 9 metabolic pathways, with linoleic acid and glycerophospholipid metabolism being most prominent (Fig. 5C), further supporting the amelioration of linoleic acid and glycerophospholipid metabolism facilitates the elimination of tophi. A heatmap of the hub metabolites abundance across the Control, Preop, and Postop groups was generated (Fig. 5D). The results indicated that organic acids, fatty acids (FA), and heterocyclic compounds constituted the three most abundant classes of differential metabolites (Fig. 5D), implying that surgical tophi removal instigates comprehensive metabolic reprogramming encompassing energy and lipid metabolism. Together, these findings demonstrate that surgery alleviates gout through systemic reprogramming of key metabolic pathways, particularly linoleic acid and glycerophospholipid metabolism.

A: Upset plots identified the hub differential metabolites associated with gout pathogenesis and surgery treatment in serum. The hub metabolites were labeled in red. B: Scatter plot displays the KEGG pathway enrichment results based on hub metabolites associated with gout pathogenesis and surgery treatment in serum. The various color levels displayed different levels of significance of terms from low (blue) to high (red). C: Venn diagram identified the overlapped pathways identified in synovial tissue and serum. The disorders of nine pathways were improved by surgery-removed tophi. D: Heat map showing the relative contents of hub metabolites associated with gout pathogenesis and surgery treatment in Control, Postop and Preop groups. The high and low levels of metabolites are shown in red and green, respectively. Scale bar represents the normalized peak areas of related metabolites in each biological replicate.

### **3.7. Surgery reduces the expression of inflammation-related genes in serum of gout patients**

To investigate molecular mechanisms, we analyzed the expression of genes associated with key metabolic pathways. RT-qPCR analysis revealed that in the linoleic acid metabolism pathway, the expression of the gene ALOX15, which is associated with inflammation removal, was significantly up-regulated in Postop compared with Preop (Fig. 6A). Conversely, the expression of PLA2G3 in the glycerophospholipid metabolism pathway was significantly downregulated in Postop compared with Preop (Fig. 6C), indicating that surgery alleviates serum inflammation and promotes tissue repair. Moreover, pro-inflammatory factors TNF $\alpha$  and NF- $\kappa$ B1 were also significantly down-regulated expression in Postop compared with Preop (Fig. 6B, D). These results demonstrate that surgery markedly reduces the inflammatory response in serum. Subsequently, we examined the protein accumulation of inflammation-related genes in serum before and after surgery. The results showed that protein accumulation of PLA2G3, TNF $\alpha$ , and NF- $\kappa$ B1 in serum were significantly higher in Postop than that those of Preop, whereas ALOX15 protein accumulation was significantly lower before surgery than after surgery (Fig. 6E-H). These findings further support that surgery reduces the expression of inflammation-related genes in serum.

A-D: RT-qPCR assay detected the expression of ALOX15, PLA2G3, TNF $\alpha$  and NF- $\kappa$ B1 genes in serum of post-operation (Postop) and pre-operation (Preop). The GAPDH gene was used as an internal reference. Values are mean  $\pm$  SD of three independent biological replicates. Asterisks represent significant differences (\* $P < 0.05$ , Student's  $t$  test). E-H: The protein accumulations of ALOX15, PLA2G3, TNF $\alpha$  and NF- $\kappa$ B1 in serum of Postop and Preop were detected by western blotting. Actin (Act) as an internal control indicates equal loading.

## 4. Discussion

Tophi are not merely local destructive lesions but also drivers of systemic metabolic dysregulation in gout [23, 24]. In this study, integrated metabolomic analyses of synovial tissue and serum demonstrate that surgical tophi removal reshapes both local and systemic metabolic networks. Our findings indicate that surgery not only eliminates the physical insult of urate crystals but also reverses gout-associated pathological states at the metabolic level, primarily through modulation of lipid metabolism pathways, notably linoleic acid and glycerophospholipid metabolism.

Gout is a common metabolic disease triggered by hyperuricemia and characterized by monosodium urate (MSU) crystal deposition [25]. Poorly controlled acute gout progresses to tophaceous disease [26], for which surgery remains a mainstay [10]. Beyond simple excision, modern surgical management incorporates tissue repair techniques—such as vacuum sealing drainage (VSD) and platelet-rich gel—to restore anatomical and metabolic homeostasis [20]. In chronic gout, persistent MSU deposition maintains synovial tissue in a chronically activated metabolic state. Our PCA and volcano plot analyses revealed a distinct synovial metabolic landscape in gout patients after tophi removal. KEGG and heatmap analyses further identified linoleic acid and glycerophospholipid metabolism as the most significantly altered pathways. These results suggest that gout induces extensive metabolic reprogramming in synovial tissue, and that tophi removal can fundamentally reverse serum metabolic

profiles, particularly within these two lipid pathways. Collectively, our findings establish a novel mechanistic link between surgery and the regulation of linoleic acid/glycerophospholipid metabolism.

Linoleic acid, an essential polyunsaturated fatty acid, reduces the risk of chronic cardiovascular and malignant diseases [27, 28]. Its metabolites can exert proatherogenic and anti-inflammatory effects via endothelial activation [29–31] and modulate oxidative stress and inflammatory responses [32]. For instance, linoleic acid activates PI3K/Akt and p38 MAPK/ERK1/2 signaling, leading to NF- $\kappa$ B activation and expression of inflammatory mediators such as VCAM-1 [27, 28]. Kim et al. [33] showed that linoleic acid regulates inflammatory mediator release in activated mast cells. Meanwhile, glycerophospholipids are major cell membrane constituents; their dysregulation compromises membrane integrity, fluidity, and signal transduction. We propose that surgical tophi removal eliminates persistent MSU-driven inflammation, thereby breaking a vicious cycle of metabolic dysregulation and allowing these pathways to normalize. Supporting this, RT-qPCR revealed that post-surgery expression of inflammation-related genes—including PLA2G4A, NF- $\kappa$ B1, and TNF $\alpha$ —was significantly down-regulated, whereas ALOX15 expression was markedly up-regulated compared to controls. This shift indicates that surgery reduces systemic inflammatory burden, likely by resetting lipid mediator profiles.

A deeper interpretation emerges tophi function as metabolic “reservoirs” that continuously fuel both local synovitis and systemic metabolic disturbances. Their surgical removal not only relieves mechanical compression but also disrupts a positive feedback loop among MSU deposition, chronic inflammation, and lipid dysmetabolism. The coordinated restoration of linoleic acid and glycerophospholipid metabolism suggest a common upstream regulatory node possibly involving PPARs or AMPK—that warrants future investigation. From a clinical perspective, our findings imply that early surgical intervention, before irreversible joint or metabolic damage occurs, might offer dual benefits: anatomical salvage and systemic metabolic correction. This adds a novel therapeutic rationale for considering surgery not merely as a last resort but as a disease-modifying strategy in selected tophaceous gout patients.

## 5. Conclusions

We propose a mechanistic model: as a persistent inflammatory nidus, tophi continuously disrupt lipid metabolic homeostasis in synovial tissue and serum, prominently affecting linoleic acid and glycerophospholipid metabolism. Surgical removal of this nidus alleviates these disruptions. These findings deepen our understanding of the metabolic benefits of surgery in gout and provide potential targets for the future adjuvant therapies aimed at modulating these specific pathways.

## Abbreviations

BMI body mass index

CG Control group

DEMs Differential metabolites

GP glycerophospholipids

HCA Hierarchical cluster analysis

KEGG Kyoto Encyclopaedias of Genes and Genomes

LC–MS Liquid Chromatography–Mass Spectrometry

MSU monosodium urate

OG Tophus group

OPLS-DA Orthogonal Partial Least Squares Discriminant Analysis

PCA principal component analysis

PCC Pearson correlation coefficients

VSD vacuum sealing drainage

RT-qPCR Reverse Transcription quantitative real-time Polymerase Chain Reaction

## Declarations

**Supplementary Information:** The following supporting information can be downloaded online

**Author Contributions:** C.Y.Q., W.R., H.L.F. was the overall principal investigator who conceived the study, revised the manuscript and obtained financial support. C.Y.Q., T.L.H. and D.J.J. and J.C.K. M.D. performed the experiments and wrote the manuscript. C.Y.Q., and D.J.J., collected the data and drew the figures and tables. All authors critically reviewed the article and approved the final manuscript.

**Funding:** This research received partial funding from several sources, including the Special project of Diagnosis and treatment technology for key clinical diseases of Suzhou Municipal Health Commission grants number LCZX202240, Science Innovation Fund-General Project of the People's Hospital of Suzhou New District (No. SGY2023A05).

**Data Availability Statement:** The raw metabolomics data from this study have been submitted to Open Science Framework repository and can be accessed via OSF DOI:10.17605/OSF.IO/52XPV. Additional data supporting the findings of this study can be obtained from the corresponding author upon request.

### Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki and its subsequent amendments. Ethical approval was obtained from the Ethics Committee of The People's

Hospital of Suzhou New District (protocol code.2023-123). Written informed consent was obtained from all participants or their legally authorized representatives, in line with the Declaration of Helsinki.

### **Consent for publication**

Not applicable.

### **Conflict of interest**

The authors declare no competing interests

## **References**

1. Dalbeth N, Choi HK, Joosten LAB, et al. Gout Nat Rev Dis Primers. 2019;5(1):69.
2. Zhang W, Feng Q, Gu J, et al. Carpal tunnel syndrome caused by tophi deposited under the epineurium of the median nerve: A case report. Front Surg. 2023;9:942062.
3. Hao H, Kong W, Li H. Carpal tunnel syndrome caused by tophi in the superficial flexor tendon: a case report. Front Surg. 2023;10:1282202.
4. Camurdan A, Riemann C, Brasch F, et al. Case Report: A rare case of bilateral middle ear tophaceous gout. Front Surg. 2024;11:1353116.
5. Kuo CF, Grainge MJ, Mallen C, et al. Comorbidities in patients with gout prior to and following diagnosis: case-control study. Ann Rheum Dis. 2016;75(1):210–7.
6. Dehlin M, Jacobsson L, Roddy E. Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors. Nat Rev Rheumatol. 2020;16(7):380–90.
7. Kuo CF, Grainge MJ, Zhang W, et al. Global epidemiology of gout: prevalence, incidence and risk factors. Nat Rev Rheumatol. 2015;11(11):649–62.
8. Colantonio LD, Saag KG, Singh JA, et al. Gout is associated with an increased risk for incident heart failure among older adults: the REasons for Geographic and Racial Differences in Stroke (REGARDS) cohort study. Arthritis Res Ther. 2020;22(1):86.
9. Oh YJ, Moon KW. Presence of tophi is associated with a rapid decline in the renal function in patients with gout. Sci Rep. 2021;11(1):5684.
10. Pan YC, Lin SS, Pan NF, et al. Tophus severity grading and surgical treatment: Chinese Medical Doctor Association Multidisciplinary Expert Consensus Statement and Recommendations, 2025. Int J Surg. 2026;112(1):42–54.
11. Stewart S, Dalbeth N, Otter S, et al. Clinically evident tophi are associated with reduced muscle force in the foot and ankle in people with gout: a cross-sectional study. J Foot Ankle Res. 2017;10:25.
12. Sun C, Qi X, Tian Y, et al. Risk factors for the formation of double-contour sign and tophi in gout. J Orthop Surg Res. 2019;14(1):239.
13. Ma L, Sun R, Jia Z, et al. Clinical characteristics associated with subcutaneous tophi formation in Chinese gout patients: a retrospective study. Clin Rheumatol. 2018;37(5):1359–65.

14. Dalbeth N, Gosling AL, Gaffo A, Abhishek A. Gout Lancet. 2021;397(1):1843–55.
15. Zhang Y, Zhang H, Chang D, et al. Metabolomics approach by <sup>1</sup>H NMR spectroscopy of serum reveals progression axes for asymptomatic hyperuricemia and gout. *Arthritis Res Ther*. 2018;20(1):111.
16. Yang Q, Xu Y, Zhang J, et al. From soluble uric acid to sodium urate crystal: immune metabolic inflammation driven by uric acid morphological transformation and mechanism-oriented therapy. *Front Immunol*. 2026;17:1794782.
17. Zhang F, Liu Z, Jiang L, et al. A Randomized, Double-Blind, Non-Inferiority Study of Febuxostat Versus Allopurinol in Hyperuricemic Chinese Subjects with or Without Gout. *Rheumatol Ther*. 2019;6(4):543–57.
18. Öztürk R, Atalay İB, Bulut EK, et al. Place of orthopedic surgery in gout. *Eur J Rheumatol*. 2019;6(4):212–5.
19. Yen PS, Tu HP, Huang SH, Lee SS. Timely Shaver Treatment Removes Chronic Tophaceous Mass Improve Surgical Outcomes. *Int J Med Sci*. 2024;21(10):1799–805.
20. Wang A, Ma WG, Wang CD, et al. Clinical effects of autologous platelet rich plasma gel combined with vacuum sealing drainage technology in repairing refractory wounds. *Zhonghua Shao Shang Za Zhi*. 2021;37(1):42–8.
21. Neogi T, Jansen TL, Dalbeth N, et al. 2015 Gout classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis*. 2015;74(10):1789–98.
22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>(-Delta Delta C(T))</sup> Method. *Methods*. 2001;25(4):402–8.
23. FitzGerald JD, Gout. *Ann Intern Med*. 2025;178(3):ITC33–48.
24. Zhang QB, Zhu D, Dai F, et al. MicroRNA-223 Suppresses IL-1 $\beta$  and TNF- $\alpha$  Production in Gouty Inflammation by Targeting the NLRP3 Inflammasome. *Front Pharmacol*. 2021;12:637415.
25. Zhang M, Zhu X, Wu J, et al. Prevalence of Hyperuricemia Among Chinese Adults: Findings from Two Nationally Representative Cross-Sectional Surveys in 2015-16 and 2018-19. *Front Immunol*. 2022;12:791983.
26. Singh G, Lingala B, Mithal A. Gout and hyperuricaemia in the USA: prevalence and trends. *Rheumatology (Oxford)*. 2019;58(12):2177–80.
27. Cheng Y, Zhang L, Wang H, et al. Integrated metabolomic profiling of synovial tissue and serum reveals pivotal roles of linoleic acid and glycerophospholipid metabolism in the therapeutic efficacy of total knee arthroplasty. *J Orthop Translation*. 2018;14:37–45.
28. Wylenzek F, Bühling KJ, Laakmann E. A systematic review on the impact of nutrition and possible supplementation on the deficiency of vitamin complexes, iron, omega-3-fatty acids, and lycopene in relation to increased morbidity in women after menopause. *Arch Gynecol Obstet*. 2024;310(4):2235–45.

29. Berkowitz L, Araneda P, Cofré G, et al. The controversial role of linoleic acid in cardiometabolic health: from molecular pathways to human studies. *Front Nutr.* 2026;12:1728865.

30. Chistyakov DV, Chistyakov VV, et al. Oxylipins in Atherosclerosis: Their Role in Inflammation, Diagnosis, and Therapeutic Perspectives. *Int J Mol Sci.* 2025;26:10577.

31. Belury MA. Linoleic acid, an omega-6 fatty acid that reduces risk for cardiometabolic diseases: premise, promise and practical implications. *Curr Opin Clin Nutr Metab Care.* 2023;26(3):288–92.

32. Innes JK, Calder PC. Omega-6 fatty acids and inflammation. *Prostaglandins Leukot Essent Fat Acids.* 2018;132:41–8.

33. Kim HY, Rho SH, Lim JH, Park HJ, Jeong HJ. Protective effect of linoleic acid against inflammatory reactions by mast cell via caspase-1 cascade pathways. *J Food Biochem.* 2019;43(8):e12932.

# Figures

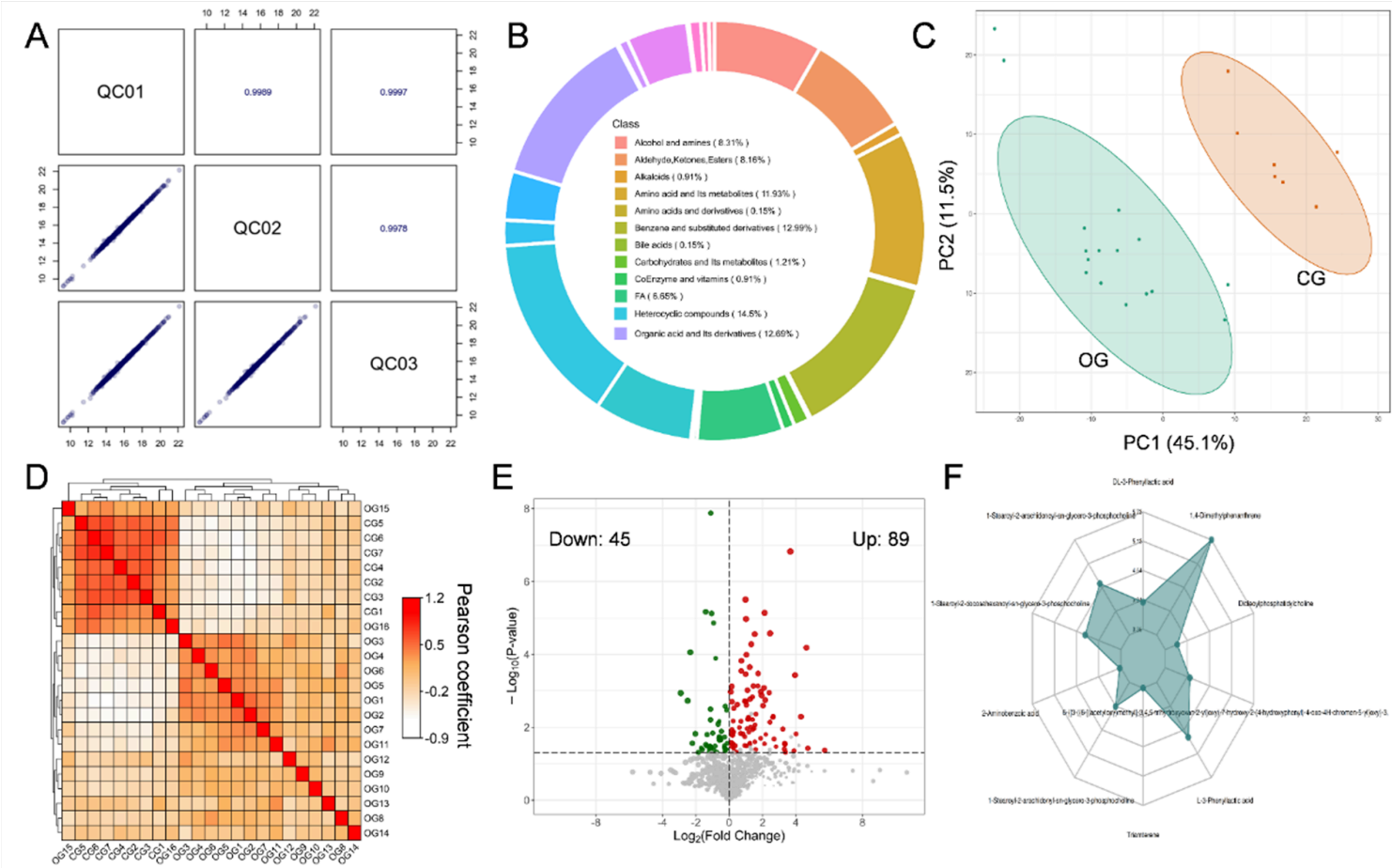


Figure 1

## Metabolic landscape of synovial tissue from gout patients after surgery for removing tophi

A: Correlation analysis on QC samples showing credibility of metabolomics data. B: Category analysis of all metabolites identified in metabolomics profiles of synovial tissue. C: Principal component analysis

(PCA) plots of metabolic compositions of synovial tissue of gout patients after (OG) surgery and the chronic ligament injuries or meniscus injuries patients during arthroscopic repair surgeries (CG). Ellipses show 95% confidence intervals. The samples with different treatments were shown in different colour. The PCA plots are constructed using top 2 principal components (PC1 and PC2). D: Unsupervised hierarchical clustering displays the separation in metabolic composition CG and OG samples. The correlation value between each sample is calculated using Pearson algorithm. The higher correlation relationship between samples is shown in red. E: Volcano plots of differential metabolites in OG vs. CG comparison. Each plot represented a metabolite, and the up-regulated metabolites ( $\text{Log}_2.0$  Foldchange  $> 1.0$ ;  $P < 0.05$ ) were labelled red, whereas the down-regulated metabolites were shown in green. The number of differential metabolites of each comparison are shown in the figure panel. F: Top 10 differential metabolites in OG vs. CG comparison. The top 10 metabolites are identified based on foldchange.

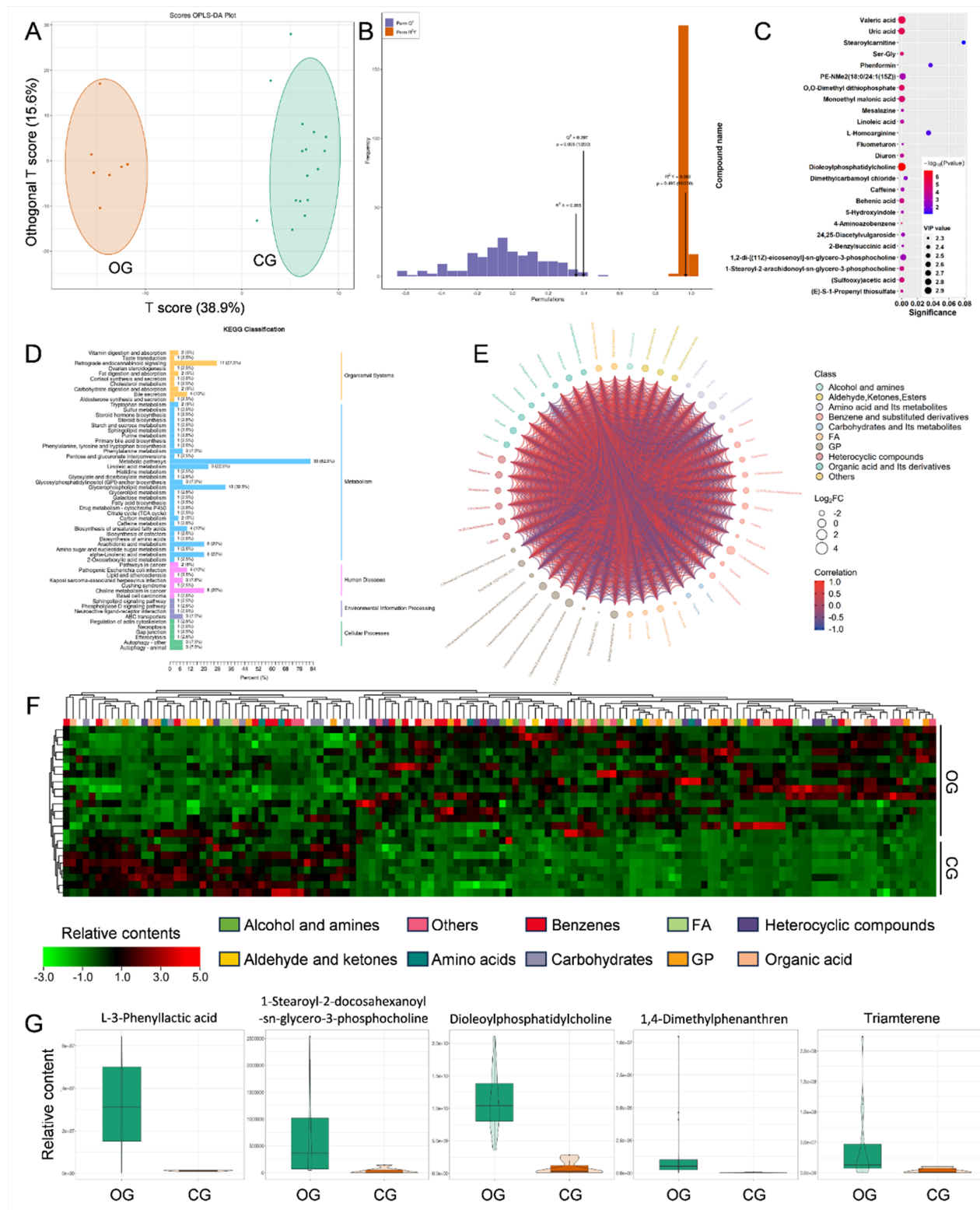


**Figure 2**

## Gout patients cause metabolic disorders related to gout in synovial tissue

A: OPLS-DA scatter plots derived from metabolic profiles of CG vs. OG comparison. The OPLS-DA model was validated using a permutation test that was randomly permitted 1000 times with the first component extracts. B: Permutation test of the OPLS-DA model for CG vs. OG comparison. C: Scatter

plot displays the top 30 differential metabolites from CG vs. OG comparison. The differential metabolites were identified using  $VIP > 1.0$  from OPLS-DA model. D: KEGG pathway enrichment analysis on differential metabolites from CG vs. OG comparison. Each plot in the diagram represents a metabolic pathway, and the abscissa coordinate and the plot size represent the metabolite number of related pathway. The vertical coordinate and plot colour represent the P-value  $[-\ln(P\text{-value})]$  of enrichment analysis. E: Correlation analysis on all differential metabolites from CG vs. OG comparison. All differential metabolites involved in a complex correlation network. F: Heat maps display the relative changes in contents of differential metabolites in CG vs. OG comparison. The up- and down-regulated metabolites were shown in red and green, respectively. The scale represents the normalized peak area of metabolite in each biological replicates. G: Box plots display the contents of hub metabolites in CG and OG samples.

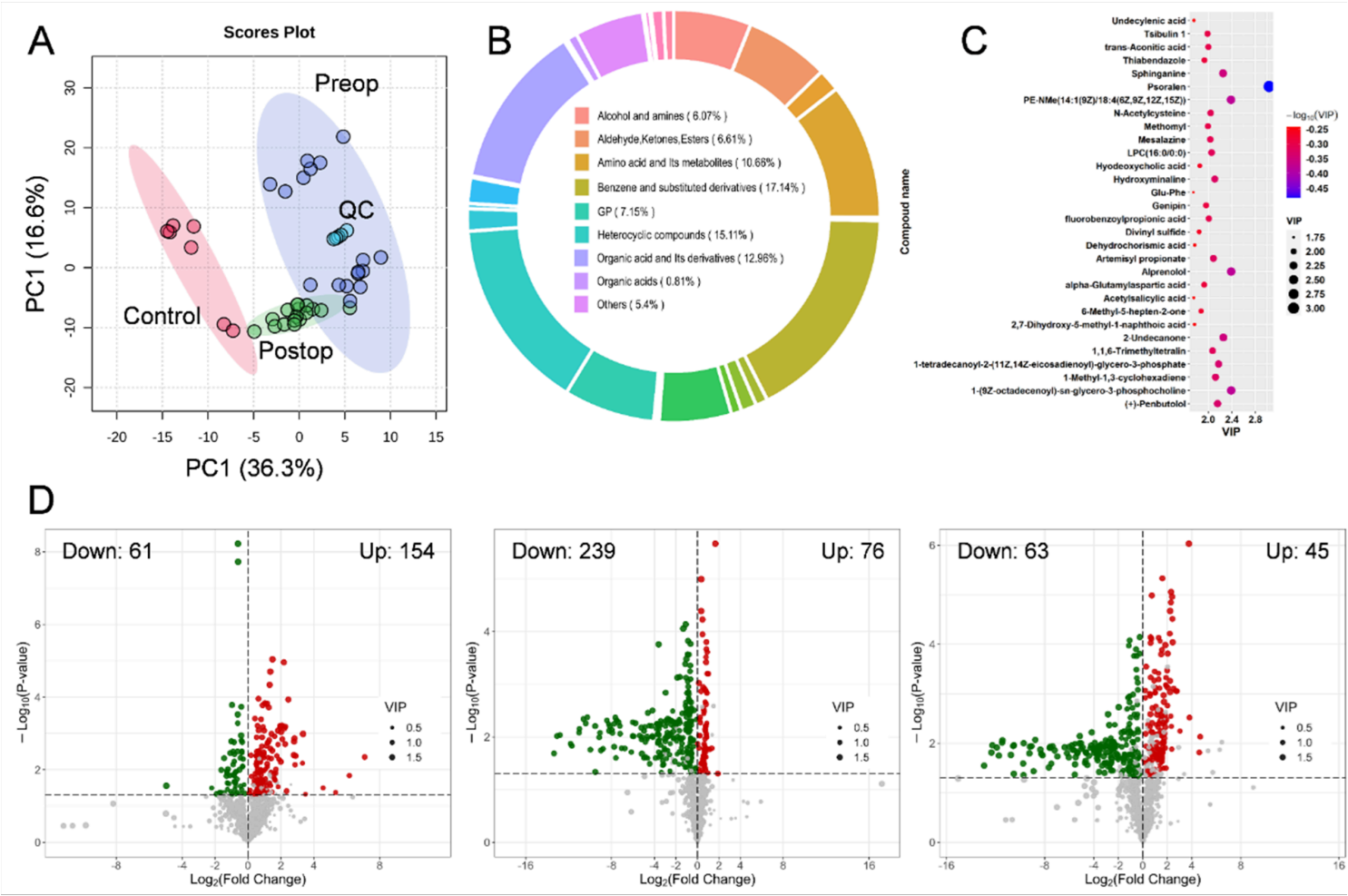


**Figure 3**

### Metabolic landscape of serum from gout patients before and after surgery for removing tophi

A: Principal component analysis (PCA) plots of metabolic compositions of serum from normal (Control), postoperation (Postop) and preoperation (Preop) groups. Ellipses show 95% confidence intervals. The samples with different treatments were shown in different color. The PCA plots are constructed using

top 2 principal components (PC1 and PC2). B: Category analysis of all metabolites identified in metabolomics profiles of serum from all groups. C: Scatter plot displays the top 30 differential metabolites among Control, Postop and Preop groups. The differential metabolites were identified using VIP > 1.0 from PLS-DA model. D: Volcano plots of differential metabolites in Preop vs. Control (left), Postop vs. Preop (medium) and Postop vs. Control (right) comparisons. Each plot represented a metabolite, and the up-regulated metabolites (Log2.0 Foldchange > 1.0; P < 0.05) were labeled by red, whereas the down-regulated metabolites were shown in green. The number of differential metabolites of each comparison are shown in the figure panel.

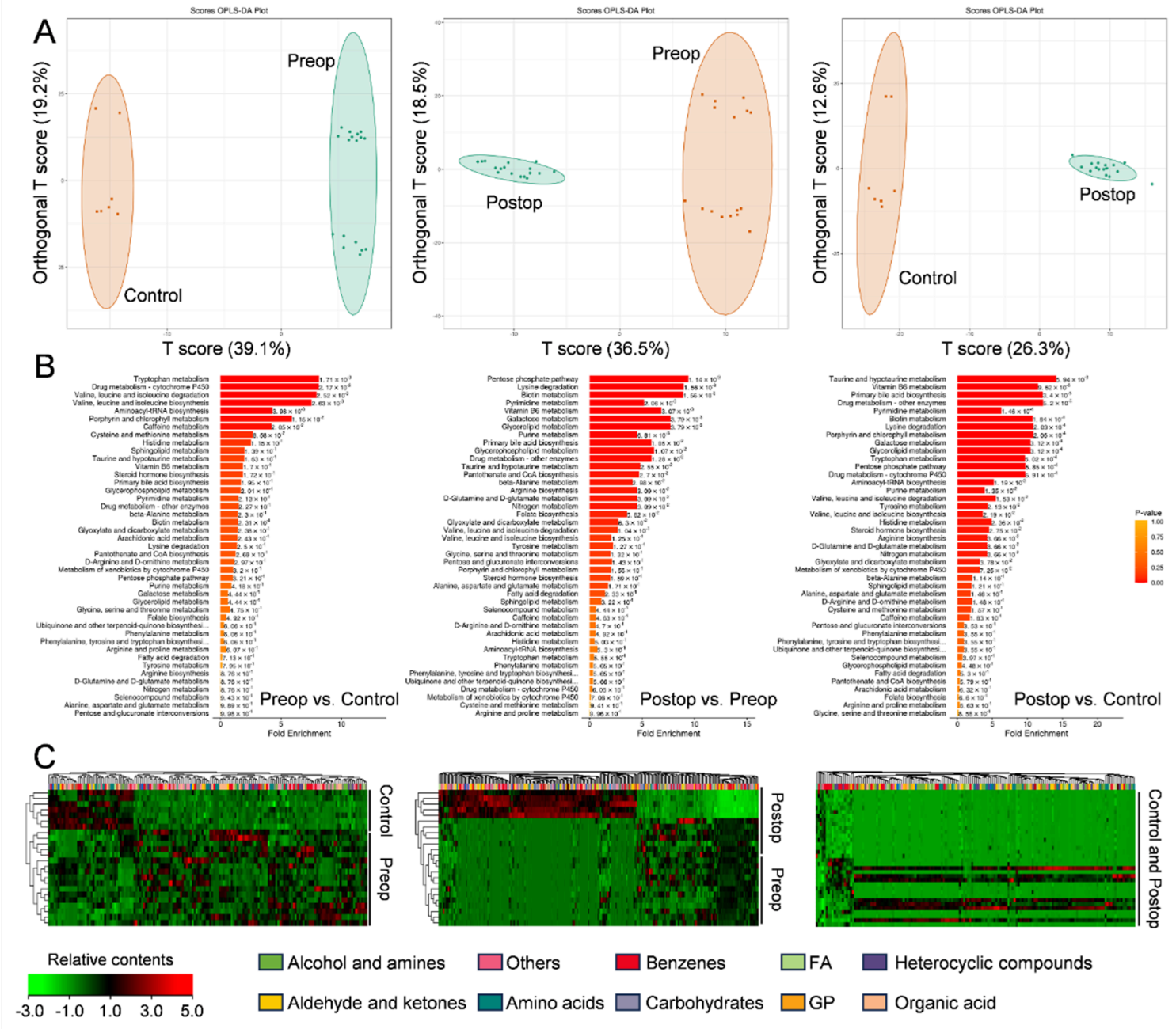


Figure 4

Surgery to remove tophi improved gout-associated metabolic disorders in patient serum

A: OPLS-DA scatter plots derived from metabolic profiles about Preog vs. Control (left), Postog vs. Preog (medium) and Postog vs. Control (right) comparisons. B: KEGG pathway enrichment analysis on differential metabolites from Preog vs. Control (left), Postog vs. Preog (medium) and Postog vs. Control (right) comparisons. Each plot in the diagram represents a metabolic pathway, and the abscissa coordinate and the plot size represent the metabolite number of related pathway. The vertical coordinate and plot color represent the P-value  $[-\ln(P\text{-value})]$  of enrichment analysis. C: Heat maps display the relative changes in contents of differential metabolites in Preog vs. Control (left), Postog vs. Preog (medium) and Postog vs. Control (right) comparisons. The up- and down-regulated metabolites were shown in red and green, respectively. The scale represents the normalized peak area of metabolite in each biological replicates.

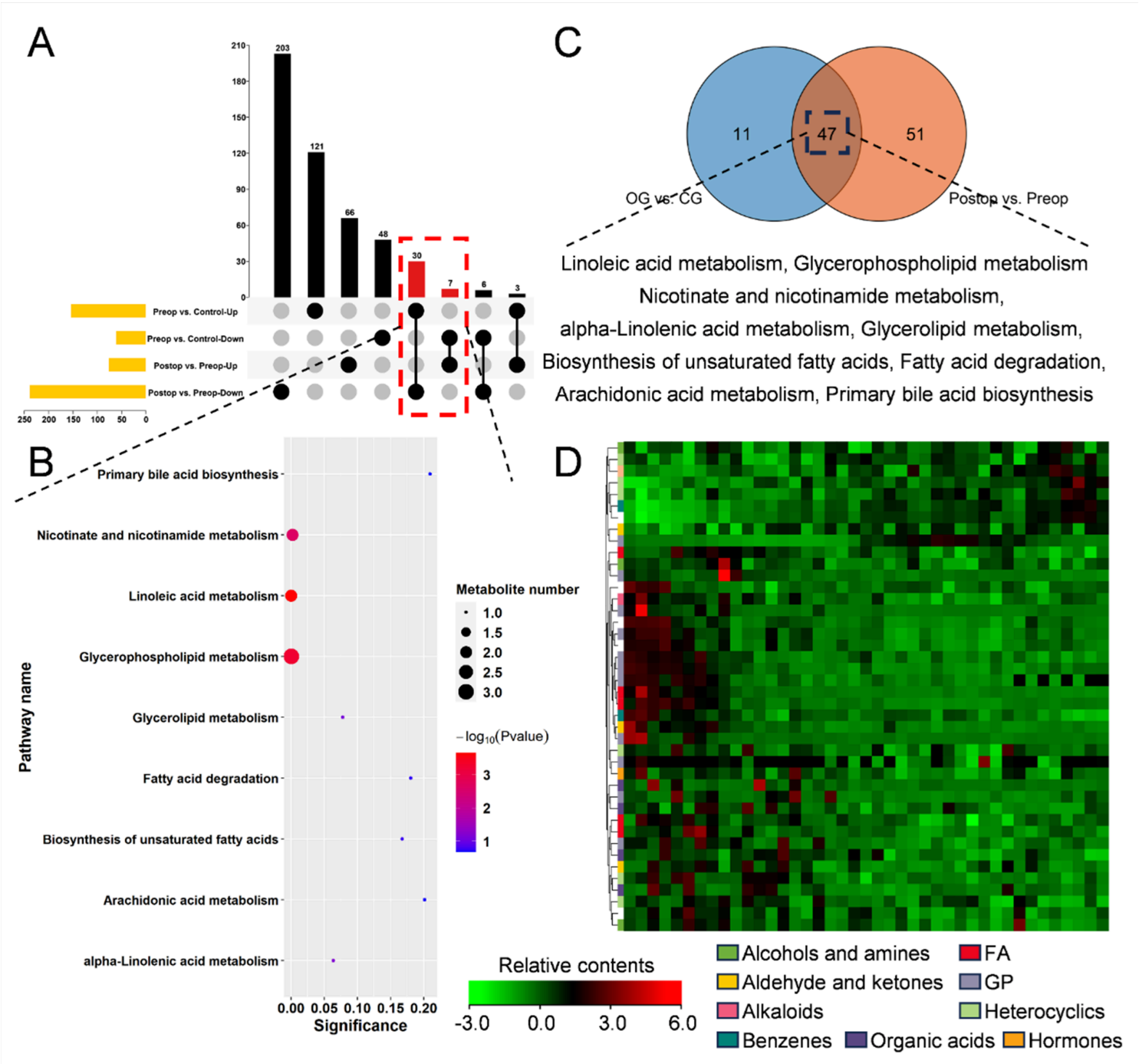


Figure 5

**Improvement of linoleic and glycerophospholipid metabolisms involved in mechanisms by surgery for improving gout**

A: Upset plots identified the hub differential metabolites associated with gout pathogenesis and surgery treatment in serum. The hub metabolites were labeled in red. B: Scatter plot displays the KEGG pathway enrichment results based on hub metabolites associated with gout pathogenesis and surgery treatment in serum. The various color levels displayed different levels of significance of terms from low (blue) to high (red). C: Venn diagram identified the overlapped pathways identified in synovial tissue and serum. The disorders of nine pathways were improved by surgery-removed tophi. D: Heat map showing the relative contents of hub metabolites associated with gout pathogenesis and surgery treatment in Control, Postog and Preog groups. The high and low levels of metabolites are shown in red and green, respectively. Scale bar represents the normalized peak areas of related metabolites in each biological replicate.

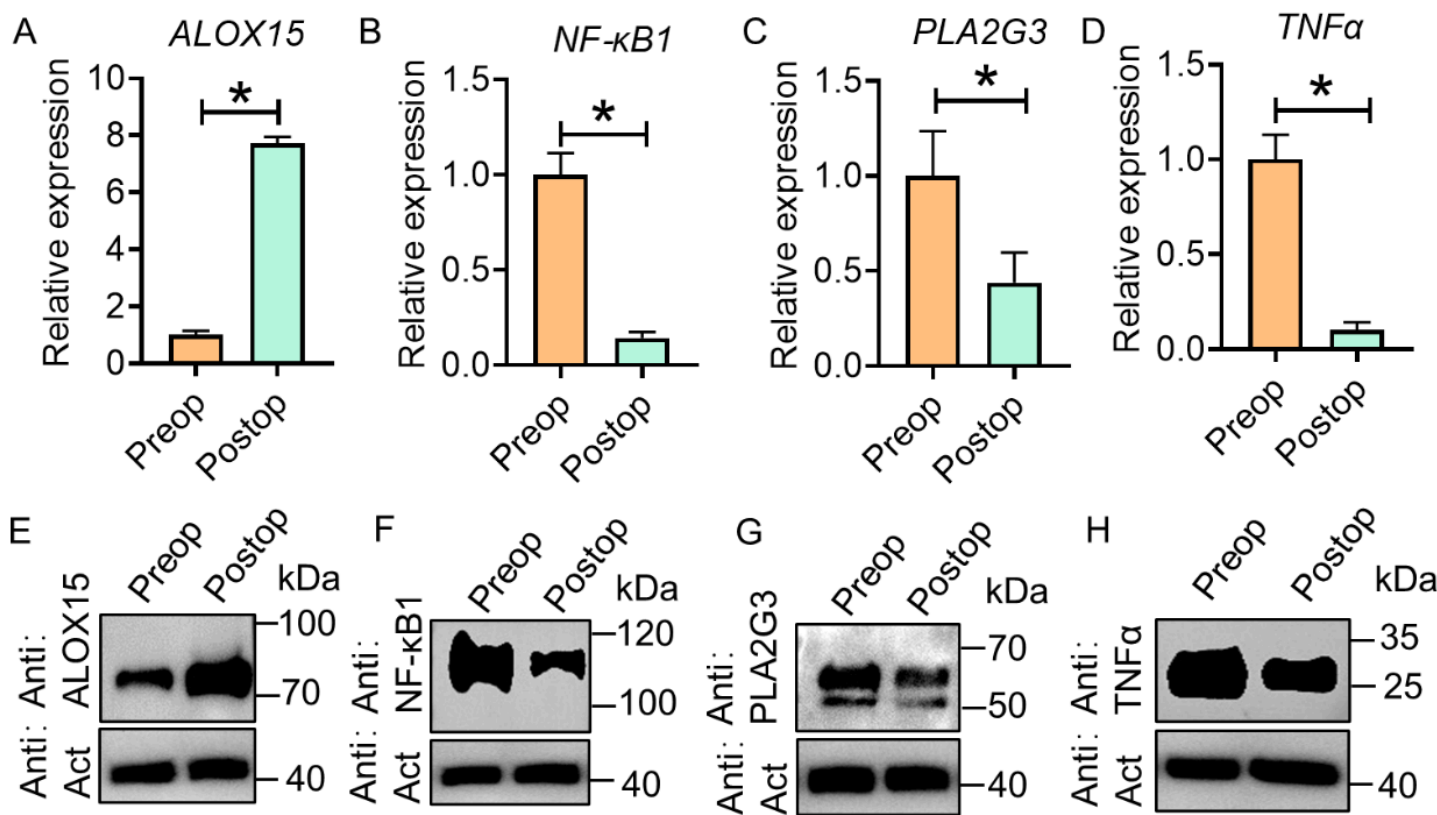


Figure 6

**The expression of inflammation-related genes in serum of Postop and Preop**

A-D: RT-qPCR assay detected the expression of ALOX15, PLA2G3, TNF $\alpha$  and NF- $\kappa$ B1 genes in serum of post-operation (Postop) and pre-operation (Preop). The GAPDH gene was used as an internal reference. Values are mean  $\pm$  SD of three independent biological replicates. Asterisks represent significant

differences (\*P < 0.05, Student's t test). E-H: The protein accumulations of ALOX15, PLA2G3, TNF $\alpha$  and NF- $\kappa$ B1 in serum of Postop and Preop were detected by western blotting. Actin (Act) as an internal control indicates equal loading.

## Supplementary Files

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