

Proteome analysis of lysine 2-hydroxyisobutyrylation in the peripheral blood of systemic lupus erythematosus patients

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Research

Keywords: SLE, 2-hydroxyisobutyrylation, Mass spectrometry, Bioinformatics

Posted Date: August 31st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-18679/v2>

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Abstract

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that affects multiple organs, the pathogenic mechanism is related to many factors, but the specific pathogenic mechanism has not been clarified yet. Protein lysine modifications play important roles in gene regulation, transcription, metabolism and other biological processes. The lysine 2-hydroxyisobutyrylation (K_{hib}) histone mark has recently been discovered as a novel protein modification. In this study, patients in the active SLE group were examined (N=8), while the control group was healthy (N=20). Utilizing antibody-based affinity enrichment and nano-HPLC/MS/MS analyses of K_{hib} peptides, we identified 156 upregulated proteins (fold change >1.5), 124 downregulated proteins (fold change <1/1.5), including 220 K_{hib} sites that were upregulated and 187 K_{hib} sites that were downregulated. Our data demonstrate that proteins with K_{hib} sites were localized in the cytoplasm. Functional enrichment analysis revealed that proteins with K_{hib} sites are broadly involved in a wide range of biological processes, cellular components and molecular functions. The 03010 Ribosome pathways may exert important influence on the SLE pathogenic mechanism, according to a KEGG analysis. The functional analysis of K_{hib} is of value for important future investigations of SLE pathogenesis.

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease caused by many factors, such as heredity, medications, and the environment, and clinical manifestations of the disease involve many organs and the adaptive immune system [1]. Posttranslational modifications (PTMs) promote the formation of covalent bonds between chemical groups/peptides and target proteins and constitute a mechanism that ultimately results in changes in protein structure and function [1]. Lysine 2-hydroxyisobutyrylation (K_{hib}) is a newly identified PTM found in animal and yeast cells. This newly discovered posttranslational modification has been poorly reported in relation to lupus and other diseases such as cancer, cardiovascular disease, diabetes and other immune disorders, and the underlying mechanisms need further elucidation. There have also been studies on prokaryotes and plants. Previous research suggested that histone K_{hib} is involved in male cell differentiation and plays a critical role in the regulation of chromatin functions in animals [3]. Posttranslational lysine 2-hydroxyisobutyrylation of human sperm tail proteins affects motility, that may be one of the causes for asthenozoospermia [4]. The removal of the K_{hib} group from H4K8 is mediated by the histone lysine deacetylases Rpd3p and Hos3p in vivo. In addition, eliminating modifications at this site by alanine substitution alters transcription in carbon transport/metabolism genes and results in a reduced chronological life span (CLS). Furthermore, consistent with the glucose-responsive H4K8hib regulation, proteomic analysis of K_{hib} revealed a large set of proteins involved in glycolysis/gluconeogenesis [5]. Dong reported comprehensive identification of K_{hib} in *Proteus mirabilis*, with K_{hib} sites involved in metabolic pathways, such as the pentose phosphate pathway, and in purine metabolism and glycolysis/gluconeogenesis [6]. The modification of carbon sources can affect the occurrence of K_{hib} . Furthermore, they observed that K_{hib} on K343 had a negative regulatory effect on enolase (ENO) activity,

and K_{hib} may change the binding formation of ENO and its substrate, 2-phospho-D-glycerate (2PG), to prevent the substrate from coming close to the active sites of the enzyme[6]. He's study revealed that EP300-catalyzed K_{hib} and further indicated that EP300 has the intrinsic ability to select short-chain acyl-CoA-dependent protein substrates by regulating cellular glucose metabolism. Meng and coworkers[7] provided the first systematic analysis of K_{hib} modifications in developing rice (*Oryza sativa*) seeds, and functional annotation analyses indicated that proteins modified by K_{hib} are preferentially targeted in glycolysis/gluconeogenesis, TCA cycle metabolism, starch biosynthesis, lipid metabolism, protein biosynthesis and other biological processes. Research has revealed that K_{hib} sites are conserved in the histone proteins H3 and H4 in humans, mice and *Physcomitrella patens* and are novel sites in the histone proteins H1, H2A and H2B in *Physcomitrella patens*[3].

Here, we identified that 156 upregulated proteins(fold change>1.5),124 downregulated proteins(fold change<0.66), including 220 K_{hib} sites that were upregulated and 187 K_{hib} sites that were downregulated. In addition, functional enrichment analysis revealed that proteins modified by K_{hib} were broadly involved in a wide range of biological processes, cellular components and molecular functions. Our research on the K_{hib} modifications of the histones in SLE will facilitate the understanding of the pathogenic mechanism of SLE.

Materials And Methods

With the informed consent of SLE patients and healthy controls, this activity was carried out through the Helsinki Declaration under the guidance of the program approved by the Key Laboratory of Guangxi Metabolic Disease Research Ethics Committee. , Wecollected 3.5 ml peripheral blood (PBMC) samples from 8 SLE patients and the normal control group, which consisted of 20 healthy people; the information regarding these patients is displayed in Table 1. PBMCs were isolated by density gradient centrifugation using Ficoll-Hypaque and sonicated three times on ice using a high-intensity ultrasonic processor (Scientz) in lysis buffer (8 M urea and 1% protease inhibitor cocktail). (Note: For PTM experiments, the inhibitors were also added to the lysis buffer, e.g., 50 mM NAM and 3 μ M TSA for acetylation). The remaining debris was removed by centrifugation at 12,000 \times g and 4 $^{\circ}$ C for 10 min. Finally, the supernatant was collected after centrifugation and the protein concentration was determined by a BCA kit according to the manufacturer's instructions.

Trypsin Digestion

The protein solution was reduced with 5 mM dithiothreitol (56 $^{\circ}$ C for 30 min) and alkylated with 11 mM iodoacetamide (room temperature in darkness, 15 min). The protein sample was then diluted by adding 100 mM TEAB to a urea concentration that was less than 2 M. Finally, trypsin was added at a 1:50 trypsin-to-protein mass ratio for the first digestion, which was completed overnight, and a 1:100 trypsin-to-protein mass ratio for a second digestion, which lasted 4 h.

Antibody-based PTM enrichment:

To enrich the K_{hib} -modified peptides, tryptic peptides dissolved in NETN buffer (100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, and 0.5% NP-40 at pH 8.0) were incubated with prewashed antibody beads at 4 °C overnight with gentle shaking. Then, the beads were washed four times with NETN buffer and twice with double distilled water (ddH₂O). The bound peptides were eluted from the beads with 0.1% trifluoroacetic acid. Finally, the eluted fractions were combined and vacuum dried. For the LC-MS/MS analysis, the resulting peptides were desalted with C18 Zip Tips (Millipore) according to the manufacturer's instructions.

LC-MS/MS Analysis

Tryptic peptide were dissolved in 0.1% formic acid and analyzed in a reversed-phase column. They were run through an Easy-NLC 1000 system with a constant flow rate set at 400 nL/min. The mobile phase A aqueous solution was prepared with 0.1% formic acid and 2% acetonitrile. The mobile phase B aqueous solution was prepared with 0.1% formic acid and 90% acetonitrile aqueous solution. The following liquid gradient setting was used: 8%-22% B phase, 0-38 min; 32%-35% B phase, 8-52 min; 35%-80% B phase, 52-56 min; 80% B phase, 56-60 min. The flow rate was maintained at 800 nL/min.

The peptides were isolated by an ultra-high-performance liquid phase system and subsequently ionized in an NSI source at an ion source voltage of 2.0 kV. The ionized peptides were analyzed by Q Exactive TM Plus mass spectrometry (MS/MS). The high-resolution Orbitrap was used to detect and analyze the peptide parent ions and their secondary ion fragments. The peptide ions were first scanned by first-order mass spectrometry at a scanning range of 350-1800 m/z and a resolution of 70,000. The secondary mass spectrometry scanning was performed at a scanning range of 100 m/z and a resolution of 17,500. Data were collected using a data-dependent scan (DDA) program. The mass spectrometer had a set automatic gain control (AGC) parameter of 5e4 with a signal threshold of 5,000 ions/s, a maximum injection time of 200 ms and a dynamic exclusion time of 30 s for the tandem mass spectrum scanning. The aim was to improve the efficiency of the mass spectrometry and avoid repeated scanning of the parent ions.

Database Search

The secondary mass spectrometry data were determined by Maxquant (v1.5.2.8). The SwissProt Human database (20,130 sequences) was used. Considering the problem of the false positive rate (FDR), an inverse library was added during retrieval. In addition, to eliminate the influence of contaminant proteins affecting the identification results, a common contamination database was added to the analysis. Trypsin/P was set as the enzyme digestion mode. The number of missing cut positions was set to 4. The minimum length of each peptide should be restricted to 7 amino acid residues. The maximum number of modifications for a peptide was set at 5. The tolerance of the quality error for the primary parent ion of the first search was set to 20 PPM, that of the primary parent ion for the main search was set to 5 PPM and that of the secondary fragment ion was set to 0.02 Da. Carbamidomethyl on Cysteine was specified as a fixed modification, and the K_{hib} modification and oxidation on Met were specified as variable

modifications. The FDR was adjusted to $< 1\%$, and the minimum score for the modified peptides was set to > 40 .

Bioinformatics Methods

- 1) We used WoLFPSORT, subcellular localization prediction software, to predict the subcellular localization. WoLFPSORT is an updated version of PSORT/PSORT II that is used for the prediction of eukaryotic sequences.
- 2) The Gene Ontology (GO) annotation proteome was derived from the UniProt-GOA database ([www.http://www.ebi.ac.uk/GOA/](http://www.ebi.ac.uk/GOA/)). Through the UniProt-GOA database ([www.http://www.ebi.ac.uk/GOA/](http://www.ebi.ac.uk/GOA/)), the proteomics level is reported as GO analysis comments. First, the system converts the protein ID into the UniProt ID that is detected by UniProt-GOA, and then, the UniProt ID is used to match the GO ID to be analyzed, and finally, the annotation information of the corresponding protein is retrieved in the database according to the GO ID. InterProScan can be used to predict the GO function of a protein that is not retrieved from the UniProt-GOA database. The retrieved protein information was classified into three categories: cell composition, molecular function and physiological process. For each category, two-tailed Fisher's exact test was employed to test the enrichment of the differentially modified proteins against all the identified proteins. GO data with corrected p-values < 0.05 are considered significant.
- 3) The KEGG pathway annotation tools were used. First, KEGG online KAAS service tools were used to annotate the KEGG database description. Then, the annotation results from the KEGG pathway database were mapped using the KEGG online service tool, KEGG mapper. The KEGG database was used to identify enriched pathways by a two-tailed Fisher's exact test, which is used to determine the enrichment of the differentially modified proteins against all the identified proteins. The pathway with a corrected p-value < 0.05 was considered significant. These pathways were classified into hierarchical categories according to the KEGG website.
- 4) The functional descriptions of the identified protein domains were annotated by InterProScan (a sequence analysis application) based on the protein sequence alignment method, and the InterPro domain database was used. InterPro (<http://www.ebi.ac.uk/interpro/>) is a database that integrates diverse information about protein families, domains and functional sites, and it is made freely available to the public via web-based interfaces and services. The diagnostic models, known as signatures, are central to the database and can be used to search protein sequences to determine their potential function. InterPro has utility in the large-scale analysis of whole genomes and meta-genomes, as well as in characterization of individual protein sequences. For each category of proteins, InterPro database (a resource that provides functional analysis of protein sequences by classifying them into families and predicting the presence of domains and important sites) was searched, and a two-tailed Fisher's exact test was employed to determine the enrichment of the differentially modified proteins against all identified proteins. Protein domains with corrected p-values < 0.05 were considered significant.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[8] partner repository with the dataset identifier PXD015351.

Results

K_{hib} protein detection and identification

Based on the criteria, P value < 0.05 , we identified that 156 upregulated proteins (fold change > 1.5), 124 downregulated proteins (fold change < 0.66), including 220 K_{hib} sites that were upregulated and 187 K_{hib} sites that were downregulated. The proteins with higher fold change and multiple upregulated modifications were hemoglobin subunit gamma-2 (P69892), 40S ribosomal protein S9 (P46781), and 60S ribosomal protein L6 (Q02878). The proteins with higher fold change and multiple downregulated modifications were GRIP1-associated protein 1 (Q4V328), heterogeneous nuclear ribonucleoprotein A/B (Q99729), and heterogeneous nuclear ribonucleoproteins A2/B1 (P22626), the differentially expressed protein summary in table 2. The number of modification sites per protein in the Figure 1a, The proteins with > 7 K_{hib} modification sites were Spectrin alpha chain, nonerythrocytic 1 (Q13183), Talin-1 (Q9Y490), Spectrin beta chain, erythrocytic (P11277) Figure. The 897 2-hydroxyisobutylated proteins, most contain 1-2 modification sites, some have 3-7 modification sites, and a few contain 8 or more modification sites. Most peptides vary in length from 8 to 20 amino acids, consistent with trypsin digestion (Figure 1b). To investigate the presence of K_{hib} in SLE, Western blotting with K_{hib} antibody was carried out in the total protein, we can see a large number of protein bands, then a wide protein mass range was observed, revealing that K_{hib} is highly abundant with the protein (Figure 2a). To identify K_{hib} sites in SLE, we randomly selected H3K79_{hib} site and H3K14_{hib} loci for Western blotting analysis, and detected the expression of H3K79_{hib} site and H3K14_{hib} antibodies in SLE with H3 as a reference (Figure 2b).

Subcellular localization information and GO classifications of proteins with K_{hib}

According to the subcellular localization analysis, the largest percentage of proteins with K_{hib} was in the cytoplasm, and proteins with higher fold change modifications were localized in the cytoplasm. For the upregulated proteins with K_{hib} shown in Figure 3(a), 49% were located in the cytoplasm, 19% in the nucleus, and 10% in the mitochondria. Among the downregulated proteins in Figure 3(b), 42% were located in the cytosol, 30% in the nucleus, and 14% in the extracellular fraction. The GO classification indicated that the identified proteins with K_{hib} fell into three categorizations (the cellular component, molecular function, and biological process). Of the upregulated proteins in the cellular component category, as shown in Figure 4(a), 20% were in the cell, 19% were in the organelle, 15% were in the extracellular region, and 13% were in the membrane. Among the downregulated proteins shown in Figure 4(b), 20% were in the cell, 20% were in the organelles; 14% were in the membrane and 14% were in the membrane-enclosed lumen. Based on their molecular function, of the upregulated proteins shown Figure 4(c) and the downregulated proteins shown Figure 3(d), 51% and 55%, respectively, were in the largest category: binding. Based on their biological process, of the upregulated proteins shown in Figure 4(e),

13% were involved in cellular processes, 12% were involved in biological regulation, and 11% were involved in single-organism processes. The percentages of the downregulated proteins were involved in the following biological processes: cellular processes, 14%; biological regulation, 12%; metabolic processes, 11%; and single-organism processes, 11% Figure 4(f).

Functional enrichment of proteins with K_{hib}

Functional enrichment analysis revealed that the proteins with K_{hib} were broadly involved in biological processes. Figure 4 shows the functional enrichment description based on the GO analysis (cellular component, molecular function, biological process), the Fisher exact test p-value was determined after the logarithmic transformation. The longer the bar in the graph, the more significant the enrichment of differentially expressed proteins in this classification or function. Based on the upregulated proteins with the red bar of Figure 5(a), the proteins were enriched for the adherens junctions, anchoring junctions, and cell-substrate junctions in the cellular components category. The proteins were enriched for the domain-specific binding, structural constituent of ribosome, and structural molecule activity in the molecular function categories. As determined by the biological process categories, the proteins localized at the endoplasmic reticulum, for actin cytoskeleton reorganization, and for the regulation of the viral life cycle were enriched. Based on the downregulated proteins, signified by the blue bar in Figure 5(b), for the cellular component category, the proteins were enriched in the cytosolic ribosomes, ribosomal subunits, and the cytosol; for the molecular function category, these proteins were enriched in the structural constituents of ribosomes for structural molecule activity and DNA binding; and for the biological process category, these proteins were enriched for ribosomal large subunit biogenesis, viral gene expression, and protein localization to the endoplasmic reticulum.

KEGG organically combines genomic information and gene function information, and is a powerful tool for metabolic analysis and metabolic network research in vivo. By analyzing metabolic pathways and interaction networks of disease-related genes, it is helpful to understand the molecular pathological basis of diseases. Of the 9 different signaling pathways revealed by the KEGG pathway enrichment analysis, the hsa03010 Ribosome pathway was the most relevant, as shown in Figure 6. The multiple modifications with an fold change > 2.0 for the proteins with the upregulated proteins of the hsa03010 Ribosome pathway analysis were 40S ribosomal protein S9(P46781), 60S ribosomal protein L6(Q02878), 60S ribosomal protein L26(P61254), 40S ribosomal protein SA(P08865), 40S ribosomal protein S16(P62249), 40S ribosomal protein S3a(P61247). The 40S ribosomal protein S9(P46781) K_{hib} modification sites locus was predicted by STRING (Figure 7), the 40S ribosomal protein S9(P46781) had 6 K_{hib} modification sites with K121, K139, K93, K52, K91 and K180 .

The protein domain functional descriptions identified for K_{hib} were annotated by InterProScan (Figure 8). Upregulated proteins with K_{hib} were significantly enriched for the Calponin homology domain, the Nucleotide-binding alpha-beta plait domain and FERM, and the N-terminal. The downregulated proteins with K_{hib} were enriched for Globin-like, Globin/Protoglobin, Globin domains and so on.

Discussion

Since the discovery of phosphorylation, many new PTMs have been identified over the years, such as crotonylation, propionylation, malonylation, butyrylation, succinylation, glutarylation, β -hydroxybutyrylation and 2-hydroxyisobutyrylation[9]. PTMs have been found in different pathologic conditions in SLE[10, 11]. Lu's studies showed that FOXO3a was a downregulated molecule in SLE patients, while FOXO3 was upregulated by glucocorticoids (GCs), which relied on the suppression of p13K/AKT-mediated FOXO3a phosphorylation. In one of their other studies, FOXO3a plays an important role in the GC-mediated inhibition of NF- κ B activity, which might involve FOXO3a interaction with NF- κ B p65 protein[11]. The study reported that B-cell epitopes, named 2-1, an altered isoform, showed lower levels of phosphorylation in SLE patients, which may be related to the pathogenesis of SLE; it also demonstrated that higher concentrations of phosphoproteins are found in the cell membrane and nucleus and that 50 nucleic acid metabolic pathways are modified during the pathogenesis of SLE, specifically the MAPK signaling pathway, which leads to abnormal intracellular signaling[12]. Zieve[13] studied the methylation of Smd1 protein (sDMA) in SLE patients. H2B and H4 of nucleosomes were shown to undergo apoptosis-induced acetylation in mice with lupus and patients with SLE[14]. K_{hib} is one of the newly discovered PTM types that influences protein properties, including biological processes, such as cellular localization, protein interaction, structure stability, and enzymatic activity. Dai identified K_{hib} on histones in HeLa cells and mouse embryonic fibroblast cells, which were the first to be reported in the world, and the research shows that histone Kac and Kcr is involved during male germ cell differentiation in distinct genomic distributions[15]

Currently, there are no reports of K_{hib} PTMs in SLE. In our study, The significantly differentially expressed proteins were screened out and their functional characteristics were analyzed by comparing SLE with the normal control group. Step 1: Annotate the functions of all identified proteins at different levels. Step 2: Analyze the proportion of differentially expressed proteins in different functions; Step 3: Enrichment analysis was used to find the function of significant enrichment with differential expression. Step 4: The functional similarities and differences of the differentially expressed proteins obtained under different experimental treatment conditions were analyzed by functional rich cluster analysis. We identified 156 upregulated proteins (fold change > 1.5), 124 downregulated proteins (fold change < 0.66), which included 220 K_{hib} sites that were upregulated and 187 K_{hib} sites that were downregulated. Hemoglobin subunit gamma-2 (P69892) is an upregulated protein with higher fold and multiple change modifications that has 3 K_{hib} sites and a subcellular localization in the cytoplasm. The 40S ribosomal protein S9 (P46781) has 3 K_{hib} sites and subcellular localization in the cytoplasm.

Ribosomes are remarkable ribonucleoprotein complexes that are responsible for protein synthesis in all forms of life. Ribosomal protein (RP) is the main component of ribosomes, with ribosomal RNA forming ribosomes, roots. According to the derivation, the size subunits were named Ribosomal Protein Large (RPL) and Ribosomal Protein Small (RPS), respectively. Recent studies have shown that RP is abnormally expressed in a variety of tumors, and it has become a hotspot in recent years because of its influence on

the apoptosis, aging, growth, invasion, drug and radiotherapy resistance of tumor cells through various mechanisms.[16]. In our study, it is the differential proteins such as ribosomal proteins 40S ribosomal S9, 60S Ribosomal L6, 60S Ribosomal L26, 40S Ribosomal SA, 40S Ribosomal S16, and has 03010 ribosome signaling pathway that have been screened out. Ribosomal proteins act as immune regulatory targets to regulate the body's immune response and cells. For example, ITGA6 and RPSA synergistically promote pancreatic cancer invasion and metastasis via PI3K and MAPK signaling pathways[17]. Ribosomal proteins can be modified by phosphorylation to alter their activity and mode of action. Akt inhibits apoptosis by phosphorylating RPS3 and leading to the dissociation of RPS3-E2F1[18]. RPS6 is closely related to protein synthesis, cell proliferation, cell volume, and glucose homeostasis, and phosphorylated RPS6 promotes cell senescence[19]. In lung cancer, RPS6 is activated by phosphorylation, and the migration ability of human lung cancer H1650 and Skms-1 cells with RPS6 knocked out is significantly decreased, while the dephosphorylation of RPS6 can reduce the migration ability of esophageal cancer cells[20]. We believe that the screened ribosomal proteins and hsa03010 ribosomes play the important roles in immune regulation, inflammation regulation, complement activation and other aspects, and are closely related to the pathogenesis of SLE, so potential links need to be explored. The 40S ribosomal protein S9(P46781), as an both upregulated and downregulated protein, which regulates ribosomal function and the protein production mechanism in the hsa03010 ribosome pathway. Our results showed that proteins with K_{hib} were enriched in the hsa03010 ribosome, and research on venous thromboembolism (VTE) showed that module genes were mainly enriched in the pathways of the hsa03010 ribosome[21]. It has been reported that the number or activity of ribosomes could be increased upon exposure to cytokines released at the site of inflammation. The immune response of the human body is related to cell necrosis. Cell apoptosis and necrosis can induce an immune response. Therefore, appropriate treatment of inflammation is conducive to the occurrence and development of autoimmune diseases. SLE is an autoimmune multisystem injury disease in which thrombosis is a likely symptom, and its mechanism in the endothelium is caused by the activation of a variety of immune inflammatory factors where there is cellular damage and vasculitis. Inflammation is an important factor in SLE pathogenesis. Vasculitis prevalence in patients with SLE is reported to be between 11% and 36%[23]. The presence of antibodies against vascular inflammation, which causes cell destruction, has been documented in SLE[24, 25]. SLE patients with inflammatory cytokines IL-1, IL-6 IL-8 and IL-18 and a high concentration of TNF- α and its related disease activity, which causes blood coagulation in patients, except in the fibrinolytic system, form thrombosis that is enhanced by the immunity-induced inflammation[26]. Interferon (IFN)- α and type III IFNs (IFN- λ) have also recently been associated with SLE. The levels of circulating IFN- λ 1 and IFN- α define subsets of patients with SLE with different characteristics[27]. The increased release of factors in the inflammatory network during the pathogenesis of SLE is an important factor involved in the pathogenesis of SLE immunological thrombosis, which can promote the occurrence and exacerbation of the clinical symptoms of SLE.

Conclusion

In conclusion, bioinformatics analysis based on a proteome analysis of K_{hib} in the peripheral blood of patients with SLE indicated that K_{hib} -modified proteins were distributed in various cellular compartments and were involved in a broad spectrum of processes. In addition, proteins with K_{hib} were enriched in the hsa03010 ribosome, and ribosomal proteins can regulate inflammatory cytokines to affect the pathogenesis of SLE. Our results provide novel insights into the functions of proteins with K_{hib} in SLE. However, the clinical applicability of K_{hib} -modified ribosomal proteins should be verified in the future.

Declarations

Ethics approval and consent to participate

It is verbal agreement. Guangxi Key Laboratory of Metabolic Diseases Research Ethics Committee

Consent for publication

All presentations of case reports must have consent for publication.

Availability of data and material

The datasets generated and/or analysed during the current study are available in the PRIDE repository. PXD015351, Username: reviewer73483@ebi.ac.uk, Password: 2qd6MgH1.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Project Plan Document of Guangxi Key Laboratory Construction (17-259-57).The Science and Technology Plan of Shenzhen (No. JCYJ20170307095606266), The Shenzhen Science and Technology Research Foundation(JCYJ20160422154407256)

Authors' contributions

Conceived and designed the experiments: Xianqing Zhou, Donge Tang, Yong Dai. Performed the experiments: Hua Lin, Ruohan Zhang, Weiguo Sui, Yue Zhang, Jiejing Chen. Analyzed the data: Wen Xue, Qiang Yan, Chunhong Li, Huixuan Xu, Shaoying Huang, Weier Dai. Wrote the paper: Xianqing Zhou. Revised the manuscript: Donge Tang, Yong Dai. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

Endnotes

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Tables

Please see the supplementary files section to view the tables.

Figures

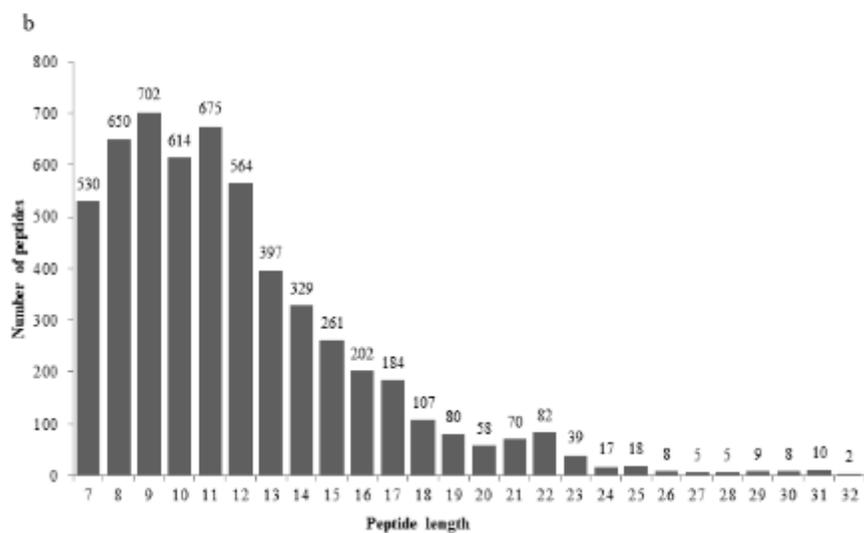
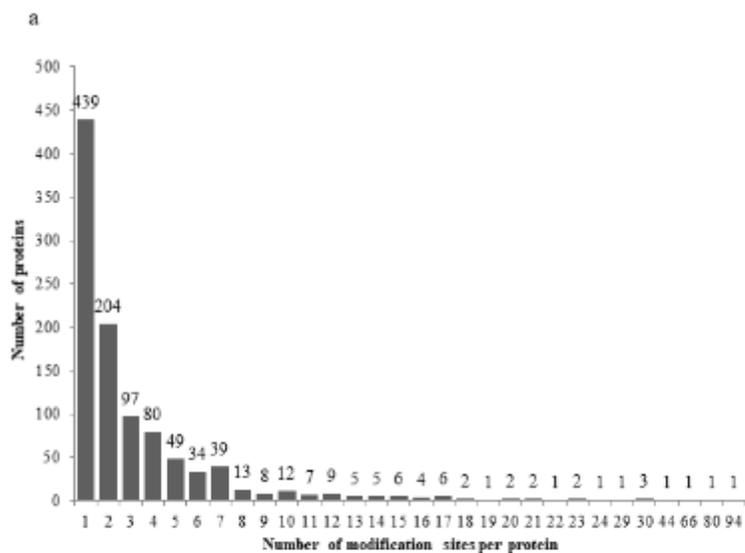


Figure 1

The analysis of protein modification site distribution and peptide length. (a) The number of modification sites per protein. (b) The peptides based on their length of Khib modified proteins.

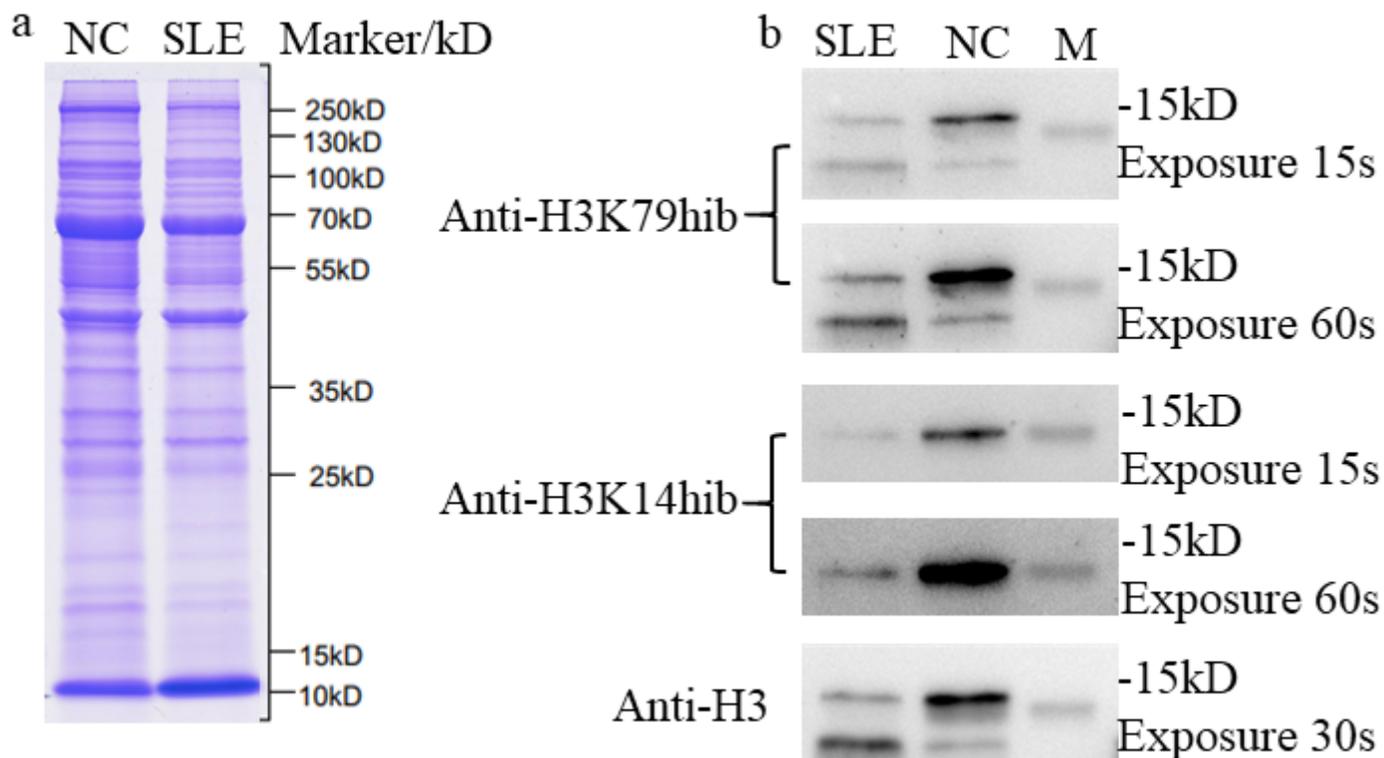
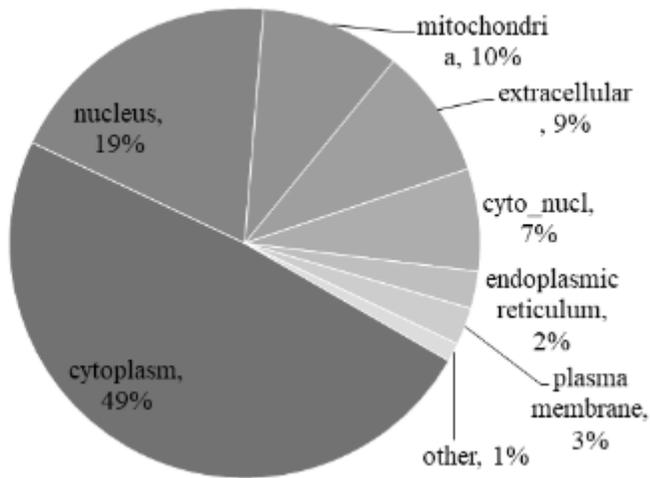


Figure 2

Western blotting analysis of Khib modified proteins and sites in SLE. (a)Coomassie brilliant blue staining to investigate the existence of Khib modified proteins in SLE and NC. (b) Western blotting with anti-H3K79hib,anti-H3K14hib antibody and anti-H3 antibody in SLE and NC.

a



b

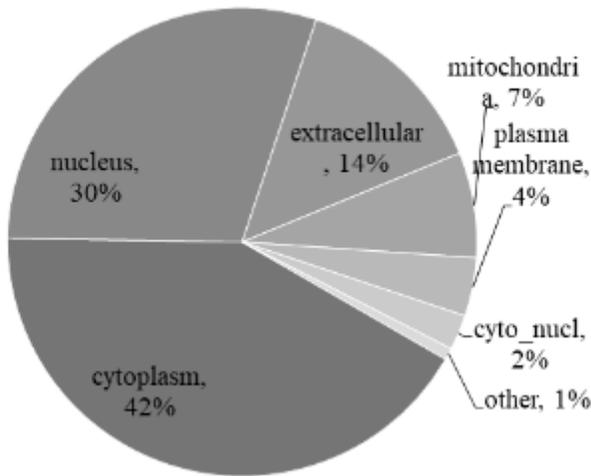


Figure 3

Functional classification of Khib in subcellular. (a) Subcellular localization of upregulated Khib proteins (b) Subcellular localization of downregulated Khib proteins.

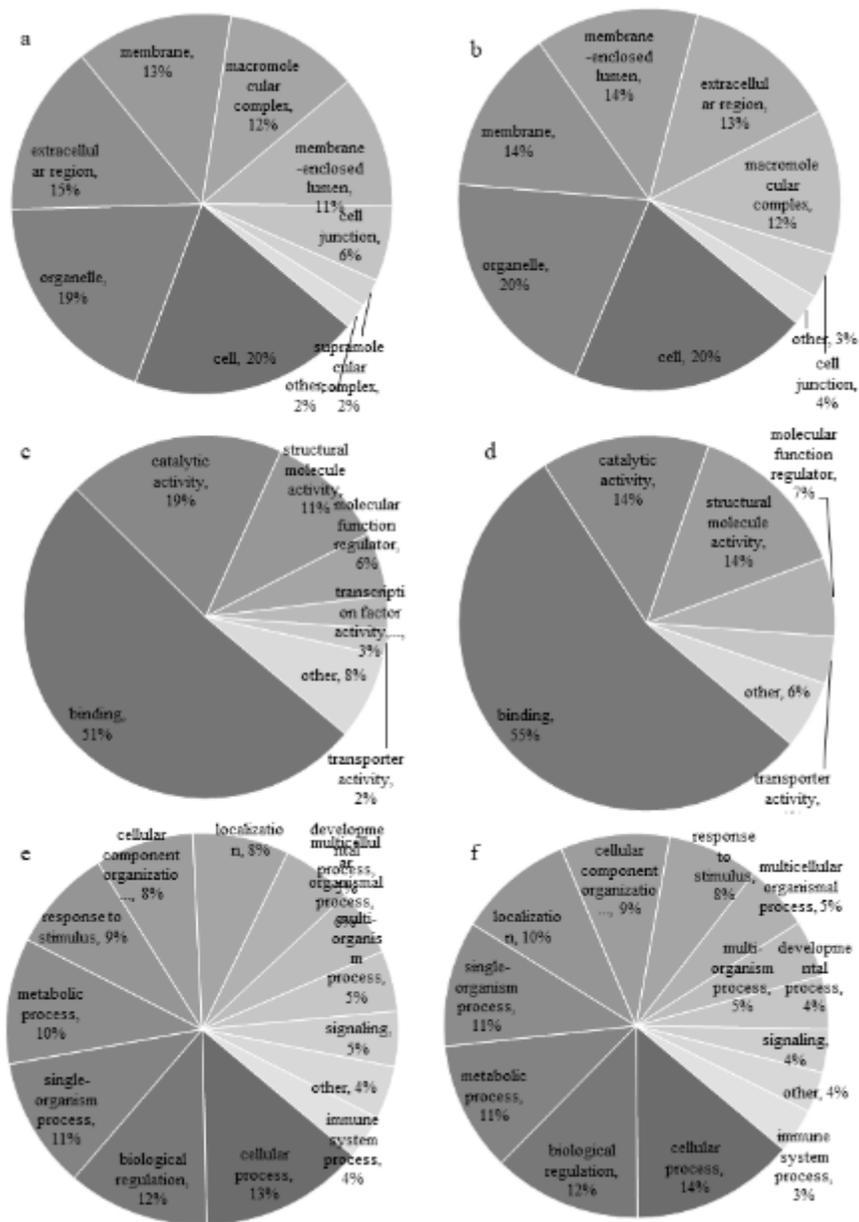


Figure 4

Functional classification of Khib in GO analysis. (a) GO analysis for upregulated Khib proteins in cellular component. (b) GO analysis for downregulated Khib proteins in cellular component. (c) GO analysis for upregulated Khib proteins in molecular function. (d) GO analysis for downregulated Khib proteins in molecular function. (e) GO analysis for upregulated Khib proteins in biological processes. (f) GO analysis for downregulated Khib proteins in biological processes.

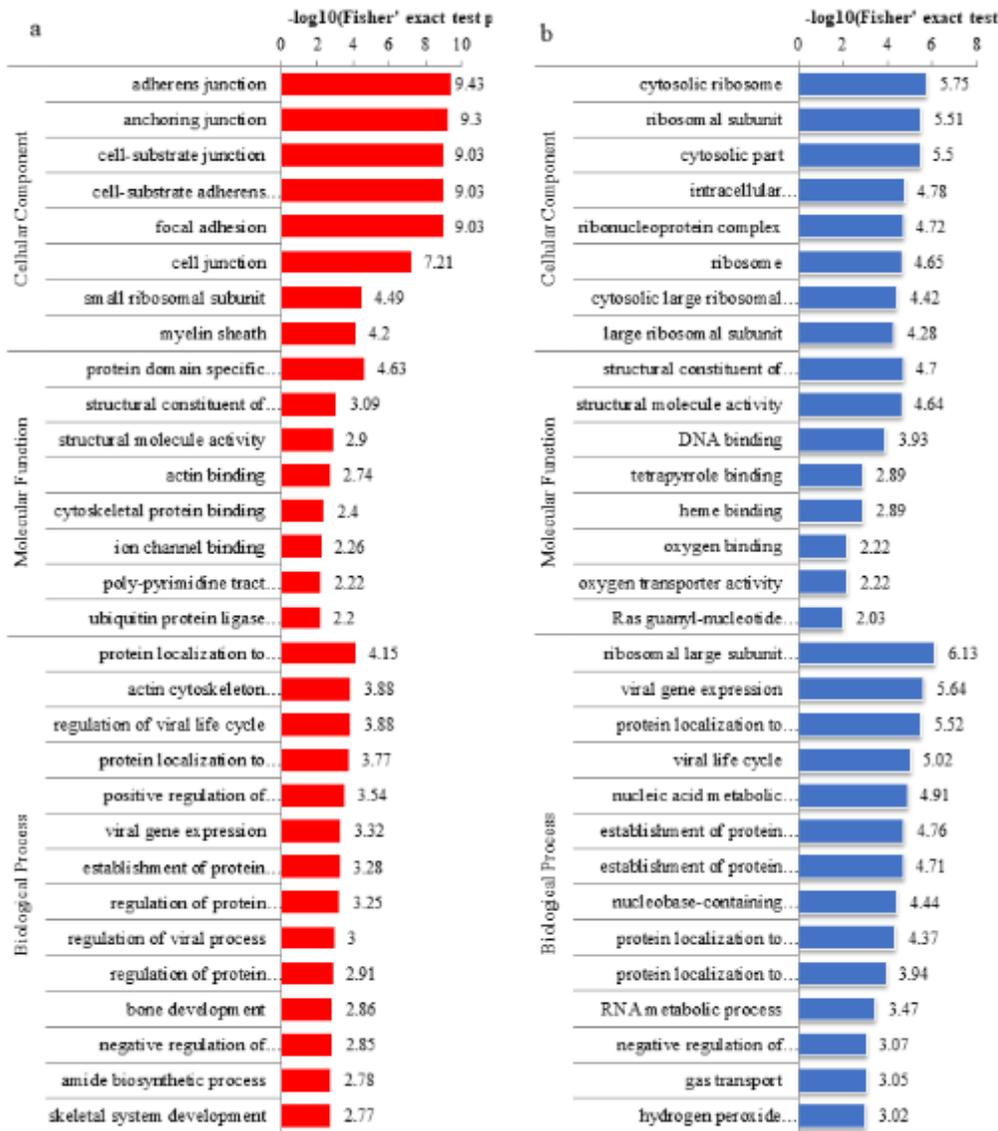


Figure 5

Functional enrichment analysis of Khib in GO. And Upregulated Khib proteins (red bar), downregulated Khib proteins (blue).

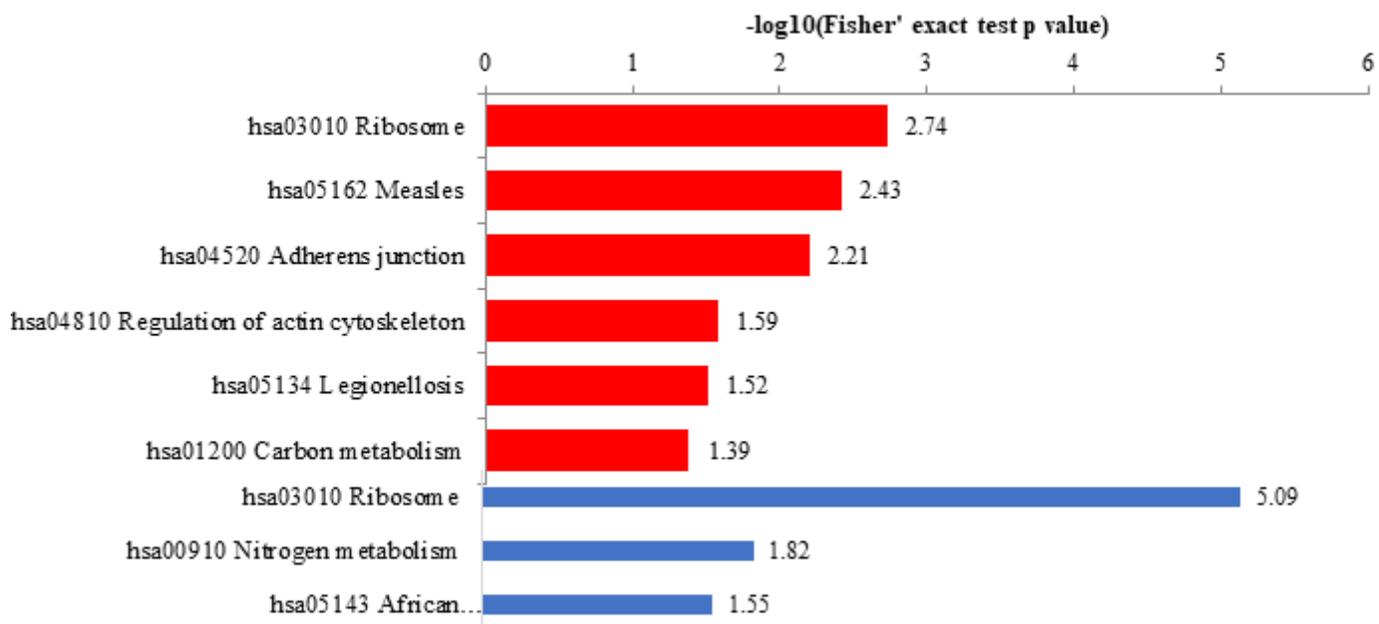


Figure 6

Functional enrichment analysis of Khib in KEGG. And Upregulated Khib proteins (red bar), downregulated Khib proteins(blue).

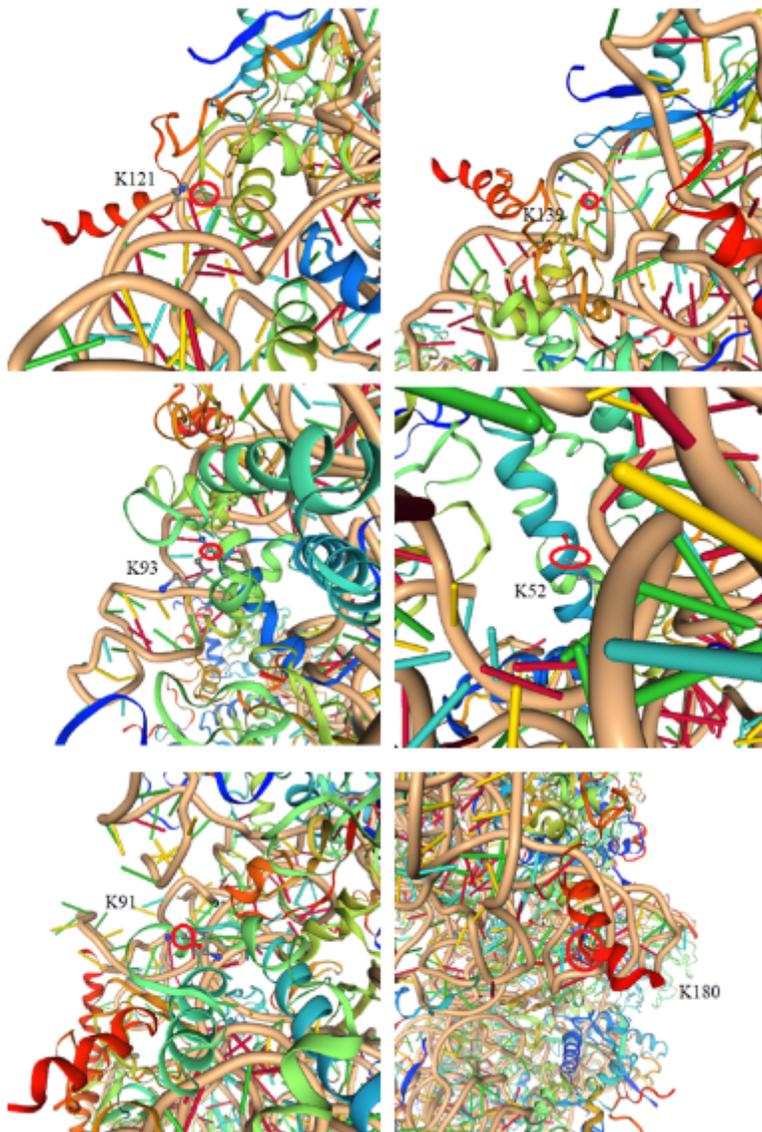


Figure 7

The 40S ribosomal protein S9(P46781) Khib modification sites (K121,K139,K93,K52,K91,K180) .

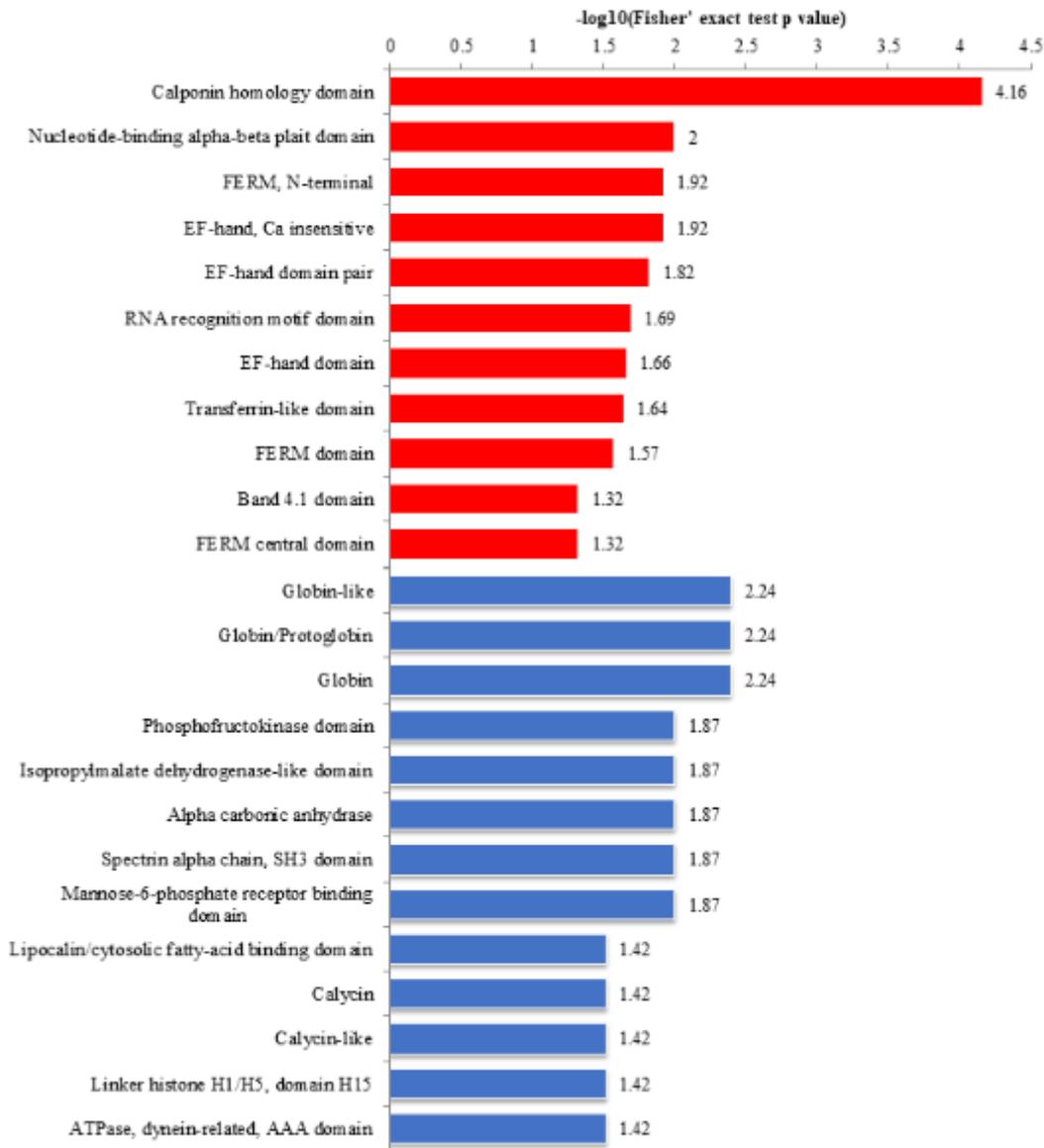


Figure 8

Functional enrichment analysis of Khib in protein domain. And Upregulated Khib proteins (red bar), downregulated Khib proteins (blue).

Supplementary Files

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