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Temporal Hierarchy and Multi-Omic Regulatory Networks Underlying Endurance Training-Induced Hepatic Adaptation in Rats

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Abstract

Purpose

This research sought to delineate the temporal dynamics and gender-specific characteristics of hepatic adaptation to endurance training by using multi-omics data from the MoTrPAC rat liver dataset.Methods: Data from proteomics, phosphoproteomics, and untargeted metabolomics obtained from rat livers after 1, 2, 4, and 8 weeks of endurance training were amalgamated. Differential analysis, time-series clustering, PTM-SEA, GSVA, cross-omics integration, and sex-stratified studies were used to comprehensively assess molecular reactions to training.Results: Hepatic multi-omics reactions demonstrated a unique temporal order. The majority of notable phosphorylation modifications transpired without corresponding changes in protein abundance, whereas AMPK- and mTOR-associated signalling profiles exhibited persistent or stage-specific enhancement, indicating that post-translational regulation serves as an initial adaptive response to exercise stimuli. Phosphorylation alterations in enzymes associated with central carbon metabolism and mitochondrial fatty acid transport were subsequently noted, with a synchronised reconfiguration of metabolites and protein levels. Subsequent phases were marked by functional reorganization that included improved lipid utilization and the preservation of protein homeostasis. Sex-differentiated analysis indicated that the majority of training-induced reactions were consistent across genders, while sex-specific responses were primarily linked to variations in metabolic regulation and signalling pathways, especially concerning lipid metabolism, amino acid metabolism, and redox balance.Conclusion: Hepatic adaptation to endurance exercise is not a straightforward accumulation of multi-omics alterations but rather a dynamic phenomenon marked by initial post-translational modifications, subsequent metabolic network reconfiguration, and eventual functional stabilization. The collective response is mostly sex-conserved, further reinforcing a temporally stratified and essentially communal regulatory architecture that underpins exercise-induced hepatic adaptation.

1.Introduction

Endurance training serves as a significant non-pharmacological approach to enhancing metabolic health and averting metabolic disorders. It restructures systemic metabolic networks via the modulation of energy metabolism, lipid consumption, inflammatory conditions, and redox equilibrium (Lindstad et al. 2026; Zhang et al. 2026). The liver, as a pivotal organ for systemic metabolic control, is critical for sustaining glucose equilibrium, metabolizing fatty acids, employing amino acids, modulating cholesterol-bile acid metabolism, and preserving cellular stability during exercise adaptation (Martino et al. 2024; Fuentes-Barría et al. 2026). Consequently, systematically delineating the dynamic changes in hepatic molecular networks throughout endurance training is essential for comprehending the mechanisms that drive exercise-induced enhancements in hepatic metabolic health and its prospective protective roles against metabolic disorders, such as fatty liver disease and insulin resistance.

Prior research examining exercise-induced hepatic adaptations has predominantly concentrated on isolated omics layers or specific time intervals, thereby constraining the understanding of the ongoing regulatory mechanism from immediate stimulus detection to prolonged functional reconfiguration (Lindstad et al. 2026; MoTrPAC Study Group Primary Authors et al. 2024; Hoffman 2017). Physiological adaptations resulting from exercise include synchronized regulation across several molecular tiers. Alterations in protein levels mostly indicate persistent structural and functional modifications, often necessitating extended stimulation and progressive control via transcriptional and translational mechanisms (Shen et al. 2026; Amar et al. 2024). Conversely, metabolite concentrations immediately indicate substrate consumption and changes in total metabolic flow, embodying the functional outcome of cellular metabolic conditions (Walzik et al. 2026; Belhaj et al. 2021). In contrast to these downstream molecular layers, post-translational modifications (PTMs) serve as swift and reversible regulatory mechanisms that can react to external stimuli prior to significant alterations in protein levels, thus quickly adjusting signal transduction pathways and metabolic enzyme functions. Phosphorylation is regarded as a crucial initial regulatory process that connects exercise stimulus to later metabolic reconfiguration among post-translational modifications (Pataky et al. 2026; Kelty et al. 2026). Consequently, evaluations that rely solely on singular omics layers, varying molecule quantities, or static pathway enrichment methods are inadequate for encompassing the dynamic process of “signal regulation-metabolic reprogramming-functional homeostasis remodeling.” Comprehensive investigations that include phosphoproteomics, proteomics, and metabolomics are essential to thoroughly clarify the time-dependent processes driving hepatic adaptation due to endurance exercise across many regulatory levels.

The Molecular Transducers of Physical Activity Consortium (MoTrPAC) has produced the most extensive publicly accessible dataset encompassing multiple tissues, time points, and omics related to endurance exercise training in rats, offering a detailed molecular map of exercise-induced changes across various tissues and omics dimensions (Nair et al. 2024; Kelty et al. 2026; Lindholm et al. 2024). The first MoTrPAC research mainly aimed to provide a comprehensive framework for exercise responses across many tissues, whereas the intricate regulation mechanisms within specific organs were outlined in a rather generalized manner. The liver serves as a pivotal regulator of energy and nutrition metabolism, significantly influencing glucose and lipid metabolic reconfiguration, substrate distribution, and the preservation of metabolic equilibrium during physical activity. Consequently, the molecular processes governing hepatic adaptation to endurance exercise require more exploration. In this research, we conducted secondary analyses of publicly accessible MoTrPAC liver datasets, which not only provide enhanced molecular understanding of exercise adaptation but also optimize the use of this extensive resource.

This research utilized rat liver datasets from MoTrPAC, integrating proteomic, phosphoproteomic, and metabolomic data to investigate the connections between post-translational modification (PTM) regulation, alterations in metabolic networks, and restructuring of functional modules during endurance training. By applying cross-omics integration, temporal dynamic analysis, and sex-stratified assessments, our goal was to develop a stage-specific molecular framework for hepatic adaptations

resulting from endurance training and to improve the understanding of the mechanisms driving liver adaptation to exercise at the organ-specific level.

2. Materials and Methods

2.1 Data source

This research utilized publicly accessible multi-omics datasets on rat endurance training, provided by the Molecular Transducers of Physical Activity Consortium (MoTrPAC) (Pass1b-06), and retrieved on April 1, 2026 (MoTrPAC Study Group Primary Authors et al., 2024). In the MoTrPAC trial, 6-month-old rats underwent incremental treadmill-based endurance training, with tissue samples collected 48 hours after the final exercise session. The training periods included durations of 1, 2, 4, and 8 weeks, while sedentary untrained rats served as controls. This study specifically focused on liver tissues and incorporated proteomic, phosphoproteomic, and untargeted metabolomic datasets to assess molecular responses during various phases of endurance training.

2.2 Data preprocessing and differential analysis

This study utilized processed datasets, sample annotation files, and differential analysis results provided by MoTrPAC. Quality control, normalization, and statistical analyses for differential expression were conducted by the MoTrPAC consortium. Processed liver proteomic, phosphoproteomic, and metabolomic datasets were extracted and organized based on sample annotation information for further analyses. The differential analysis results from these processed datasets were employed for proteomic, phosphoproteomic, and metabolomic assessments. In the case of phosphoproteomic data, protein abundance-adjusted phosphosite measurements were utilized to reduce the impact of changes in protein abundance on the interpretation of phosphorylation alterations (Many et al., 2024). For all three omics layers, molecular features with $P < 0.01$ and $|\log_2 FC| > 0.5$ were deemed differentially regulated and were included in subsequent analyses. As the analyses were exploratory and primarily focused on characterizing temporal response patterns across molecular layers, nominal P values were applied for feature selection, while adjusted P values provided in the original MoTrPAC datasets were maintained in the supplementary tables for reference.

2.3 Time-series clustering and functional enrichment analysis

Time-series clustering of differentially regulated phosphosites and metabolites was conducted using the Mfuzz package (Nair et al. 2024; Kumar and Futschik 2007). For each molecular feature, abundance values were matched to sample metadata and averaged within each training group. The resulting temporal profiles were organized in the following order: sedentary control, 1, 2, 4, and 8 weeks of training. Features with incomplete temporal profiles were excluded prior to clustering. The resulting matrices were converted into ExpressionSet objects and standardized using the standardize function in Mfuzz. The fuzzification parameter (m) was estimated separately for each omics dataset using

mestimate. Fuzzy c-means clustering was then executed with a fixed random seed of 123. Based on the structure of the temporal patterns and the interpretability of the resulting clusters, metabolites were categorized into four clusters, while phosphosites were grouped into five clusters. Cluster membership values for individual molecular features were retained and are presented in Supplementary Table S2. Following this, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed for each cluster. Phosphosite enrichment analysis utilized Entrez gene IDs, while metabolite enrichment analysis was conducted using KEGG Compound IDs (Wu et al. 2021; Kanehisa et al. 2023; Picart-Armada et al. 2018).

2.4 Phosphorylation analysis and PTM-SEA

To analyze the link between phosphorylation modifications and corresponding protein abundance changes, substantially changed phosphosites were matched with their respective proteins, and the alteration patterns of phosphosites and protein abundance were compared.

Phosphorylation profiles linked to kinases, signalling pathways, and disturbances were then assessed by PTM Signature Enrichment Analysis (PTM-SEA). Protein abundance-adjusted rat phosphosites were correlated with their human counterparts using the MoTrPAC rat-to-human phosphorylation mapping database (RAT_TO_HUMAN_PHOSPHO) (Krug et al., 2019). Human phosphosite IDs were structured as UniProt accession-site combinations and aligned using PTMsigDB v2.0.0. In cases of duplicated mapped phosphosites within the same training comparison, the entry with the lowest nominal P value was preserved. At each training time point, phosphosites were prioritized based on the calculation sign (logFC) × -log10(P), with P being the nominal differential-analysis P value. Enrichment analysis was conducted using the fgsea method with gene-set sizes limited to 3-800 mapped phosphosites and a random seed of 2026. PTM-SEA was carried out independently for the training cohorts of 1, 2, 4, and 8 weeks. Signatures exhibiting nominal $P < 0.01$ were deemed considerably enriched for the purposes of visualization and interpretation. The comprehensive PTM-SEA results, including normalized enrichment scores (NES), nominal P values, adjusted P values, and leading-edge phosphosites, are included in Supplementary Table S3. PTM-SEA outcomes signify the amplification of phosphosite signatures linked to certain kinases, pathways, or disturbances and do not explicitly indicate alterations in kinase activity.

2.5 Integrated analysis of metabolic axes

Utilising differential analysis, Mfuzz clustering, and pathway enrichment findings, key metabolites, metabolic enzymes, and relevant phosphorylation sites were identified to delineate two principal metabolic pathways: the fatty acid/acylcarnitine pathway and the TCA cycle/central carbon metabolism pathway. Cross-omics correlations were assessed by analysing temporal variations in metabolite modifications, fluctuations in protein levels of associated metabolic enzymes, and phosphorylation dynamics over various training phases.

2.6 Analysis of sex-conserved and sex-biased responses

Training-responsive molecules were classified based on differential analysis results obtained separately from male and female rats. Sex-conserved responses were defined as molecular alterations showing the same direction of change in both sexes, with statistical significance achieved in at least one sex. Male-specific and female-specific responses were defined as significant alterations observed exclusively in one sex. Sex-opposite responses were defined as significant changes occurring in both sexes but showing opposite directions of regulation.

2.7 GSVA-based protein set enrichment analysis

Gene Set Variation Analysis (GSVA) was applied to evaluate temporal alterations in proteomic functional modules (Hänzelmann et al. 2013; Aydin et al. 2023; Castanza et al. 2023; Yu et al. 2025). Protein RefSeq identifiers were converted to rat gene symbols, and GSVA scores were calculated using the Rattus norvegicus C2 KEGG gene sets obtained from the msigdbr database. Sample-level GSVA scores were aligned with the corresponding training-group metadata. For each protein set, the mean GSVA score at each training time point was compared to that of sedentary controls. The magnitude of change was determined as the difference between the mean GSVA score of the training group and that of the control group (ΔGSVA = mean_training−mean_control). Statistical comparisons between each training group and the controls were performed using two-sample Student's t-tests. For visualization purposes, protein sets were ranked based on the smallest nominal P value observed across the four training time points, and representative modules demonstrating significant temporal changes were selected for Fig. 6. Complete GSVA differential results are available in Supplementary Table S5. GSVA results indicate relative changes in protein-set abundance patterns and should not be interpreted as direct changes in pathway functional activity.

3. Results

3.1 Global characteristics of hepatic multi-omic responses induced by endurance training

To assess the impact of prolonged endurance training on hepatic molecular modifications, we first analysed the variances in the proteome, phosphoproteome, and metabolome after 1, 2, 4, and 8 weeks of training. Volcano plots of multi-omics datasets depicted the allocation of differentially regulated proteins, phosphosites, and metabolites over several training phases (Fig. 1A). Following 1, 2, 4, and 8 weeks of endurance training, 46, 79, 37, and 91 distinct differentially expressed proteins were detected, respectively. Furthermore, 696, 826, 1101, and 1122 distinct differentially regulated phosphosites, together with 103, 205, 108, and 265 unique differential metabolites, were identified at the respective time intervals (Fig. 1B; Supplementary Table S1).

Significantly, several phosphosites exhibited considerable changes as soon as one week post-training, indicating that the modulation of phosphorylation might serve as an initial adaptive response for the liver's detection of exercise-induced signals (Shen et al. 2026; Hoene et al. 2009). The differential

phosphosites escalated with extended training periods, peaking at 4 weeks and sustaining significant levels at 8 weeks. Conversely, alterations in protein levels were rather restricted, as the quantity of differential proteins rose at 2 and 8 weeks, although decreased at 4 weeks. The metabolomic response exhibited a biphasic pattern, characterised by an increase in differential metabolites at 2 weeks, followed by another rise at 8 weeks.

Phosphoproteomic changes were widespread throughout various training phases, while proteomic and metabolomic reactions seemed to lag, suggesting differing response intensities and timing across molecular levels during endurance training adaptation. To enhance the understanding of common and stage-specific chemical reactions across training phases (Fig. 1C-E), we examined the intersection of differential molecules at various time intervals. The findings indicated that modifications unique to certain time points constituted a significant fraction of the differential molecules across the proteome, phosphoproteome, and metabolome, whereas only a limited number of molecules demonstrated enduring changes throughout several training phases. The results suggest that the hepatic molecular responses elicited by endurance training do not increase in a linear fashion with time, but rather display dynamic patterns that are distinct to different stages (Kelty et al. 2025; MoTrPAC Study Group et al. 2024).

Figure 1. Global characteristics of hepatic multi-omic molecular responses following endurance training in rats (A) Multi-omic volcano plots showing differentially regulated proteins, phosphosites, and metabolites at different training time points. The x-axis represents the three omics layers, the y-axis represents log_2FC, and colors indicate the direction of molecular alterations. (B) Number of unique differentially regulated molecular features identified in the proteome, phosphoproteome, and metabolome across different training stages. (C-E) UpSet plots showing the shared and time point-specific distributions of differentially regulated phosphosites, proteins, and metabolites among different training durations

3.2 Time-series clustering reveals stage-specific remodeling of phosphorylation and metabolic profiles

To characterize the temporal trajectories of molecular alterations during endurance training, we performed time-series clustering of differentially regulated phosphosites and metabolites using Mfuzz, followed by KEGG pathway enrichment analysis to determine the functional characteristics of each cluster (Fig. 2). The phosphoproteomic dataset was categorized into five dynamic clusters (Fig. 2D), including 588, 632, 597, 461, and 521 phosphosites, respectively (Supplementary Table S2). Although the temporal patterns ranged within clusters, the elevated biological activities were generally connected with energy sensing and nutrition signaling, cellular quality control, as well as tissue architecture and cell adhesion-related processes (Fig. 2C).

Clusters 1 and 4 showed variable temporal dynamics, with enriched pathways mostly related to insulin signaling, AMPK signaling, mTOR signaling, FoxO signaling, autophagy, nucleocytoplasmic transport, and endocytosis. Cluster 3 exhibited a persistent rising trend, indicating that a subset of phosphorylation

events may contribute to the gradual development of long-term training adaptation. Functional enrichment of this cluster includes insulin signaling, AMPK signaling, autophagy, and glucagon signaling pathways.

The metabolomic dataset was divided into four dynamic clusters (Fig. 2A), including 105, 119, 117, and 114 metabolites, respectively (Supplementary Table S2). Cluster 1, which showed an overall decreasing pattern, and Cluster 3, which displayed an initial increase followed by a decline, were primarily enriched in nucleotide metabolism, purine metabolism, glutathione metabolism, amino acid metabolism, CoA-related metabolism, and aspects of central carbon metabolism. Conversely, Cluster 2 had a continuous upward trend, while Cluster 4 saw an initial decline followed by a slow rise. These clusters were predominantly involved with nicotinate and nicotinamide metabolism, bile secretion, unsaturated fatty acid biosynthesis, glycerophospholipid metabolism, choline metabolism, ATPase-related activities, and lipid transport pathways (Fig. 2B).

3.3 Decoupling between phosphorylation alterations and protein abundance changes and temporal features revealed by PTM-SEA

To ascertain if the modifications in phosphorylation due to training were influenced by variations in the associated protein levels, significantly altered phosphosites were aligned with their respective proteins, and the correlation between changes in protein abundance(log_2FC) and phosphosite modifications(log_2FC) at each training interval was analyzed (Fig. 3A).

The findings indicated that variations in phosphosite levels were not entirely aligned with changes in the associated protein levels. For the majority of regulated phosphosites, the associated proteins had log_2FC values around zero, whereas phosphosites corrected for protein abundance revealed significant differential alterations. Throughout the training periods of 1, 2, 4, and 8 weeks, the percentage of differential phosphosites linked to notably modified proteins remained consistently under 4%, while 96%-98% of regulated phosphosites manifested without substantial alterations in the levels of their associated proteins (Fig. 3B). These results suggest that phosphorylation responses were mostly unaffected by changes in protein abundance, highlighting a distinct regulatory mechanism of post-translational modification during adaptation to endurance exercise (Supplementary Fig. 1A).

To further the understanding of phosphorylation regulatory patterns, PTM-SEA was used to assess the enrichment of phosphorylation signatures linked to established kinases and signalling pathways (Fig. 3C-D; Supplementary Fig. 1B; Supplementary Table S3). Diverse temporal trends were seen across various phosphorylation signatures. AMPKα1/PRKAA1-associated signatures exhibited significant enrichment as early as one week post-training and persisted at elevated levels throughout the training duration, indicating that energy-sensing phosphorylation networks are activated during the initial adaptation phase and continuously engage in subsequent regulatory mechanisms. Conversely, mTOR/MTOR and its associated p70S6K/RPS6KB1 signatures exhibited heightened enrichment

throughout the intermediate training phase, suggesting a progressive augmentation of regulatory mechanisms linked to protein synthesis and cellular growth during metabolic adaptation.

ERK2/MAPK1-associated signatures showed greater enrichment mostly in the mid-to-late phases of training, indicating a heightened role of stress-responsive signaling and cellular adaptive restructuring. In the interim, TIE2-associated markers exhibited persistent elevation throughout advanced training, while TNF-related markers demonstrated stage-dependent variations, suggesting that the preservation of tissue equilibrium, inflammatory control, and microenvironmental adjustment could be crucial elements of prolonged training responses.

3.4 Coordinated changes in phosphorylation, protein abundance, and metabolites within the fatty acid/acylcarnitine and TCA/central carbon metabolic axes

To delve further into the molecular foundations of exercise-induced metabolic remodeling, we included key metabolites, metabolic enzymes, and their associated phosphates in the TCA/central carbon metabolism pathway and the acylcarnitine transport pathway (Fig. 4). Numerous medium- and long-chain acylcarnitines exhibited significant accumulation inside the acylcarnitine transport axis during 2–4 weeks of training (Fig. 4B). Simultaneously, essential enzymes implicated in fatty acid activation, mitochondrial transport, and lipid trafficking, such as carnitine palmitoyltransferase 1A (Cpt1a), carnitine-acylcarnitine translocase (Slc25a20), long-chain acyl-CoA synthetase 4 (Acsl4), and liver fatty acid-binding protein (Fabp1), demonstrated gradual changes in protein levels. Conversely, the phosphorylation modifications of enzymes associated with β-oxidation, including long-chain acyl-CoA dehydrogenase (Acadl) and hydroxyacyl-CoA dehydrogenase (Hadh), transpired during the first phases of training (Fig. 4C-D).

Subsequent stratified analysis of lipid metabolites indicated that phospholipid metabolites had stage-specific variations, whereas storage-associated lipid types, such as triglycerides and free fatty acids, progressively increased throughout the intermediate to advanced phases of training. These results indicate that lipid metabolic reconfiguration displays unique temporal characteristics during the response to endurance exercise (Supplementary Fig. 2A-B). In the TCA/central carbon metabolism route, citrate, isocitrate, and several intermediates from glycolysis and the pentose phosphate pathway exhibited stage-specific changes throughout training (Fig. 4E-F). Phosphorylation sites of crucial enzymes that facilitate glucose entry into mitochondria and regulate the TCA cycle, such as the E1α subunit of pyruvate dehydrogenase (Pdha1), aconitase 1 (Aco1), and isocitrate dehydrogenase 1 (Idh1), exhibited early responsiveness during training. Conversely, alterations in the levels of glycolytic enzymes, such as phosphofructokinase muscle isoform (Pfkm), hexokinase 2 (Hk2), and pyruvate dehydrogenase kinase 4 (Pdk4), transpired at a later stage.

Overall, the TCA/central carbon metabolic axis exhibited a temporal pattern characterized by early phosphorylation and metabolite responses followed by subsequent protein abundance adjustments. Collectively, these results indicate that phosphorylation alterations of metabolic enzymes generally

preceded changes in protein abundance and metabolite levels, whereas broader proteomic remodeling occurred predominantly during the later stages of training (Fig. 4G).

3.5 Sex-conserved and sex-biased patterns of hepatic responses to endurance training

To ascertain whether hepatic adaptations resulting from endurance training display sex-specific traits, we analyzed molecular response patterns in male and female rats at various training phases (Fig. 5). The findings indicated that sex-conserved responses were prevalent throughout all three omics layers, whereas male-specific, female-specific, and sex-opposite responses constituted comparatively minor fractions (Fig. 5A; Supplementary Table S4). These results suggest that most molecular changes in the liver due to endurance exercise show similar patterns across genders. Subsequent examination indicated that the temporal allocation of sex-conserved reactions across several omics layers closely mirrored the general trends of differential molecular responses. Phosphoproteomic changes manifested first and to considerable levels, whereas proteomic and metabolomic reactions were seen to be somewhat tardy (Fig. 5B). These findings indicate that the fundamental temporal mechanisms of liver adaptation to endurance exercise mostly stem from common biochemical reactions across genders rather than gender-specific changes.

Functional enrichment analysis further indicated that sex-biased responses mostly represented variations in regulatory mechanisms (Fig. 5C; Supplementary Table S4). In the proteome, responses unique to males were predominantly linked to amino acid metabolism, innate immune mechanisms, and transcriptional pathways, while responses exclusive to females were more abundant in cellular stress responses, cell cycle control, and nuclear receptor-related processes. In the phosphoproteome, reactions skewed toward males mostly pertained to integrin signalling, angiogenesis, and cytoskeletal control, while responses unique to females were largely linked to FOXO transcriptional regulation, oxidative stress responses, and TP53-related signalling. In contrast to the proteome and phosphoproteomic dimensions, sexual disparities were more pronounced at the metabolomic tier. Male-specific metabolic reactions were more concentrated in lipid-related categories such as triglycerides, diacylglycerols, free fatty acids, and bile acids, whereas female-specific reactions were more linked to acyl-CoA metabolism, amino acid metabolism, and redox-related mechanisms. In general, sexual disparities in hepatic adaptation to endurance training were mainly evident in metabolic regulation and signalling mechanisms rather than in the essential nature of training-induced adaptation.

3.6 Proteomic functional module alterations support late-stage adaptive remodeling

To assess the impact of prolonged endurance training on hepatic functional states, we used GSVA to delineate temporal changes in proteomic functional modules (Fig. 6A-B; Supplementary Table S5). The findings indicated that alterations in proteomic functional modules were mostly seen in the advanced

phases of training. Functional components related to fatty acid metabolism, unsaturated fatty acid synthesis, α-linolenic acid processing, peroxisomal activity, and amino acid/nitrogen metabolism progressively elevated over the training period, reaching notably higher levels between 4 to 8 weeks. Concurrently, modules associated with cholesterol production and terpenoid backbone biosynthesis demonstrated persistent growth. Conversely, functional modules related to DNA replication, homologous recombination, non-homologous end joining, mismatch repair, and cell cycle control exhibited a general decline (Fig. 6A-B).

The functional modules that were enhanced during late-stage training were mostly linked to lipid metabolism, oxidative processes, and proteostasis maintenance, whereas the downregulated modules were predominantly connected to cellular growth and DNA replication. These results indicate that the late-phase hepatic response to endurance training is marked not by significant cellular growth but by the progressive development of a functional remodeling condition that emphasizes metabolic adaptation and the preservation of tissue homeostasis. Through the amalgamation of phosphoproteomic, metabolomic, and proteomic data, we discerned a distinct temporal stratification pattern that elucidates hepatic adaptation to endurance exercise. Utilizing the prevalent response patterns across several omics layers at key training phases, we developed a model tailored to each step that elucidates the multi-omic adaptation of rat liver resulting from endurance training (Table 1).

Table 1
Stage-specific model of hepatic multi-omic adaptation induced by endurance training in rats

Stage	Major responsive layer	Core alterations	Stage characteristics
1 week	Phosphoproteome	Extensive phosphorylation alterations occurred rapidly, with most changes independent of protein abundance alterations	Rapid hepatic sensing and signal transduction in response to training stimuli, initiating early adaptive programs
2 week	Metabolome / Phosphoproteome	Coordinated alterations in metabolites, metabolic enzymes, and their phosphorylation sites	Adjustment of substrate utilization patterns and metabolic network remodeling
4 week	Phosphoproteome	Nutrient sensing, autophagy, and cellular quality control-related regulatory networks remained active	Maintenance of metabolic adaptation and promotion of cellular homeostatic regulation
8 week	Proteome / Metabolome	Remodeling of functional modules associated with lipid metabolism, proteostasis, and tissue functions	Establishment of a relatively stable long-term adaptive state
All stages	Sex-conserved responses	Most training-induced responses exhibited consistent directions between males and females	Hepatic adaptation to endurance training is predominantly characterized by shared responses between sexes, with sex differences mainly occurring at the metabolic regulation level

4. Discussion

4.1 Hepatic adaptation to endurance training represents a stage-dependent transition rather than linear accumulation

In this investigation, we molecular responses after 1, 2, 4, and 8 weeks of endurance training by amalgamating proteomic, phosphoproteomic, and metabolomic data from MoTrPAC. In contrast to the initial MoTrPAC investigation, which offered a comprehensive examination of multi-tissue and multi-omics reactions to exercise training, the current study concentrated exclusively on hepatic adaptation and delved into temporal dynamics, correlations between phosphorylation and protein levels, synchronized modifications across metabolic pathways, and changes in proteomic functional modules via secondary analysis of liver datasets. The quantities of differential molecules and Mfuzz-based temporal clustering studies jointly indicated that hepatic adaptation to endurance training displays

evident temporal stratification, with various omics layers fulfilling unique regulatory functions throughout the training regimen.

4.2 Phosphorylation alterations indicate early post-translational regulation largely independent of protein abundance changes

This research revealed that over 96% of notably modified phosphosites did not correlate with significant variations in the levels of their associated proteins, suggesting that the majority of phosphorylation changes induced by training were not merely secondary effects of protein abundance fluctuations but instead represented distinct post-translational regulatory mechanisms. Phosphorylation, being rapid, reversible, and energetically efficient, serves as a more appropriate regulatory mechanism for initial reactions to prolonged exercise stimulus than the processes of protein synthesis and degradation.

The PTM-SEA analysis also revealed the temporal attributes of this regulating mechanism. Phosphorylation profiles associated with AMPK were markedly enriched in the first phase of training, indicating that the liver promptly reacts to heightened energy requirements caused by extended exercise via energy-sensing pathways. AMPK functions as a crucial cellular energy detector and facilitates fatty acid oxidation, mitochondrial metabolism, and the preservation of metabolic adaptability (Langer et al. 2024; Smith et al. 2026). The initial and prolonged enhancement of AMPK-related phosphorylation patterns demonstrated temporal alignment with the latter alterations in fatty acid metabolism and cerebral carbon metabolism noted in this research. Concurrently, mTOR and its associated p70S6K-related markers exhibited increased enrichment throughout the intermediate phase of training. AMPK and mTOR serve as essential regulatory centers that manage cellular metabolism, with AMPK focusing on energy preservation and substrate use, while mTOR plays a role in protein synthesis, autophagy control, and cellular growth (Goul et al., 2023). Throughout extended training, these pathways may collaboratively regulate energy supply in conjunction with tissue adaption needs. In the current work, significant enrichment of mTOR/p70S6K-related signatures throughout the middle training period reflects a progressive change from early energy sensing toward metabolic remodeling and cellular quality control.

Moreover, ERK2, TIE2, and TNF-related markers were mostly detected in the intermediate to advanced phases of training. ERK signalling is intricately linked to cellular stress adaptation, proliferation, and metabolic control (Lavoie et al. 2020), whereas TIE2 plays a role in maintaining vascular homeostasis and regulating the tissue microenvironment (Augustin et al. 2009; Wang et al. 2022). The alterations in TNF-related signatures based on stage indicate that inflammatory signaling may not only be inhibited during prolonged training but also experience dynamic reconfiguration. These phosphorylation patterns together illustrate an ongoing adaptive mechanism whereby the liver shifts from energy detection to metabolic restructuring and the preservation of tissue equilibrium. These results support the notion that post-translational regulation constitutes a significant regulatory tier in the early and middle phases of endurance exercise adaptation. The findings indicate that initial hepatic adaptation to endurance training

depends more on fast changes in protein functional states than on variations in protein levels, with post-translational modification regulation acting as a molecular link between exercise stimulus and ensuing metabolic remodeling.

4.3 Metabolic network remodeling during training adaptation revealed by glucose metabolism and acylcarnitine transport axes

This research demonstrated that both the TCA cycle/central carbon metabolism pathway and the acylcarnitine transport pathway underwent a persistent adaptive process marked by initial phosphorylation reactions, subsequent metabolic alterations, and final protein adjustments. In the core carbon metabolic pathway, the pyruvate dehydrogenase E1α subunit (Pdha1) serves as a pivotal gateway for glucose-derived carbon flow into mitochondrial oxidation (Patel et al. 2026), whereas aconitase 1 (Aco1) and isocitrate dehydrogenase 1 (Idh1) are situated at the initial stages of the TCA cycle and play a role in producing reducing equivalents. These crucial metabolic hubs showed changes in phosphorylation during the first phase of training, whereas citrate, isocitrate, and associated glycolytic intermediates saw later modifications. These results indicate that endurance training modifies energy requirements and may potentially reallocate glucose-derived carbon flow across oxidative energy generation, biosynthetic activities, and redox balance.

Modifications in the acylcarnitine transport system indicated an increased utilization of lipid substrates throughout the adaptation to training. Carnitine palmitoyltransferase 1A (CPT1A) constitutes the rate-limiting factor for the ingress of long-chain fatty acids into mitochondria for β-oxidation, while carnitine-acylcarnitine translocase (SLC25A20) orchestrates the translocation of acylcarnitines through the inner mitochondrial membrane (Liang 2023), and long-chain acyl-CoA synthetase 4 (ACSL4) promotes the activation of fatty acids (Deng et al. 2025). Moreover, liver fatty acid-binding protein (FABP1) plays a role in the intracellular transport of fatty acids, whereas long-chain acyl-CoA dehydrogenase (ACADL) and hydroxyacyl-CoA dehydrogenase (HADH) are directly involved in β-oxidation mechanisms (Storch et al. 2023; Fang et al. 2022). The synchronized modifications of these metabolic elements indicate that endurance exercise progressively augments the liver's ability to absorb, activate, transport, and oxidize fatty acids, thereby enhancing the efficiency of lipid utilization as energy sources. Significantly, both metabolic pathways demonstrated earlier phosphorylation modifications compared to variations in protein levels. This temporal pattern indicates that metabolic remodeling caused by training may first transpire via fast PTM-mediated control of crucial metabolic enzyme states, subsequently leading to the formation of comparatively persistent proteome adaptations.

4.4 Sex-conserved responses represent the predominant pattern of hepatic adaptation

Sex-stratified evaluations indicated that the liver's adaptation to endurance training was mostly marked by sex-conserved reactions, implying that the majority of training-induced molecular changes happened

similarly across genders. This discovery aligns with the MoTrPAC concept of "shared adaptation with sex-dependent magnitude" (MoTrPAC Study Group Primary Authors et al., 2024), suggesting that the fundamental biological trajectory of training adaptation is largely preserved, while sex differences predominantly manifest in the magnitude of response rather than its direction. Sex-biased responses were detected throughout the proteome, phosphoproteomic, and metabolomic layers but were especially obvious at the metabolomic level. Responses unique to males were mostly linked to lipid mobilization and utilization mechanisms, whereas those distinct to females were primarily connected to amino acid metabolism, redox equilibrium, and stress management. Concurrently, sex-biased reactions within the proteome and phosphoproteome also included transcriptional control, cellular stress responses, and signal transduction pathways. Together, our data imply that male and female livers may employ somewhat unique regulation methods within a similar adaptive framework during endurance training.

4.5 Proteomic functional module remodeling reflects the establishment of long-term hepatic metabolic homeostasis

In contrast to the phosphoproteome, which mainly indicates rapid regulatory reactions, proteomic changes provide deeper understanding of functional conditions developed via prolonged adaptation. This investigation demonstrated through GSVA analysis that functional modules linked to fatty acid metabolism, unsaturated fatty acid biosynthesis, peroxisome activity, amino acid/nitrogen metabolism, autophagy, and proteostasis exhibited a gradual increase during late-stage training, while those associated with DNA replication, cell cycle regulation, and DNA damage repair displayed a general decline.

These modifications indicate that prolonged endurance training does not propel the liver into a highly proliferative condition, but rather facilitates a shift toward a more effective metabolic equilibrium. The augmentation of fatty acid metabolism and peroxisome-associated modules aligned with the previously noted reconfiguration of the fatty acid/acylcarnitine axis, endorsing enhanced long-term potential for hepatic lipid oxidation and substrate utilisation. Concurrently, elevated modules associated with amino acid and nitrogen metabolism indicate improved control of carbon-nitrogen metabolic equilibrium in the liver. The prolonged activation of autophagy- and proteostasis-associated pathways suggests that extended training not only modifies metabolic substrate use but may also preserve proteome integrity and metabolic well-being via improved cellular quality control systems. Significantly, the decrease in DNA replication, cell cycle, and specific DNA repair-associated modules during advanced training does not inherently signify compromised tissue functionality but may rather illustrate the liver's shift from an adaptation-establishment phase to a homeostatic maintenance phase. In the presence of ongoing training stimuli, hepatic adaptation seems to depend not on heightened cellular proliferation but on the enhancement of metabolic processes and homeostatic control within the current cells. Consequently, in contrast to the first adaption period marked by rapid phosphorylation reactions, the latter proteome changes mostly signify functional reorganisation and the restoration of tissue homeostasis.

4.6 Limitations

This work exploited the MoTrPAC multi-omics resource to assess the temporal hierarchy of hepatic adaptation produced by endurance exercise. However, the results were mostly based on correlation and temporal consistency rather than clear causative evidence. Consequently, the regulatory functions of initial phosphorylation responses in training adaptation, together with the correlation between metabolic substrate reconfiguration and modifications in late-stage functional modules, require more experimental confirmation. Furthermore, assessments conducted at the whole tissue level are unable to differentiate the roles of various hepatic cell types in exercise-induced adaptation. Future research, including single-cell omics, spatial omics, and focused functional assays, will enhance our understanding of cell-specific mechanisms and dynamic regulatory processes that govern hepatic adaptation to endurance exercise.

5. Conclusion

This research illustrates that the hepatic adaptations resulting from endurance exercise follow a distinct temporal order. Initial adaptation is chiefly defined by phosphorylation control that is not reliant on alterations in protein levels, succeeded by a transitional reconfiguration of central carbon metabolism and lipid utilization pathways, culminating in a sustained adaptive condition marked by enhanced lipid utilization, preservation of proteostasis, and metabolic equilibrium. In general, the liver's adaptation to endurance exercise is mostly marked by sex-conserved reactions, whereas minimal sex disparities are chiefly seen in metabolic control and stress-related signalling mechanisms. These results endorse a framework wherein hepatic adaptation to endurance training is not a straightforward accumulation of changes within a singular omics layer but instead a dynamic process characterized by sequential inputs from post-translational modifications, metabolic network reconfiguration, and the reorganization of functional homeostasis.

Declarations

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Author Contribution

Qingyao Wu: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing - original draft. Zhida Huang: Investigation, Validation, Writing - review & editing. Yunhao Zhang: Investigation, Validation, Writing - review & editing. Cui Meng:Methodology, Formal analysis, Writing – review & editing.Yong Guo: Conceptualization, Supervision, Project administration, Writing - review & editing. All authors read and approved the final version of the manuscript.

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Data Availability

The datasets analyzed in this study are publicly available through the Molecular Transducers of Physical Activity Consortium (MoTrPAC) Data Hub. The present study used the Pass1b-06 rat liver proteomic, phosphoproteomic, and metabolomic datasets. The processed datasets and associated metadata can be accessed through the MoTrPAC Data Hub. Data generated from the secondary analyses conducted in this study are provided in the Supplementary Materials.

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Figures

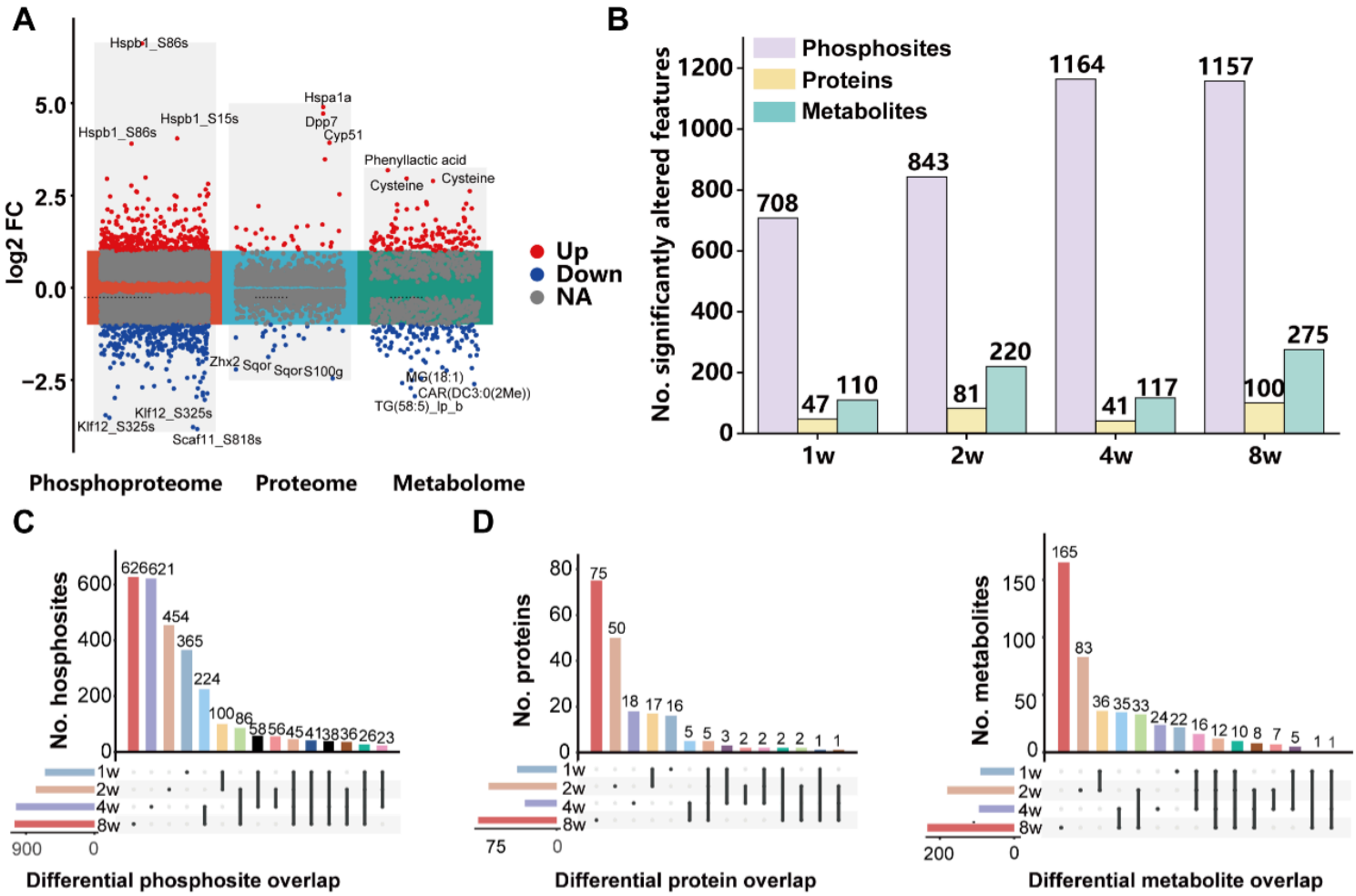


Figure 1

Global characteristics of hepatic multi-omic molecular responses following endurance training in rats(A) Multi-omic volcano plots showing differentially regulated proteins, phosphosites, and metabolites at different training time points. The x-axis represents the three omics layers, the y-axis represents log_2FC, and colors indicate the direction of molecular alterations. (B) Number of unique differentially regulated molecular features identified in the proteome, phosphoproteome, and metabolome across different training stages. (C-E) UpSet plots showing the shared and time point-specific distributions of differentially regulated phosphosites, proteins, and metabolites among different training durations

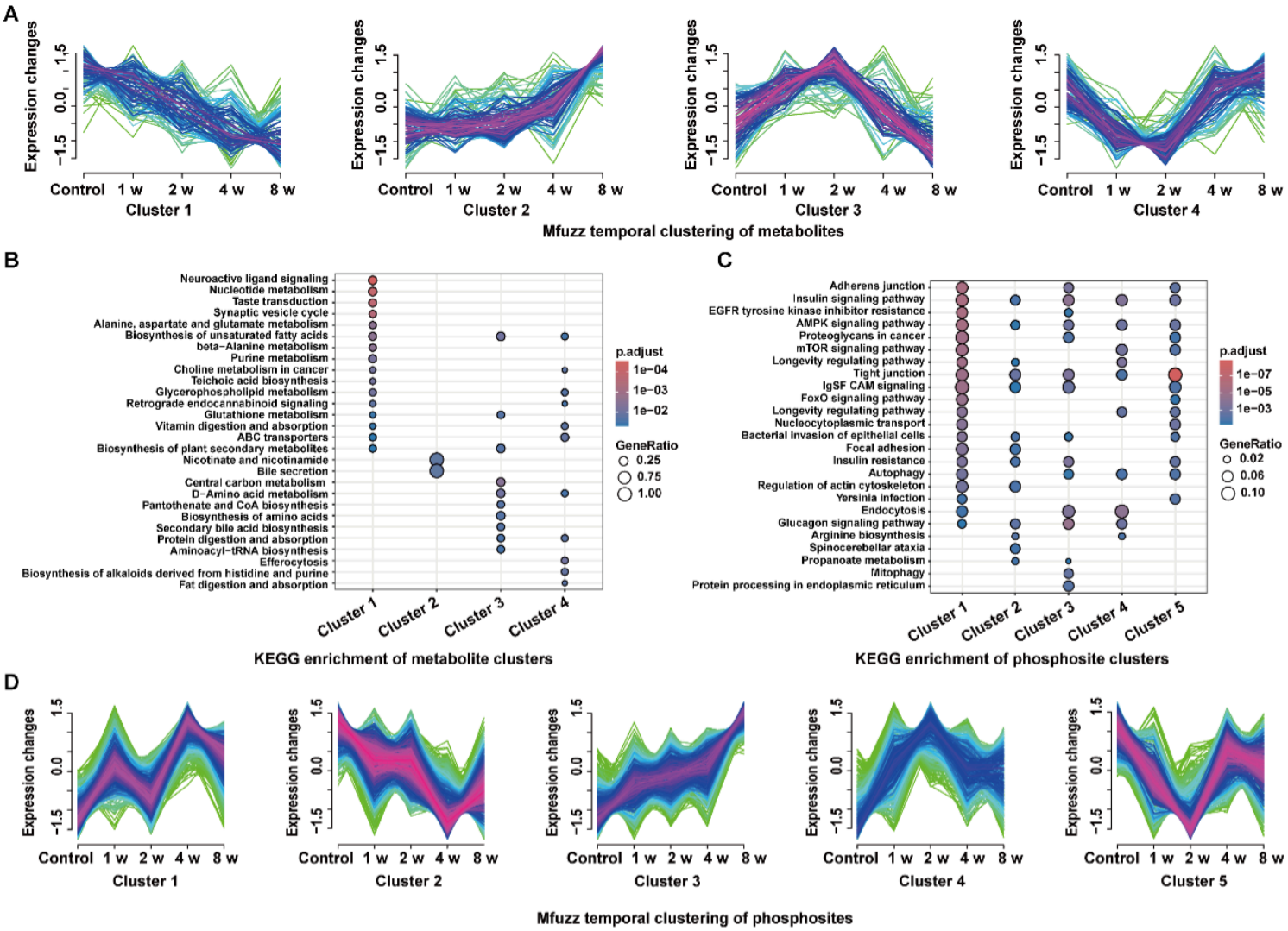


Figure 2

Temporal clustering and pathway enrichment analysis of hepatic phosphoproteome and metabolome during endurance training (A) Mfuzz time-series clustering analysis of differentially regulated metabolites, identifying four distinct temporal clusters. (B) KEGG pathway enrichment analysis of metabolite clusters. (C) KEGG pathway enrichment analysis of phosphosite clusters. (D) Mfuzz time-series clustering analysis of differentially regulated phosphosites, identifying five distinct temporal clusters. For bubble plots, the x-axis represents clusters, the y-axis represents enriched pathways, bubble size indicates enrichment significance, and color represents enrichment intensity

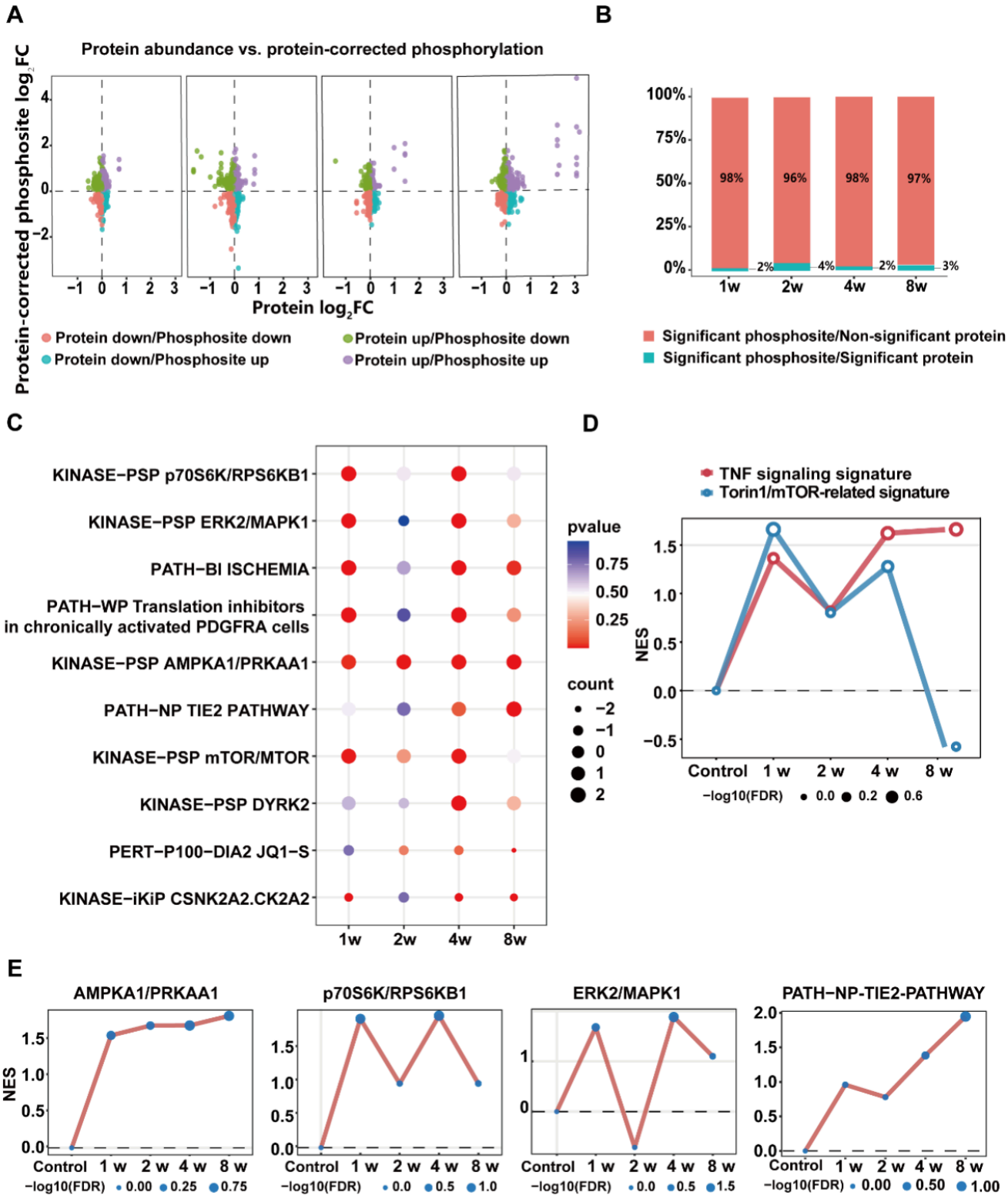


Figure 3

Decoupling between phosphorylation alterations and protein abundance changes and PTM-SEA features during endurance training (A) Four-quadrant plots showing the relationship between significantly altered phosphosites and the log_2FC values of their corresponding proteins. The x-axis represents the log_2FC of corresponding proteins, and the y-axis represents the log_2FC of phosphosites. (B) Proportion of significantly altered phosphosites with or without significant changes in the abundance

of their corresponding proteins. Significant changes were defined as $p < 0.01$ and $|\log_2 FC| > 0.5$. (C) Bubble plots showing PTM-SEA enrichment of pathway- or kinase-related phosphorylation signatures. The x-axis represents training time points, the y-axis represents kinase or pathway signatures, bubble size indicates statistical significance, and color represents normalized enrichment score (NES). (D) Temporal changes in NES values of TNF-related pathway signatures and TORIN1/mTOR-related pathway signatures during endurance training. (E) Temporal trajectories of representative kinase- or signaling-related phosphorylation signatures

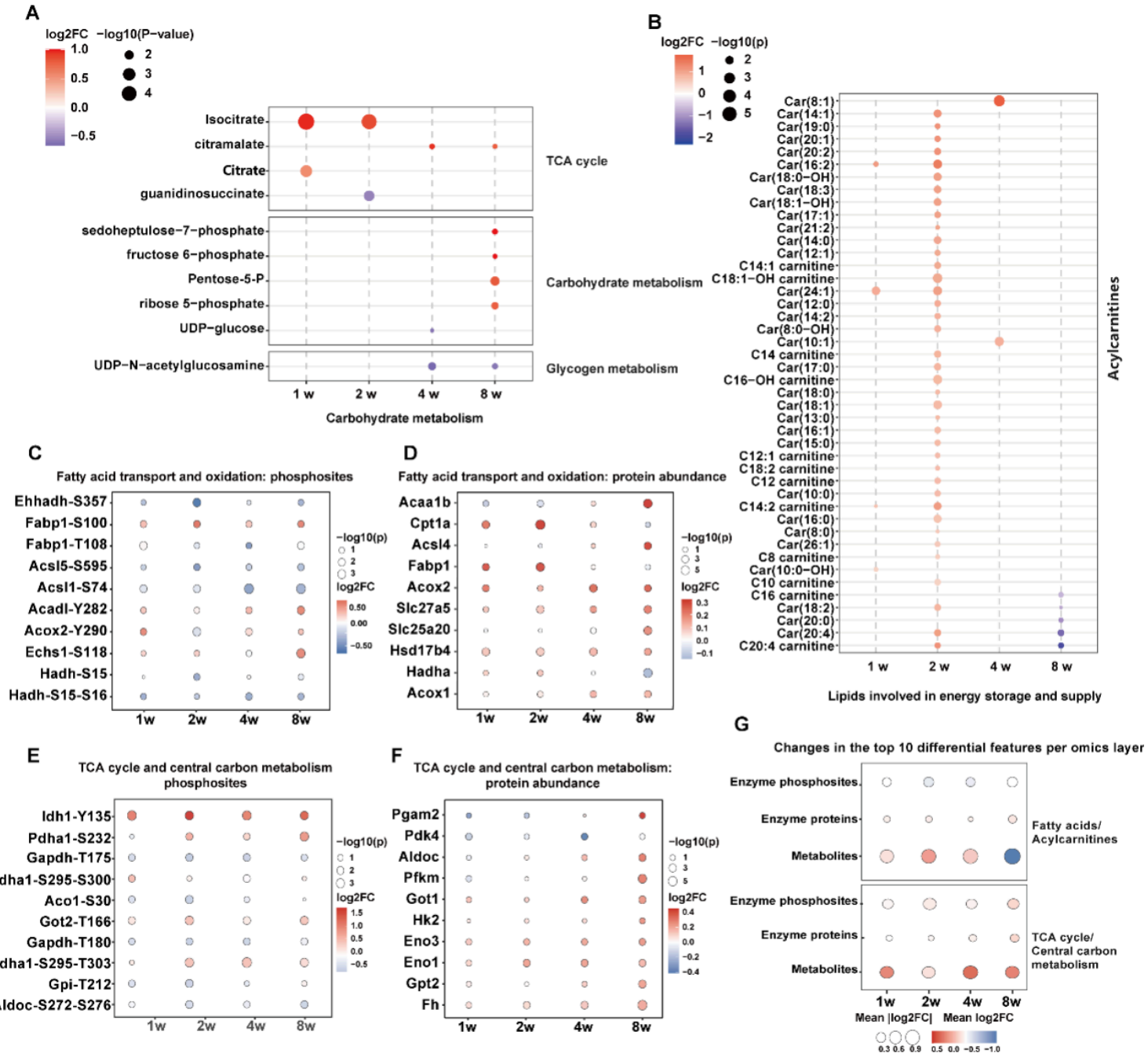


Figure 4

Coordinated changes in proteins, phosphorylation sites, and metabolites within the fatty acid/acylcarnitine and TCA/central carbon metabolic axes during endurance training (A) Bubble plot showing alterations of metabolites associated with the TCA cycle/central carbon metabolism axis. (B) Bubble plot showing alterations of metabolites associated with the fatty acid/acylcarnitine axis. (C) Bubble plot showing phosphorylation site alterations of enzymes involved in fatty acid transport and oxidation. (D) Bubble plot showing protein abundance changes of enzymes involved in fatty acid transport and oxidation. (E) Bubble plot showing phosphorylation site alterations of enzymes involved in the TCA cycle/central carbon metabolism axis. (F) Bubble plot showing protein abundance changes of enzymes involved in the TCA cycle/central carbon metabolism axis. (G) Integrated bubble plot depicting coordinated changes across the fatty acid/acylcarnitine axis and TCA/central carbon metabolism axis. Dot color represents the mean $\log_2$FC, and dot size represents the mean $|\log_2 FC|$. For bubble plots, the x-axis represents training time points, the y-axis represents metabolite, gene, or phosphosite names, color indicates $\log_2$FC, and bubble size represents $-\log_{10}(P\text{value})$

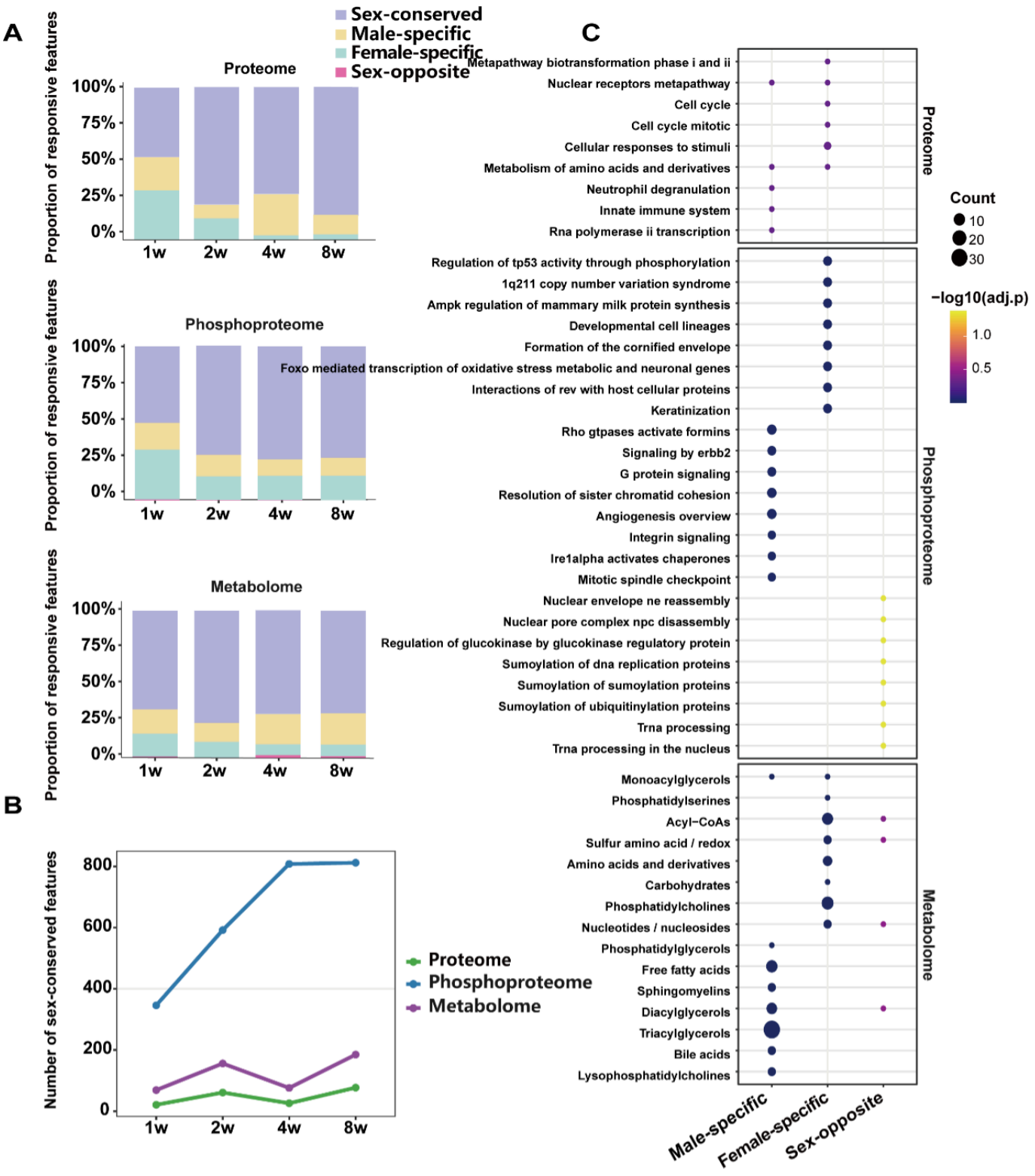


Figure 5

Sex-conserved and sex-biased responses in hepatic multi-omic adaptations to endurance training(A) Proportional distribution of sex-conserved responses, male-specific responses, female-specific responses, and sex-opposite responses across the proteome, phosphoproteome, and metabolome at different training time points. (B) Changes in the number of sex-conserved response molecules across different training stages. (C) Functional enrichment analysis of male-specific, female-specific, and sex-

opposite response molecules. Gene set-based pathway enrichment analysis was performed for proteomic and phosphoproteomic datasets, whereas metabolite category or metabolic pathway enrichment analysis was performed for metabolomic datasets. For bubble plots, the x-axis represents response types, the y-axis represents enriched pathways or functional categories, bubble size indicates enrichment significance, and color represents enrichment intensity

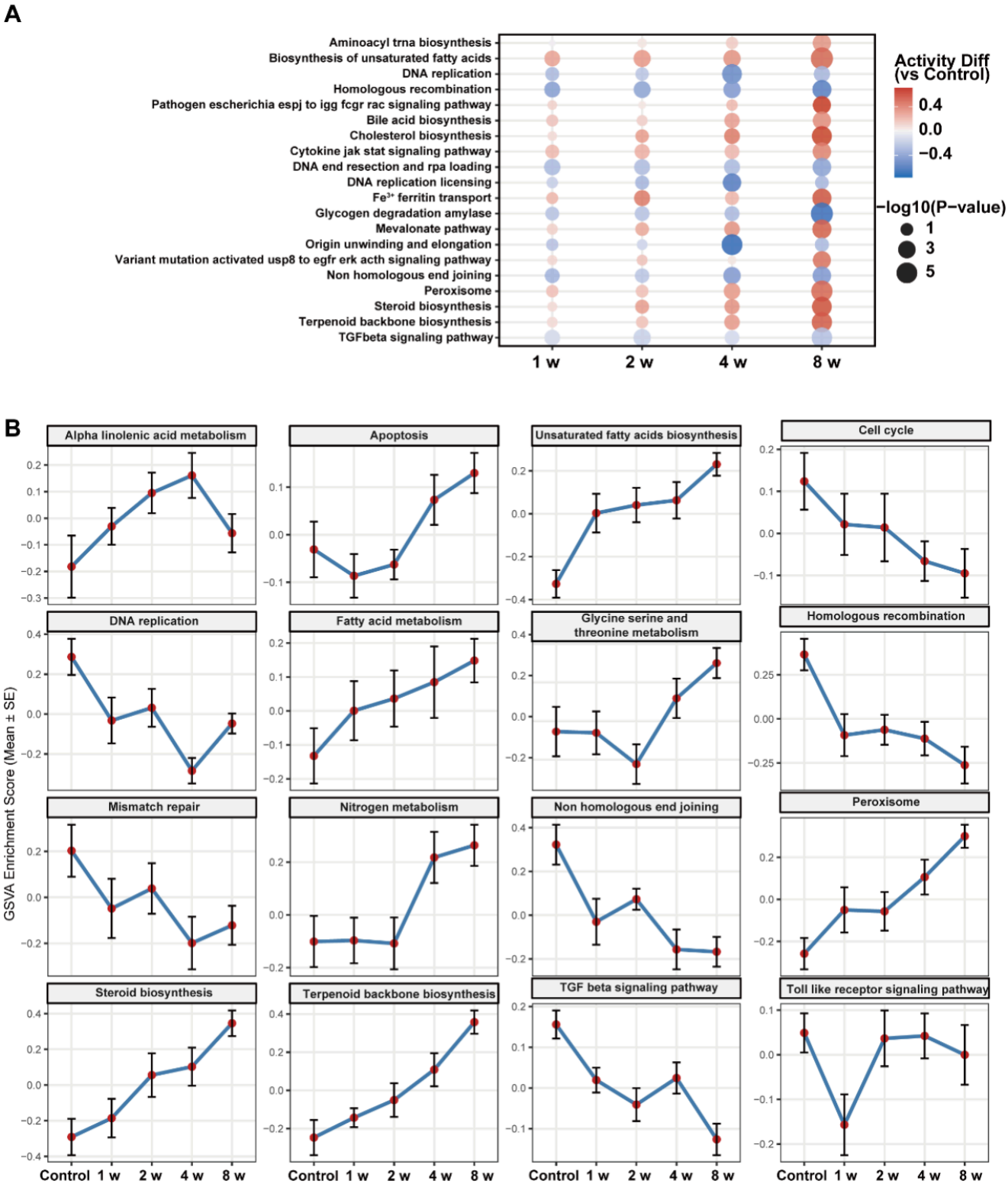


Figure 6

Proteomic functional module alterations and a stage-specific model of hepatic adaptation induced by endurance training (A) Heatmap of GSVA-based proteomic functional module scores showing alterations in fatty acid metabolism, amino acid/nitrogen metabolism, autophagy/proteostasis, peroxisome function, and cholesterol/bile acid-related modules. (B) Temporal trajectories of representative proteomic functional modules during endurance training

Supplementary Files

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