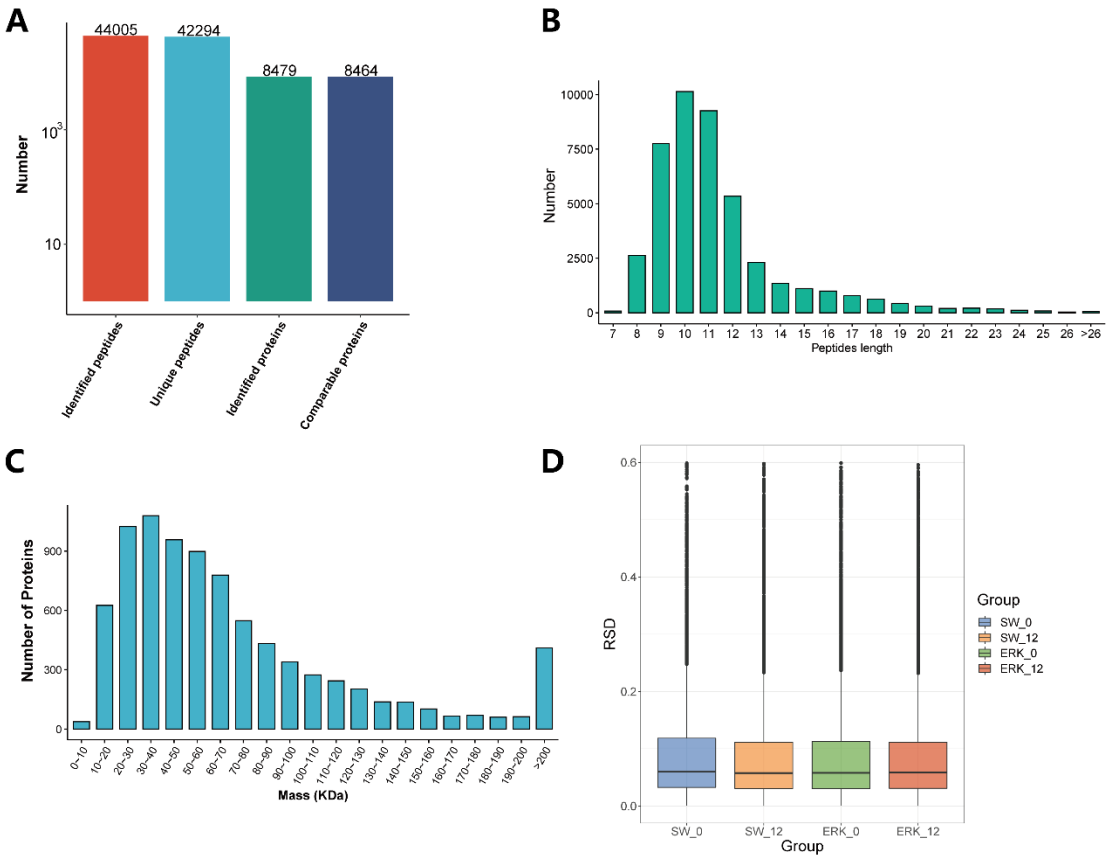

Fig. S1 Overview of the data quality control in proteome. (A) The identified peptides and proteins numbers in proteome. Identified peptides: the total number of peptide sequences detected in mass spectrometry analysis. Unique peptides: the number of distinct peptide sequences uniquely mapped to specific proteins. Identified proteins: the total count of proteins confidently detected. Comparable proteins: the number of proteins quantified by specific peptides. (B) The distribution of peptide counts per protein. Most quantified proteins were supported by more than two peptides. This multi-peptide coverage enhances quantification accuracy and reliability. (C) The molecular weight distribution of identified proteins. The identified proteins exhibited a uniform molecular weight distribution. (D) Relative standard deviation (RSD) of protein quantification values between replicates of each group in proteome. Lower RSD values reflect greater measurement precision.

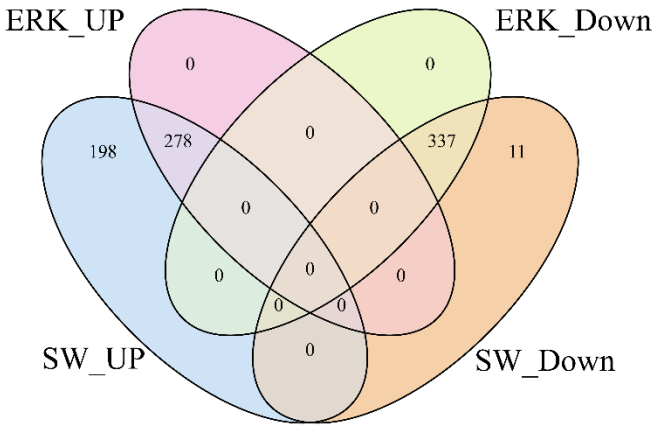

Fig. S2 The Venn diagram of significantly heat-responsive proteins regulated by ERK kinase under heat stress. SW and ERK respectively represent the group injected with sterile seawater and ERK inhibitor. SW_UP: significantly up-regulated DAPs under heat stress. SW_Down: significantly down-regulated DAPs under heat stress. ERK_UP: significantly up-regulated DAPs mediated by ERK kinase. ERK_Down: significantly down-regulated DAPs mediated by ERK kinase.

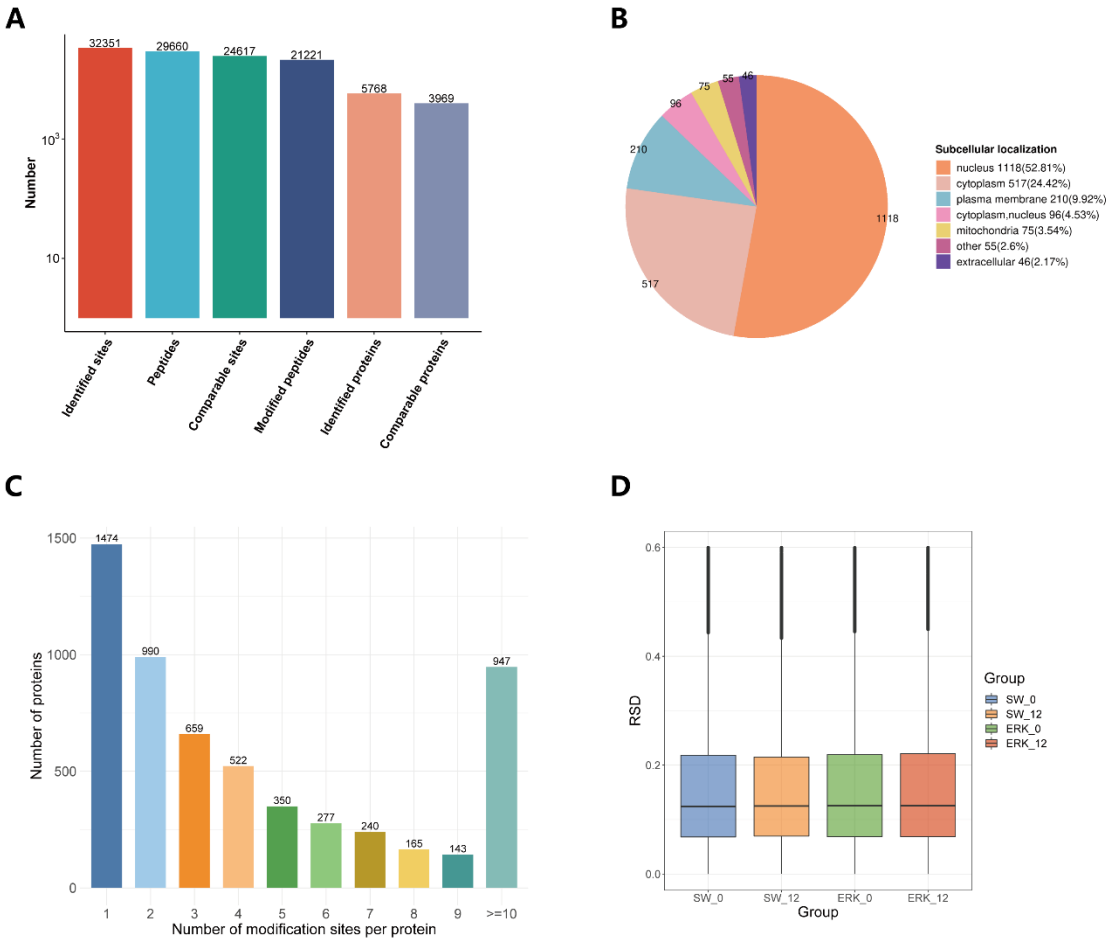

Fig. S3 Overview of the data quality control in phosphoproteome. (A) Identified peptides and proteins numbers in phosphoproteome. Identified sites: the number of phosphorylated modification sites detected by spectrum search analysis. Peptides: total number of identified peptide sequences detected in mass spectrometry analysis. Comparable sites: the number of quantitative modification sites. Modified peptides: the number of peptide sequences carrying specific PTMs. Identified proteins: the total count of proteins confidently detected. Comparable proteins: the number of proteins quantified by specific peptides. (B) Subcellular locations of differentially phosphorylated proteins. (C) The distribution of the number of protein modification sites. (D) Relative standard deviation (RSD) of protein quantification values between replicates of each group in phosphoproteome.

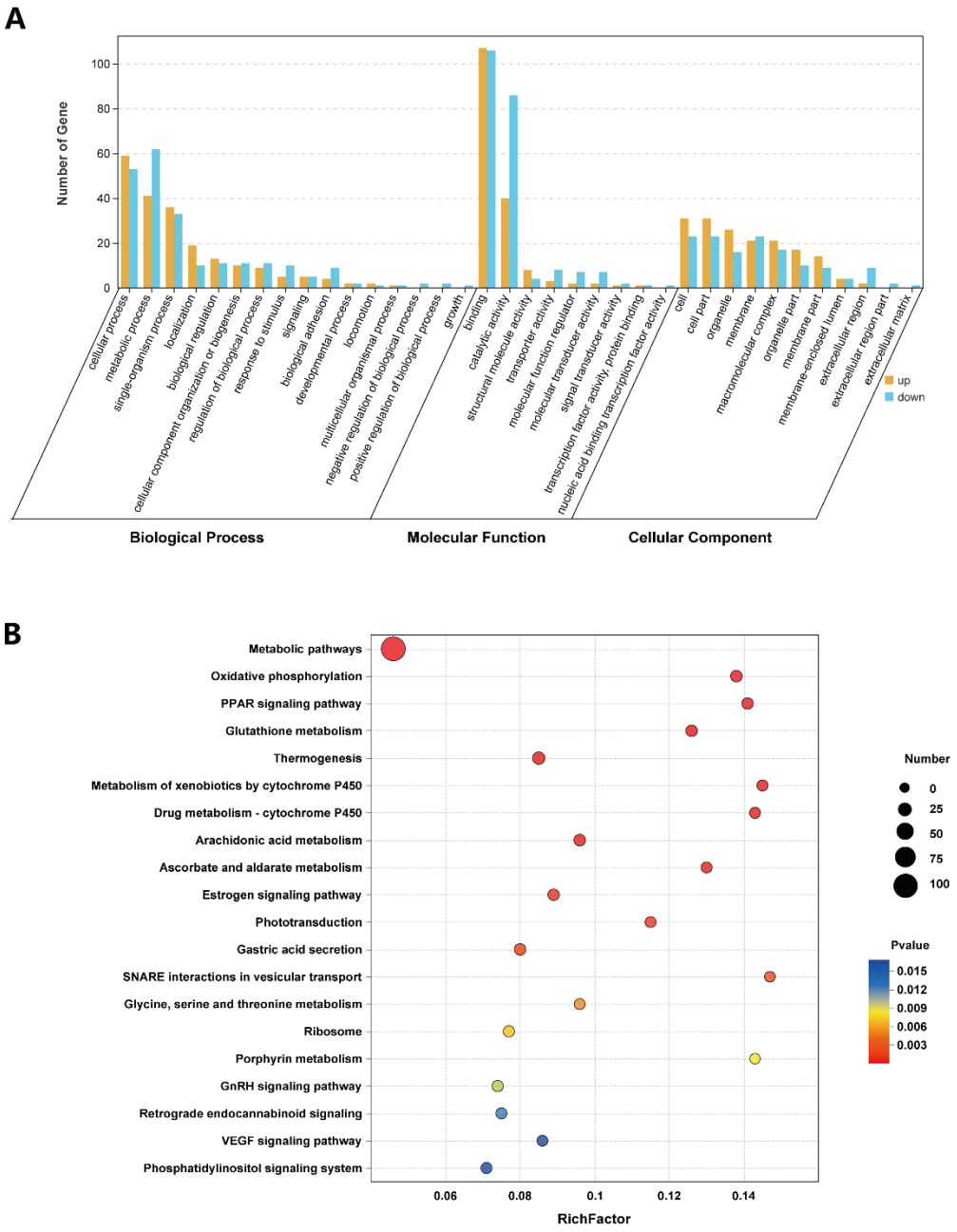

Fig. S4 Functional enrichment of significantly DAPs regulated by ERK kinases in oysters exposed to heat stress. (A) GO functional enrichment analysis of significantly DAPs regulated by ERK kinase under heat stress. (B) The Bubble plot of top 20 significant pathways by KEGG enrichment of significantly DAPs regulated by ERK kinase under heat stress.

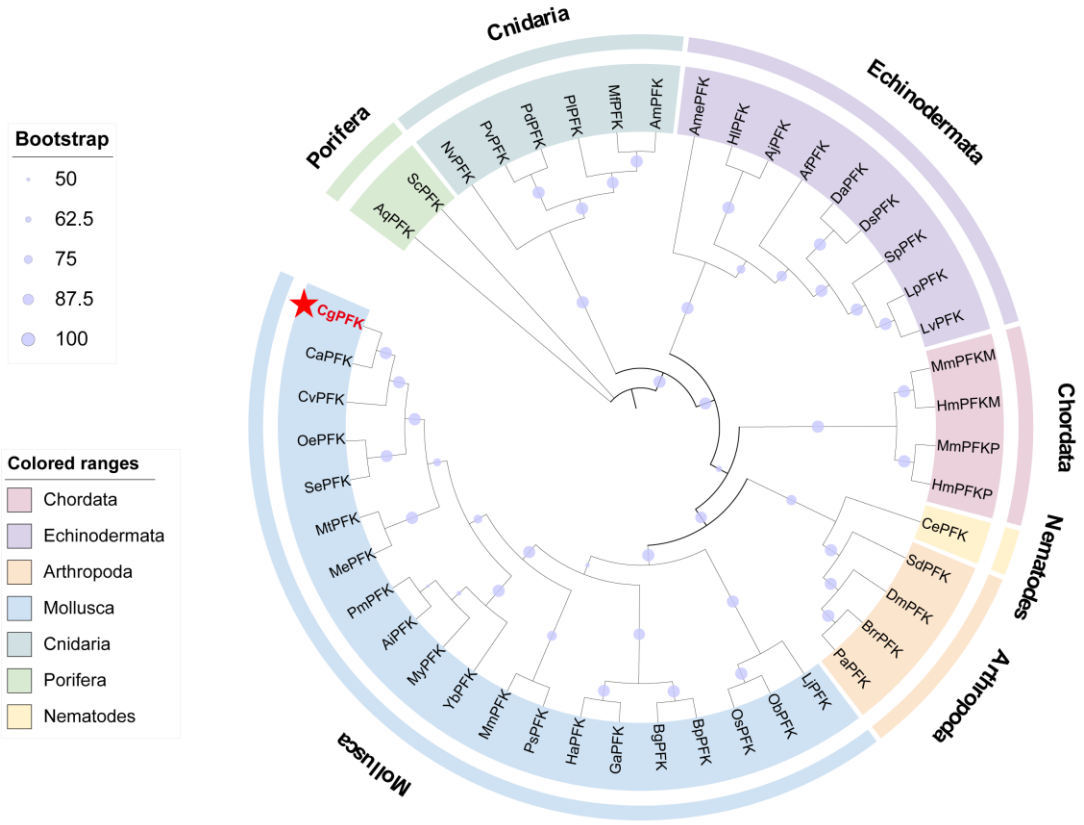

Fig. S5 Phylogenetic analysis of PFK amino acid sequences. The maximum likelihood (ML) tree was constructed using IQ-TREE, with bootstrap support values scaled by node sizes. The *Cg*PFK is marked with red bold font, and different colored ranges indicate different categories of PFK. The accession numbers and full names of representative species were listed in Table S5.