

Comparative analysis of global transcriptome, proteome and acetylome in house dust mite-induced murine allergic asthma model

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First published: 06 November 2021

<https://doi-org.bibliosan.clas.cineca.it/10.1002/ctm2.590>

Dear Editor,

Protein lysine-site acetylation (Kac) is of great importance for various cellular processes, which indicates a reversible post-translational modification of proteins. Previous studies have demonstrated that protein acetylation was involved in the pathogenesis of asthma.¹ It has been shown that lysine acetyltransferase (KAT) activity is increased in asthma, while lysine deacetylase (KDAC) activity is reduced.¹ With the development of high-throughput omics techniques, crucial processes and factors that play important roles in asthma have recently been identified. However, systematic understanding of acetylome in asthma is hampered by several major deficiencies.

In order to detect the changes of acetylated protein in allergic asthma, we used a house dust mite (HDM)-treated murine allergic asthma model, and we found that there were differences between the phosphate buffer saline (PBS)- and HDM-treated groups via immunoblotted with pan-acetyl-lysine antibody (Ac-lys)² (Figure [1A,B](#)). We isolated three lung tissues from PBS- and HDM-treated mice to further clarify the acetylation mechanism and determine the acetylome via liquid chromatography tandem mass spectrometry (LC-MS/MS) (Figure [1C](#) and Table [S1](#)). Figure [S1](#) showed the MS data validations of acetylome and indicated the mass accuracy. Principal component analysis demonstrated that the control and HDM-treated lung can be classified into two clusters (Figure [1D](#)). Pearson's correlation coefficient and relative standard deviation indicated acceptable accuracy and reproducibility (Figure [S1](#)). Proteome analysis was also performed, serving as background control for quantifying alterations of acetylation (Figure [S2](#) and Table [S2](#)). After comparative acetylome analysis, we identified 5132 Kac sites from 1860 proteins and quantified 3176 sites from 1197 proteins from these in the lung (Figure [S1A](#)). Compared to the control group, 34 sites from 31 proteins were upregulated, and 99 sites from 93 proteins were downregulated on HDM treatment (Figure [1E](#)). One thousand three hundred eighty-three

acetylated proteins and 3457 Kac sites were shared in both groups (Figure 1F,G). The five proteins with most Kac sites were myosin-9 (Q8VDD5, 44 Kac sites), spectrin alpha chain (P16546, 42 Kac sites), myosin-6 (Q02566, 33 Kac sites), filamin-A (Q8BTM8, 32 Kac sites) and fatty acid synthase (P19096, 31 Kac sites) (Table S1).

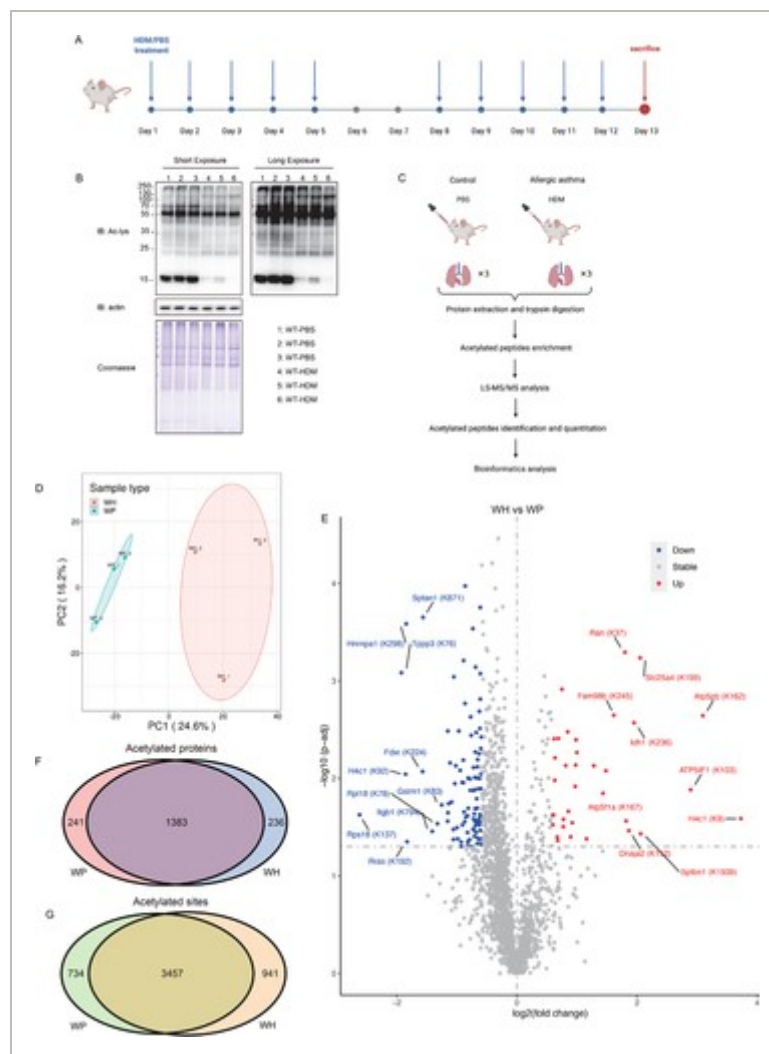


FIGURE 1

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Proteome-wide identification of lysine acetylation proteins and sites in HDM-induced murine allergic asthma model. (A) Schematic diagram of allergic asthma model. HDM, house dust mite. PBS, phosphate buffer saline. (B) Detection of lysine acetylation in mouse lungs of PBS-treated ($n = 3$) and HDM-challenged ($n = 3$) wild-type mice using pan-acetyl-lysine antibody (Ac-lys). Equal loading was verified using β -actin western blot and Coomassie blue staining. WT, wild-type. IB, immunoblot. (C) Experimental flow chart. Six 6- to 8-week-old C57BL/6 female mice were divided into two groups: PBS-treated control group ($n = 3$) and HDM-treated group ($n = 3$). All mice were sacrificed by overdose of pentobarbital 24 h after the last challenge, and lung tissues were isolated for liquid chromatography tandem mass spectrometry (LC-MS/MS). (D) Principal component analysis (PCA) of acetylome data from lung tissues. (E) Volcano plot of differentially expressed acetylated protein lysine sites between control and HDM-treated group. Venn diagram of acetylated proteins (F) and lysine sites (G) from PBS-treated and HDM-treated group mouse lung tissues. WH, HDM-treated WT mice. WP, PBS-treated WT mice.

Previously, transcriptome analysis has demonstrated that various important processes were changed in HDM-treated murine lung tissues. However, the murine strains and modelling methods used in literature reports are different from each other³⁻¹⁰ (Table [S3](#)). Therefore, in order to comprehensively analyze the molecular regulation mechanism during allergic asthma and obtain more accurate results, we also performed RNA sequence analysis of the whole lung of the same murine allergic asthma model (Figure [S4-S6](#), Table [S4](#)).

Then, we systematically compared the transcriptome and proteome data of lung tissues and found that 322 genes showed significant changes in both mRNA and protein expression. Of these, 204 gene products were upregulated, and 118 were downregulated in both datasets (Figure [2A](#)). Although limited gene products were shared between the transcriptome and proteome analyses, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses disclosed a large overlap of biological processes that were critical for immune response and inflammatory response (Figure [S7](#)). Additionally, GO analysis using lung proteomic data revealed a regulatory network of mouse lung tissues on HDM treatment which included neutrophil degranulation, actin cytoskeleton organization, regulation of cell adhesion, hemostasis, inflammatory response, cell-substrate adhesion, actomyosin structure organization and other clusters (Figure [S8](#)). As is shown in Figure [2B](#), these processes showed significant enrichment in both transcriptome and proteome datasets. These data showed that there was a significant correlation between transcriptome and proteome levels in allergic asthma.

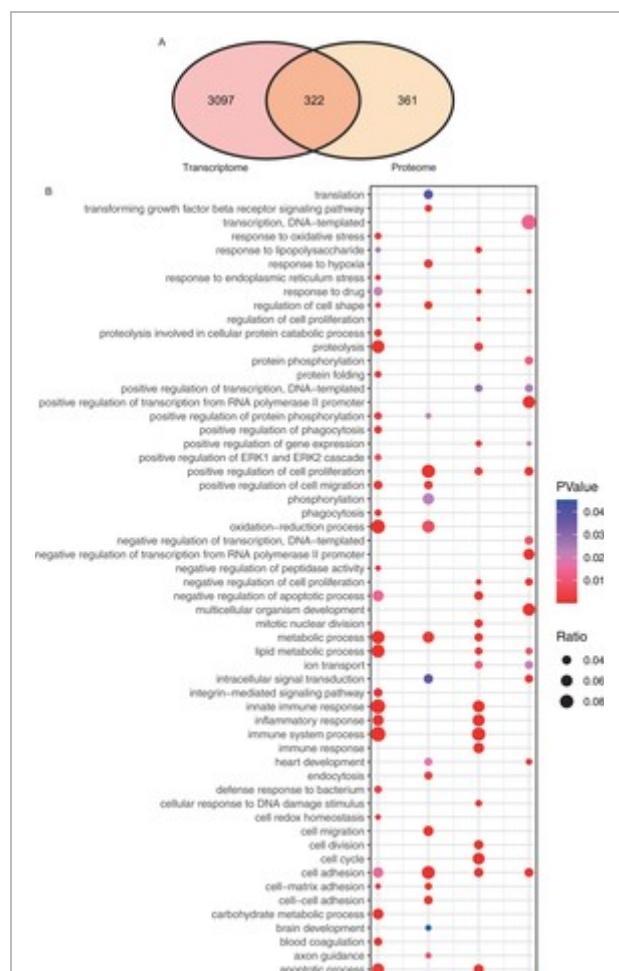




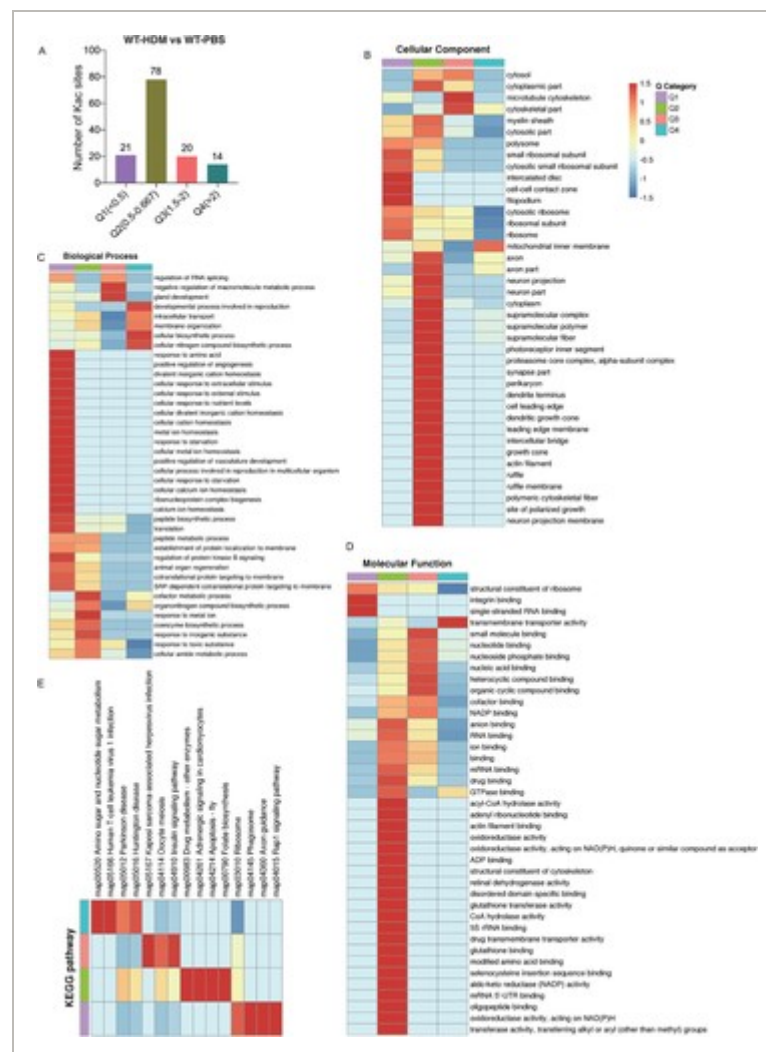
FIGURE 2

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Correlation analyses between transcriptome and proteome data from mice lung tissues. (A) Venn diagram of RNA-seq data and proteome data. (B) Comparative Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of transcriptome and proteome data. The KEGG analyses were performed using upregulated mRNAs, downregulated mRNAs, upregulated proteins and downregulated proteins. The correlation between two datasets is demonstrated. Gene ratios are indicated by circle sizes, and when $p_{adj} < 0.05$, a significant change in the KEGG pathway was recognized.

To explore the effect of protein Kac on HDM challenge, we quantified the acetylated proteins and sites in the lung. Compared to the control group, proteins or sites with $p < 0.05$ and fold changes either greater than 1.5 or less than 1/1.5 were considered significantly changed. The GO analysis, using all significantly changed acetylated proteins, showed that ATP metabolic process, substrate adhesion-dependent cell spreading, protein transport and other biological processes are regulated in lung tissues (Figure [S9A](#)). The biological processes enriched by the upregulated and downregulated acetylated proteins were in the Figure [S9](#). In addition, the cellular component and molecular function via GO analysis were in Figure [S10](#). KEGG analyses showed that there were huge differences in the biological processes of differentially expressed acetylated proteins between the two groups (Figure [S9D](#)). To detect the properties and features of acetylated sites or peptides, Motif-X program was used for motif analysis. The results showed that 10 consensus sequence motifs were enriched, including $K^{ac}H$, $K^{ac}S$, $K^{ac}Y$, $GK^{ac}T$, $K^{ac}N$, $AK^{ac}T$, $K^{ac}F$, $K^{ac}R$, $K^{ac}T$, $K^{ac}K$, $TK^{ac}V$, TK^{ac} , $GK^{ac}G$, $K^{ac}V$ and $K^{ac}W$ (K^{ac} indicates the acetylated lysine [Table [S5](#) and Figure [S9E](#)]).

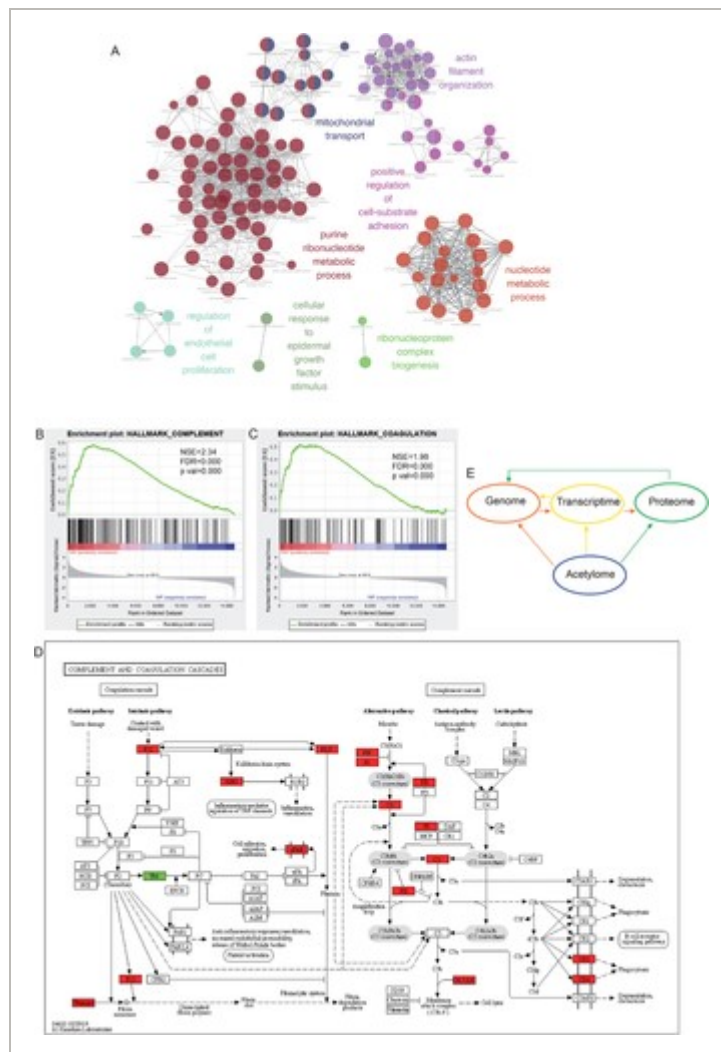
Then, we sorted all quantifiable protein Kac sites into four quantitative groups (down-regulated by 0.5 times or less as Q1, down-regulated by 0.5–0.667 times as Q2, up-regulated by 1.5–2 times as Q3 and up-regulated by more than two times as Q4) for the following GO and KEGG analysis. There were 21, 78, 20 and 14 proteins Kac sites in Q1, Q2, Q3 and Q4, respectively (Figure [3A](#) and Table [S6](#)). Proteins in Q1 mainly localized in small ribosomal subunit, intercalated disc, cell-cell contact zone and filopodium via cellular component analysis as described in Figure [3B](#). Proteins in Q3 mainly localized in microtubule cytoskeleton and cytoskeletal part, while proteins in Q4 in mitochondrial inner membrane (Figure [3B](#)). The biological process enrichment of acetylation was shown in Figure [3C](#), and Figure [3D](#) showed the molecular function analysis. KEGG-based pathway analysis showed that Q1 proteins are enriched in ribosome, phagosome, axon guidance and Rap1 signaling pathway; Q2 proteins in drug metabolism, adrenergic signaling in cardiomyocytes, apoptosis and folate biosynthesis; Q3 proteins in Kaposi sarcoma-associated herpesvirus infection, oocyte meiosis and insulin signaling pathway; and Q4 proteins in amino sugar and nucleotide sugar metabolism, human T-cell leukemia virus 1 infection, Parkinson disease and Huntington disease (Figure [3E](#)).

**FIGURE 3**

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Functional enrichment-based clustering analysis for the quantified acetylome. (A) The numbers of proteins Kac sites in each cluster. (B) Cellular component analysis. (C) Biological process analysis. (D) Molecular function analysis. (E) Kyoto Encyclopedia of Genes and Genomes (KEGG)-based functional enrichment analysis for the quantified acetylome.

Additionally, we executed a protein–protein interactions (PPI) network analysis to delineate the PPI with differentially acetylated proteins, and 124 proteins were employed in the reactome network (Figure 4A). The acetylated proteins associated with HDM treatment could fall into several groups: metabolic process group (purine ribonucleotide metabolic process and nucleotide metabolic process), cellular component organization group (actin filament organization and cellular component organization), mitochondrial transport group, regulation of endothelial cell proliferation group, positive regulation of cell-substrate adhesion group and cellular response to epidermal growth factor stimulus group (Figure 4A). These networks indicate that HDM treatment modulates the acetylation status of proteins in lung tissues, and those proteins are involved in organelle localization, metabolic process and cellular component organization and other biological processes in the murine allergic asthma model.

**FIGURE 4**

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(A) Acetylome Gene Ontology (GO) analyses of house dust mite (HDM)-treated mouse lungs. The GO reactome analysis was performed using differentially expressed acetylated proteins in phosphate buffer saline (PBS)-treated and HDM-treated mouse lung tissues. Gene Set Enrichment Analysis (GSEA) of gene sets associated with complement (B) and coagulation (C). (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway engine analysis of the complement and coagulation cascade pathway for PBS-treated and HDM-treated mouse lung tissues. (E) Schematic diagram of acetylation regulating gene expression, transcriptome and acetylome.

With the help of advanced bioinformatic analysis, we compared transcriptome, proteome and acetylome data, which revealed critical biological process and pathway related to HDM-induced allergic airway inflammation (Figure S11). Both the complement system and coagulation cascade participate in the pathogenesis of asthma. In our data, upregulated genes of HDM-treated mouse lung tissues were enriched in complement- and coagulation-related genes (Figure 4B,C). Figure 4D showed the results of mapping the screen hit data for the proteome data onto the complement and coagulation cascade KEGG pathway and indicated the proteins in this pathway that were screen hits. However, there is a little information about complement and coagulation cascade in acetylome data (Table S1). The

relationship between acetylome and transcriptome or proteome is complicated (Figure 4E). Histone acetylation to regulate the transcriptional activity is of great importance. Studies in recent years have confirmed that RNA can be acetylated, indicating that acetylation maybe a kind of post-transcriptional modification. RNA binding to the catalytic domain of KATs can stimulate the enzyme activity of KATs, thereby affecting gene expression. Acetylation modification participates in biological processes by affecting various functions of proteins. Transcriptional factors (TFs) can also regulate gene expression through acetylation, and a variety of KATs themselves act as transcription co-activators. Therefore, we need more research to clarify the acetylation in asthma, such as the acetylation modification of non-coding RNA, the effect of acetylation on the activity of TFs and the presence or changes of specific acetylated lysine sites and its role in asthma.

In summary, we employed RNA sequence technology, proteome and acetylome MS analyses to systematically determine the molecular modulation mechanisms of mouse lung tissues in response to HDM treatment. These findings highlighted the importance of incorporating different measurements of gene expression. Finally, our data are open to the vast number of scientific researchers. Combined with other approaches, we can have a deeper understanding of the pathogenesis of asthma.

ACKNOWLEDGEMENTS

This work was supported by National Science Foundation of China (grant numbers: 81970020 and 81770025).

CONFLICT OF INTERESTS

The authors declare no competing interests.

Supporting Information



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1 **Supplemental materials for**
2 **Comparative analysis of global transcriptome, proteome and acetylome in house**
3 **dust mite-induced murine allergic asthma model**

4
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6 Li^{2*}, Guochao Shi^{1*}

7
8 **Materials and methods**

9 **1. Reagents and antibodies**

10 The following antibodies were used: acetylated lysine antibody (9441, Cell Signaling Technology
11 (CST)), mouse anti- β -actin (60008, Proteintech, 1:10,000 for western blot), β -tubulin rabbit
12 monoclonal antibody (2128, CST, 1:1,000 for western blot), IL-33 antibody (AF3626, R&D
13 systems, 0.4 μ g/mL for western blot), HDAC4 (7628, CST, 1:2,000 for western blot), HDAC5
14 (20458, CST, 1:1,000 for western blot), HDAC6 (7612, CST, 1:1,000 for western blot), STAT3
15 (12640, CST, 1:1,000 for western blot), Ac-Histone H3 (sc-56616, Santa Cruz Biotechnology,
16 1:200 for western blot), Histone H3 (4499, CST, 1:2,000 for western blot), Histone H4 (13919,
17 CST, 1:1,000 for western blot), Ac-Histone H4 (sc-377520, Santa Cruz Biotechnology, 1:1000 for
18 western blot), Collagen I (ab270993, abcam, 1:1,000 for western blot), TIP60 (sc-166323, Santa
19 Cruz Biotechnology, 1:1000 for western blot), goat anti-rabbit IgG HRP-linked antibody (7074,
20 CST 7074, 1:3,000 for western blot), and goat anti-mouse IgG (peroxidase conjugated, (H+L),
21 31430, Invitrogen, 1:5000 for western blot). House dust mite (HDM) extract was purchased from
22 Greer Laboratories (Boston, Massachusetts, USA). Proteinase inhibitor cocktail (P8340) was
23 purchased from Sigma-Aldrich (Darmstadt, Germany).

24 **2. Mice**

25 All animal experiments were performed in accordance with the National Institutes of Health
26 Guide for the Care and Use of Laboratory Animals and with the approval of the Institutional
27 Animal Care and Use Committee in Institute Pasteur of Shanghai. All mice were maintained under
28 specific pathogen-free conditions.

29 Female 6- to 8- week-old C57BL/6 mice were used for experiments. For the allergic asthma model,
30 mice were challenged for five consecutive days in two weeks with intranasal administration of 35

μL (0.7 mg/mL phosphate buffered saline (PBS)) of whole HDM protein extract. Control animals received only PBS. All mice were sacrificed by overdose of pentobarbital 24 hours after the last challenge.

3. Western blot

Lung tissues of PBS-treated (n = 3) and HDM-challenged mice (n = 3) were homogenized in lysis buffer (50 mM Tris-HCl, PH 7.4, 150 mM NaCl, 0.5 mM EDTA, 1mM DTT, 1% Triton X-100, and 0.5% sodium deoxycholate) which was supplemented with 1% proteinase inhibitor cocktail, 1 mM PMSF, 1 mM NaF, and 1 mM Na₃VO₄, and centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant containing the tissue lysates was separated by SDS-PAGE and analyzed by immunoblotting. Proteins were visualized using enhanced chemiluminescence (WBKLS0500, Millipore).

4. Acetylome analysis

4.1 Protein extraction: The sample of PBS-treated (n = 3) and HDM-challenged mice (n = 3) was grinded by liquid nitrogen into cell powder and then transferred to a 5-mL centrifuge tube. After that, four volumes of lysis buffer (8 M urea, 1% Protease Inhibitor Cocktail, 3 μM TSA and 50 mM NAM) was added to the cell powder, followed by sonication three times on ice using a high intensity ultrasonic processor (Scientz). The remaining debris was removed by centrifugation at 12,000 g at 4 °C for 10 min. Finally, the supernatant was collected and the protein concentration was determined with Bradford Protein Assay Kit according to the manufacturer's instructions.

4.2 Trypsin digestion: The protein solution was reduced with 5 mM dithiothreitol for 30 minutes at 56°C and alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The sample was then diluted by adding 100 mM TEAB to urea concentration less than 2M. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h-digestion.

4.3 Acetylated peptides enrichment and LC-MS/MS analysis: The samples were reconstituted in 1.4 mL of precooled IAP Buffer, added pretreated Anti-Ac-K antibody beads (PTMScan Acetyl-Lysine Motif (Ac-K) Kit, Cell Signaling Technology), then incubated at 4 °C for 1.5 h, centrifuged at 2,000 ×g for 30s, then discarded the supernatant. Anti-Ac-K antibody beads were washed with 1mL precooled IAP Buffer for 3 times, than washed with precooled water for 3 times. Forty μL 0.15% TFA was added to the washed beads, incubated for 10 min at room temperature,

then added 0.15% TFA again, centrifuged at 2,000 ×g for 30s, the supernatant was desalted by C18 STAGE Tips.

The tryptic peptides were dissolved in 0.1% formic acid (solvent A), directly loaded onto a home-made reversed-phase analytical column (15-cm length, 75 µm i.d.). The gradient was comprised of an increase from 6% to 23% solvent B (0.1% formic acid in 98% acetonitrile) over 26 min, 23% to 35% in 8 min and climbing to 80% in 3 min then holding at 80% for the last 3 min, all at a constant flow rate of 400 nL/min on an EASY-nLC 1000 UPLC system. The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q Exactive™ Plus (Thermo) coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The m/z scan range was 350 to 1800 for full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 28 and the fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans with 15.0s dynamic exclusion. Automatic gain control (AGC) was set at 5E4. Fixed first mass was set as 100 m/z.

4.4 Database search

The resulting LC-MS/MS data were processed using MaxQuant search engine (v.1.5.2.8). Tandem mass spectra were searched against uniprot database concatenated with reverse decoy database. Trypsin/P was specified as cleavage enzyme allowing up to 4 missing cleavages. The mass tolerance for precursor ions was set as 20 ppm in First search and 5 ppm in Main search, and the mass tolerance for fragment ions was set as 0.02 Da. Carbamidomethyl on Cys was specified as fixed modification and acetylation modification and oxidation on Met were specified as variable modifications. FDR was adjusted to < 1% and minimum score for modified peptides was set > 40.

4.5 bioinformatic analysis

Enrichment of Gene Ontology analysis: Proteins were classified by GO annotation into three categories: biological process, cellular compartment and molecular function. For each category, a two-tailed Fisher's exact test was employed to test the enrichment of the differentially expressed protein against all identified proteins. The GO with a corrected *p value* < 0.05 is considered significant.

Enrichment of pathway analysis: Encyclopedia of Genes and Genomes (KEGG) database was used to identify enriched pathways by a two-tailed Fisher's exact test to test the enrichment of the

differentially expressed protein against all 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.

Enrichment of protein domain analysis: For each category proteins, InterPro database was researched and a two-tailed *Fisher's* exact test was employed to test the enrichment of the differentially expressed protein against all identified proteins. Protein domains with a corrected p value < 0.05 were considered significant.

For further hierarchical clustering based on differentially expressed protein functional classification. We first collated all the categories obtained after enrichment along with their p values, and then filtered for those categories which were at least enriched in one of the clusters with p value < 0.05 . This filtered p value matrix was transformed by the function $x = -\log_{10}(p \text{ value})$. Finally, these x values were z-transformed for each functional category. These z scores were then clustered by one-way hierarchical clustering (Euclidean distance, average linkage clustering) in Genesis. Cluster membership were visualized by a heat map using the “heatmap.2” function from the “gplots” R-package.

The protein-protein interaction (PPI) information of the studied proteins was retrieved from IntAct molecular interaction database (<http://www.ebi.ac.uk/intact/>) by their gene symbols or STRING software (<http://string-db.org/>). The results were downloaded in the XGMML format and imported into Cytoscape software (<http://www.cytoscape.org/>, version 3.2.1) to visualize and further analyze functional protein-protein interaction networks. Furthermore, the degree of each protein was calculated to evaluate the importance of the protein in the PPI network.

Kac motif analysis: The model of sequences constituting with amino acids in specific positions of modify-21-mers (10 amino acids upstream and downstream of the Kac site) in all protein sequences were downloaded at www.uniprot.org and analyzed. Statistical significance for the enrichment of Kac motifs were calculated using Fisher test. In addition, all the database protein sequences were used as the background database parameter, and the other parameters remained as defaults.

5. Proteome analysis: Upon dissection, lung tissues from three PBS-treated and three HDM-treated mice were used for proteome analysis. The methods of protein extraction, trypsin digestion, LS-MS/MS analysis, database search and bioinformatic analysis for proteome were

similar to those for acetylome. All LS-MS/MS analysis in this study was performed by Jingjie PTM Bio (China).

6. RNA sequence

6.1 Sample collection and preparation for RNA sequence: Total RNA was extracted from whole mouse lungs of PBS-treated (n = 3) and HDM-challenged mice (n = 3) using TRIzol as described previously¹. Snap-freeze the tissue to be extracted, and using a precooled mortar and pestle, grind the frozen tissue under liquid nitrogen.

RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA), and RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

6.2 Library preparation for transcriptome sequencing: 3 µg RNA of each lung sample was used as input material for RNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample.

6.3 Clustering and sequencing: The clustering of index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina HiSeq platform and 125 bp/150 bp paired-end reads were generated.

6.4 Data analysis: Index of the reference genome was built using STAR and paired-end clean reads were aligned to the reference genome using STAR (v2.5.1b). STAR used the method of Maximal Mappable Prefix (MMP) which can generate a precise mapping result for junction reads. HTSeq v0.6.0 was used to count the reads numbers mapped to each gene. Differential expression analysis of two conditions/groups (two biological replicates per condition) was performed using the DESeq2 R package (1.10.1). DESeq2 provide statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting *p* values were adjusted using the *Benjamini* and *Hochberg's* approach for controlling the false discovery rate. Genes with an adjusted *p* value <0.05 found by DESeq2 were assigned as differentially expressed. Gene Ontology (GO) enrichment analysis of

differentially expressed genes was implemented by the clusterProfiler R package, in which gene length bias was corrected. GO terms with corrected *p value* less than 0.05 were considered significantly enriched by differential expressed genes. In addition, we used clusterProfiler R package to test the statistical enrichment of differential expression genes in KEGG pathways.

7. Quantitative real-time PCR: Total RNA was extracted from mouse lungs using TRIzol. RNA was quantified and complementary DNA was reverse transcribed with 1 µgRNA using the PrimeScript RT Reagent Kit (Takara, RR037A). PCR reactions were run in an ABI Prism 7500 Sequence Detection System. Quantification of relative mRNA expression was determined by the comparative CT method. Relative gene expression was determined via normalization to the housekeeping gene β -actin. Primers are as follows:

β -actin forward, GGCTGTATTCCCCTCCATCG;

β -actin reverse, CCAGTTGGTAACAATGCCATGT;

Il13 forward, CGGCAGCATGGTATGGAGTGTG;

Il13 reverse, GGAGGCTGGAGACCGTAGTGG;

Il33 forward, CTGGCCTCACCATAAGAAAGGAGA;

Il33 reverse, AGGGAGGCAGGAGACTGTGTATAA;

Tlr4 forward, ATGGCATGGCTTACACCACC;

Tlr4 reverse, GAGGCCAATTTGTCTCCACA;

Hif1a forward, ACCTTCATCGGAAACTCCAAAG;

Hif1a reverse, CTGTTAGGCTGGGAAAAGTTAGG;

Esco2 forward, ATGGCAACTTGTACTCCAAGAAA;

Esco2 reverse, GTTGGTGGGAGCAAGACTTGT;

Muc5ac forward, CAGGACTCTCTGAAATCGTACCA;

Muc5ac reverse, AAGGCTCGTACCACAGGGA;

Il5 forward, CTCTGTTGACAAGCAATGAGACG;

Il5 reward, TCTTCAGTATGTCTAGCCCCTG;

Il6 forward, TAGTCCTTCCTACCCCAATTTCC;

Il6 reward, TTGGTCCTTAGCCACTCCTTC.

8. Immunohistochemistry: Immunohistochemistry was performed as described previously ².

Mouse lung tissue sections were incubated with primary antibody overnight at 4°C.

Peroxidase-conjugated secondary antibody was then used for one hour at 37°C, followed by addition of the streptavidin-HRP conjugate and substrate chromogen mixture. Finally, all slides were counterstained with hematoxylin.

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Figure legend of supplemental materials

Figure S1. (A) Survey of the acetylomes and Kac sites. (B) Identified peptide length distribution. (C) Peptide mass tolerance distribution. Pearson's correlation analysis (D) and relative standard deviation (RSD) analysis (E) of acetylome data from PBS- and HDM-treated mouse lung tissues. WH, HDM-treated wild type (WT) mice. WP, PBS-treated WT mice.

Figure S2. (A) Survey of the proteome. (B) Identified peptide length distribution. (C) Identified protein mass distribution. (D) Protein sequence coverage distribution. (E) Protein mass and coverage distribution. (F) The number of peptides per protein distribution. (G) Principal component analysis (PCA) of proteome data from PBS- and HDM-treated mouse lung tissues. Pearson's correlation analysis (H) and RSD analysis (I) of proteome data from PBS- and HDM-treated mouse lung tissues. WH, HDM-treated wild type (WT) mice. WP, PBS-treated WT mice.

Figure S3. Verification of proteome and acetylome data. (A) Nine proteins, namely HDAC4, STAT3, HDAC5, TIP60, Collagen I, HDAC6, IL33, Histone H3 and Histone H4 were detected by Western blot. The pan-acetylation levels of Histone H3 and Histone H4 were detected by Western blot. Tubulin was used as a control for protein quantification. (B) Hematoxylin and eosin (HE) staining, Periodic acid–Schiff (PAS) staining, immunohistochemistry staining (Collagen I, TIP60, IL33 and KAT1) of PBS-treated and HDM-treated mouse lung tissue sections.

Figure S4. (A) Schematic diagram of allergic asthma model. (B) Volcano plot of differentially expressed genes between PBS-treated and HDM-treated group mouse lung tissues. (C) Pathway and process enrichment analysis were performed and top 20 clusters were selected. Network of enriched terms was colored by cluster ID, where nodes that shared the same cluster ID were typically close to each other. WH, HDM-treated wild type (WT) mice. WP, PBS-treated WT mice.

Figure S5. Verification of transcriptome data. * < 0.05, ** < 0.01, *** < 0.001.

Figure S6. Heatmap of enriched terms across differentially expressed genes from the PBS-treated and HDM-treated mouse lung tissues, colored by *p* values.

Figure S7. GO analysis of biological process, cellular component, and molecular function of upregulated proteins (A), downregulated proteins (B), upregulated genes (C), and downregulated genes (D).

Figure S8. Pathway and process enrichment analysis using proteome data were performed and top 20 clusters were selected. (A) Network of enriched terms was colored by cluster ID, where nodes that shared the same cluster ID were typically close to each other; and by colored by *p* value (B), where terms containing more genes tend to have a more significant *p* value. (C) Network of enriched terms represented as pie charts, where pies are color-coded based on the identities of the gene lists.

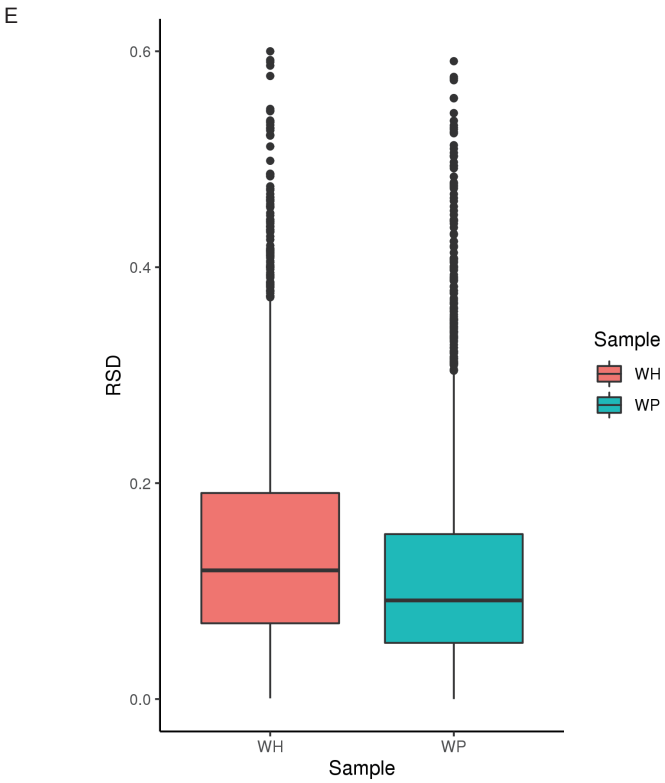
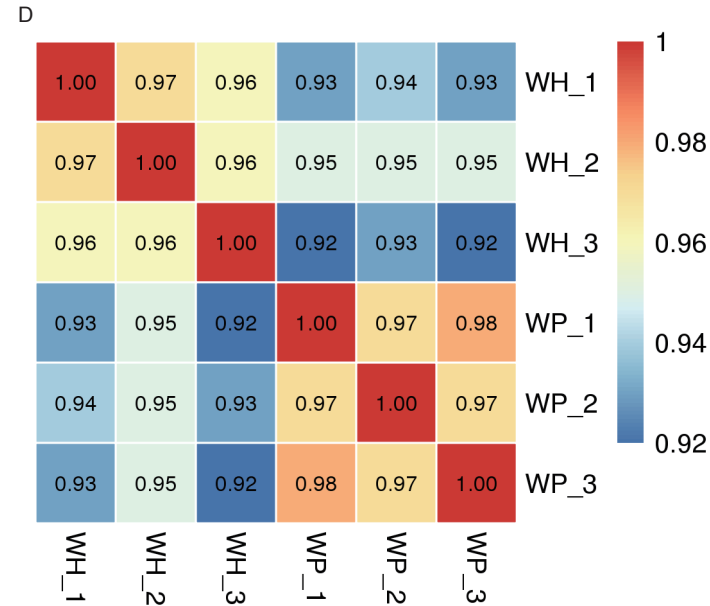
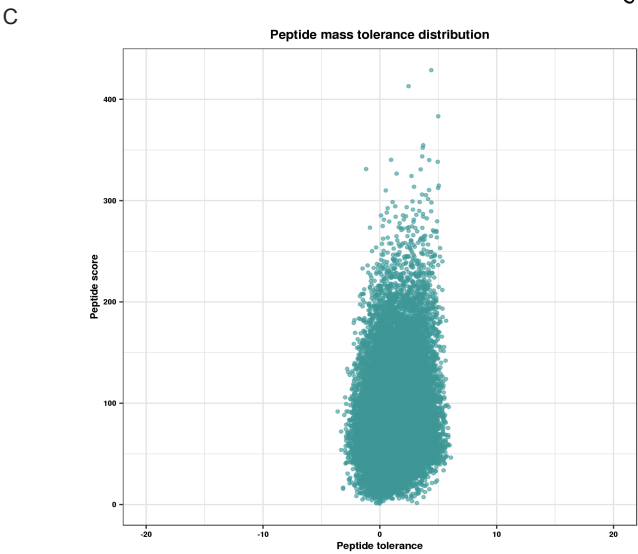
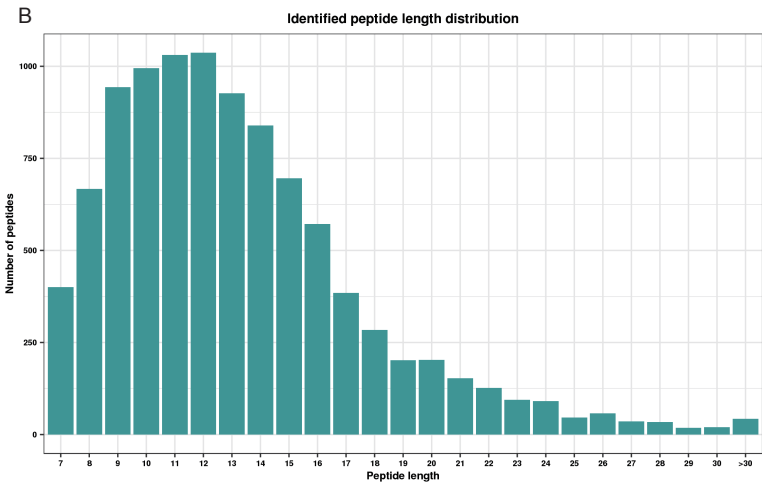
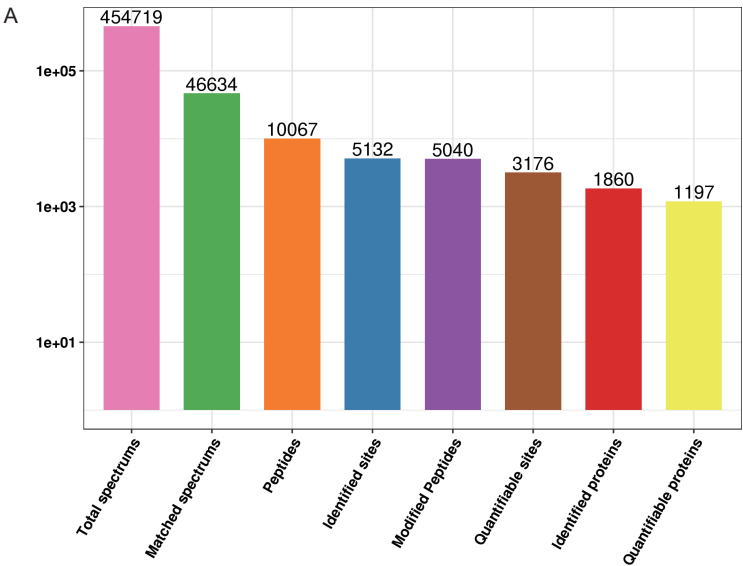
Figure S9. (A) GO-based functional enrichment analysis for the quantified acetylome. The biological processes enriched by the upregulated (B) and downregulated (C) acetylated proteins. (D) The analysis of KEGG pathway of all the quantified acetylome. (E) Heat map of the amino

230 acid compositions of the acetylated peptides.

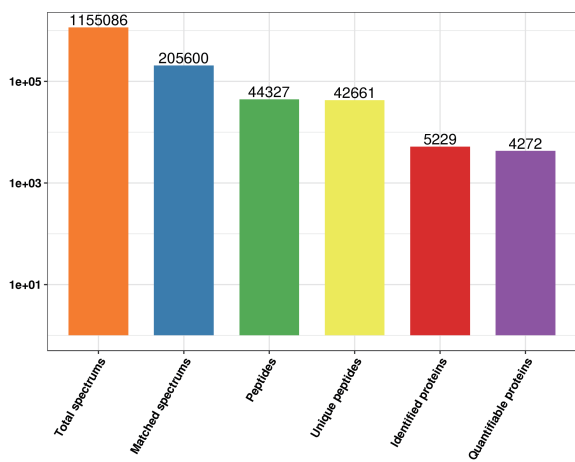
231 **Figure S10.** The cellular component and molecular function via GO analysis.

232 **Figure S11.** GO analysis of acetylome, proteome and transcriptome data. Significantly enriched

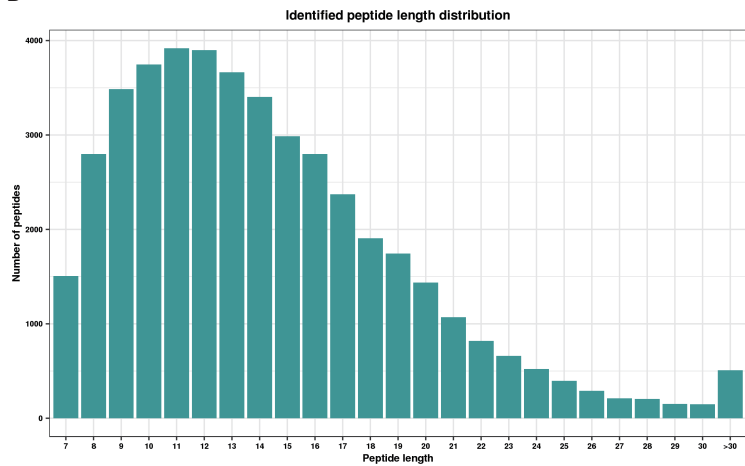
233 GO terms are shown, and the GO processes in all three datasets were shown.



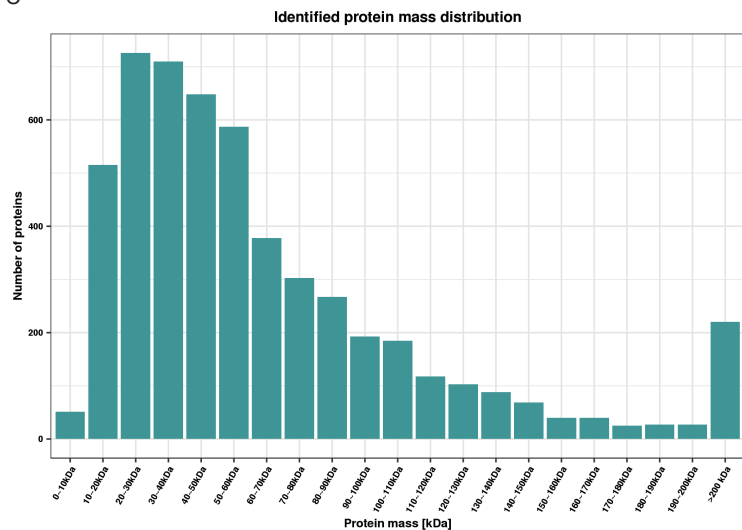
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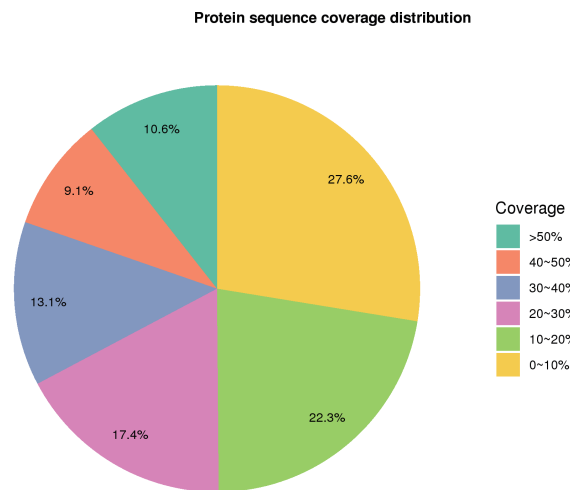
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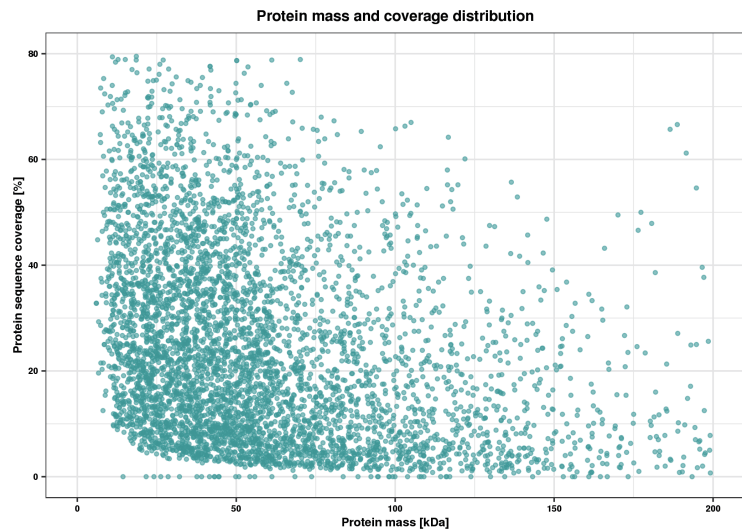
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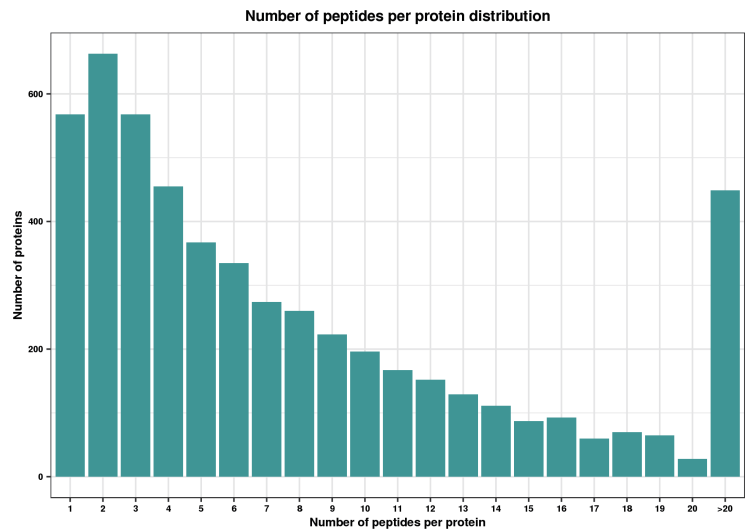
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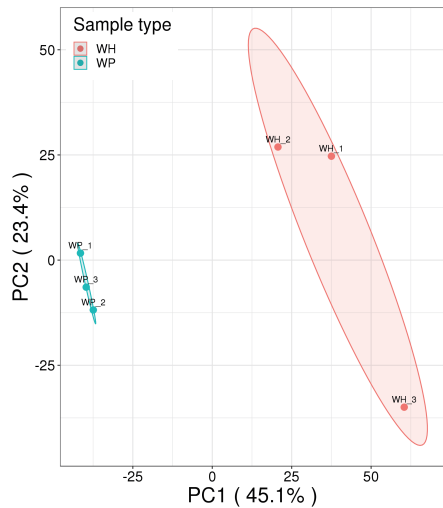
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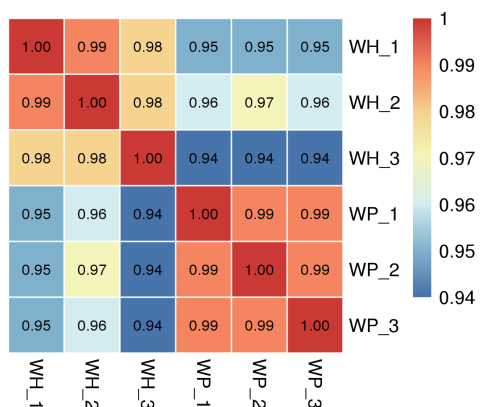
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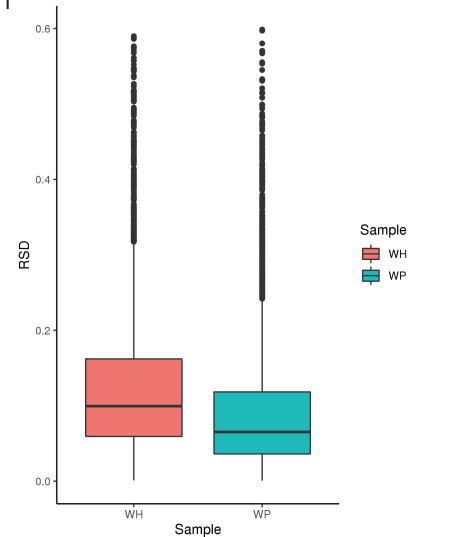
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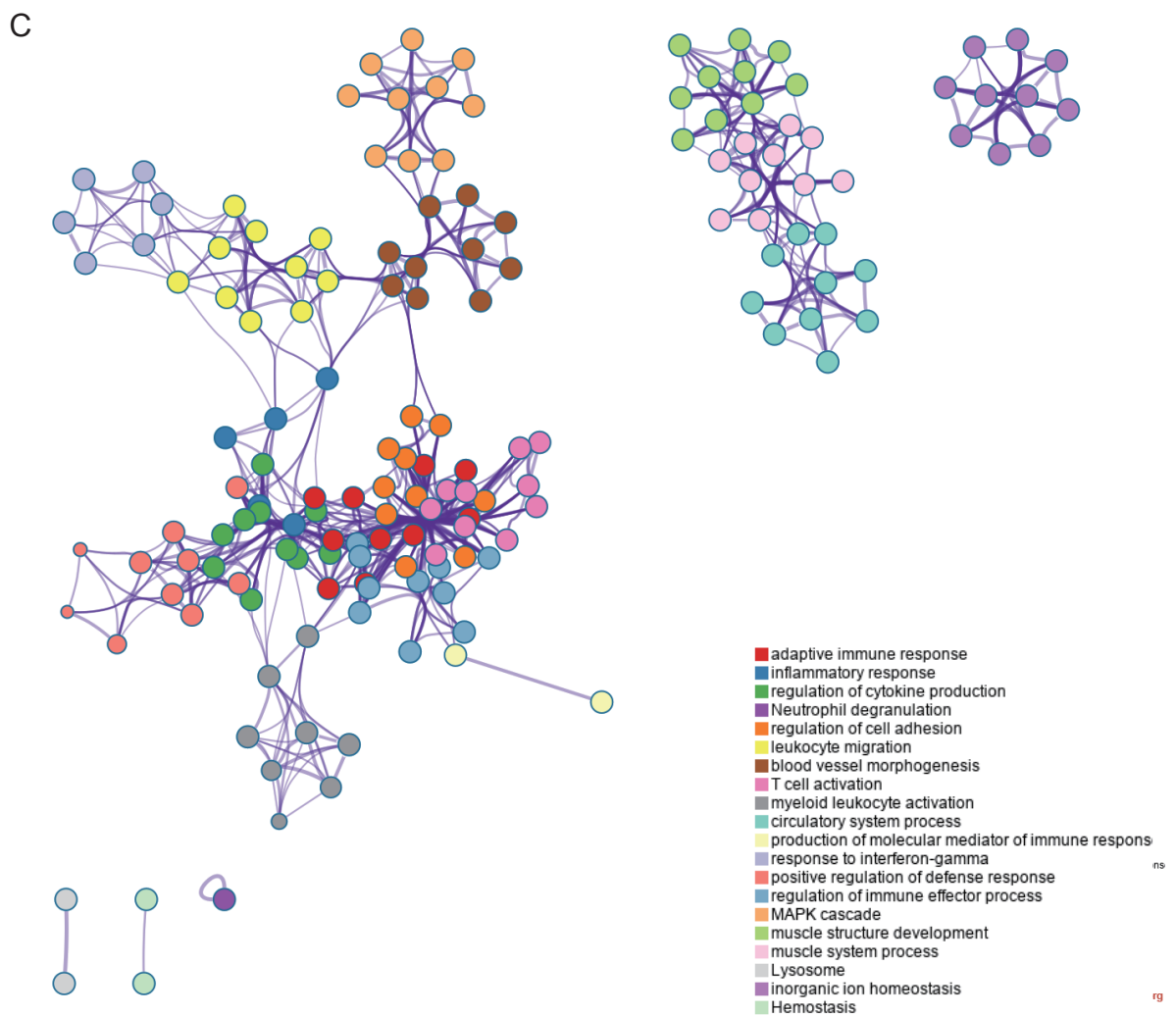
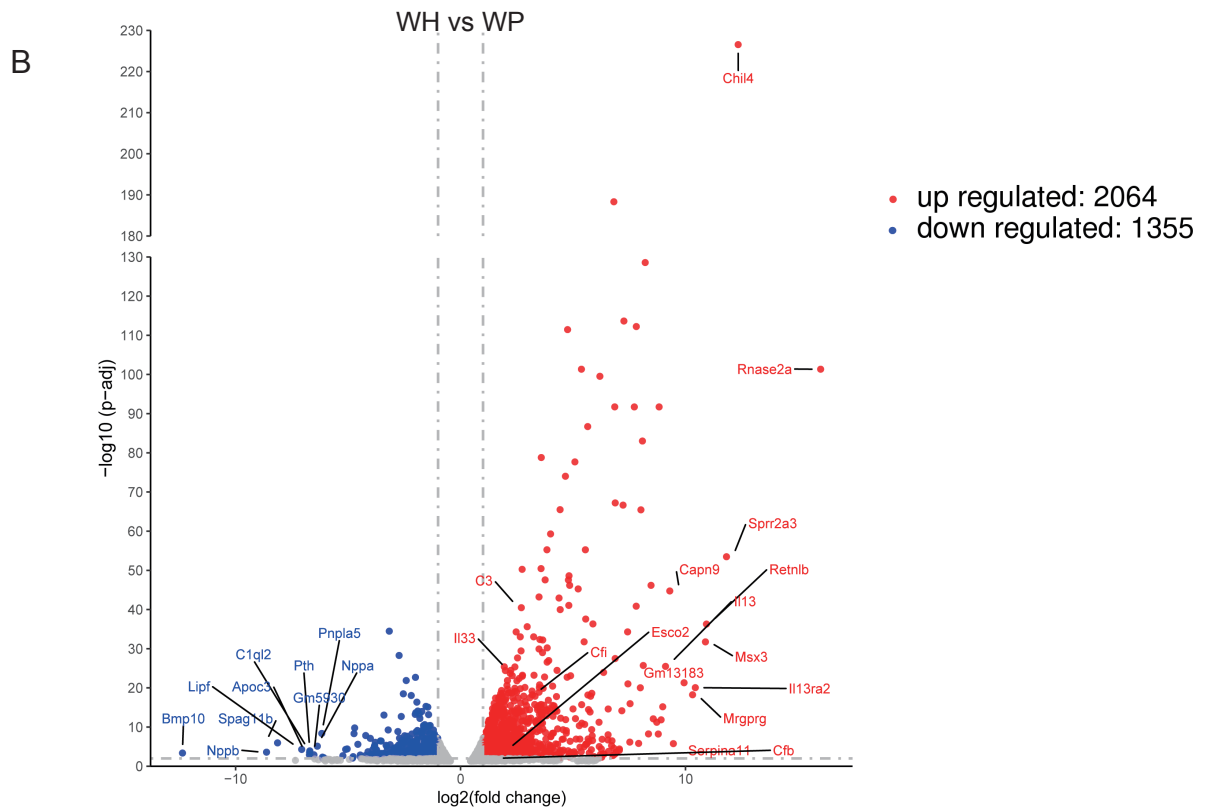
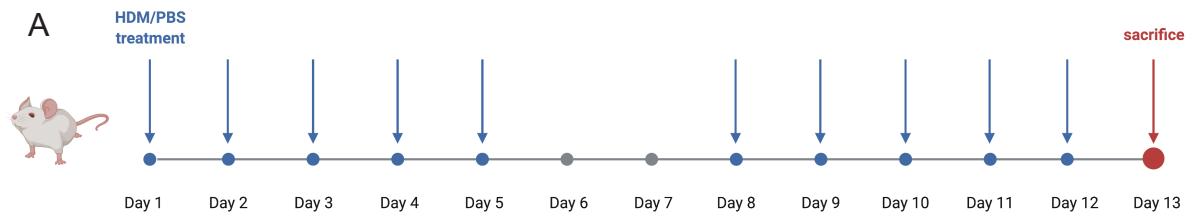


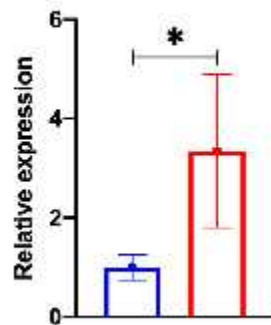
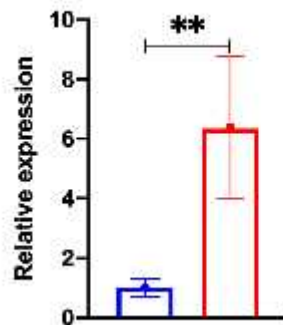
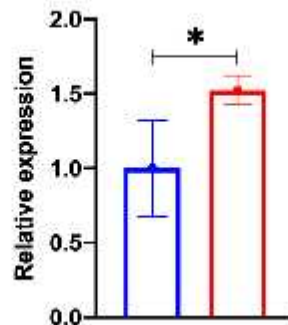
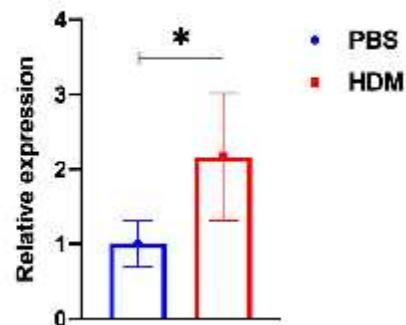
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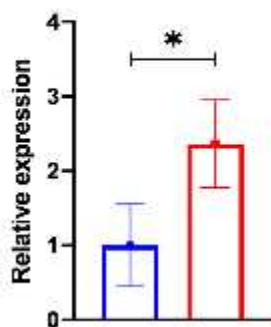
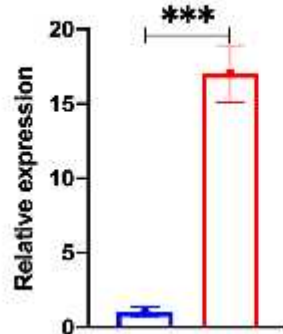
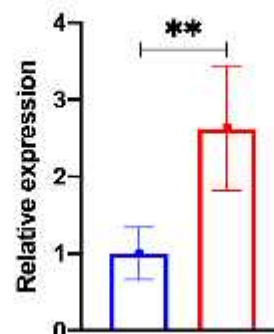
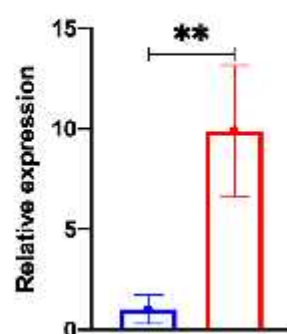
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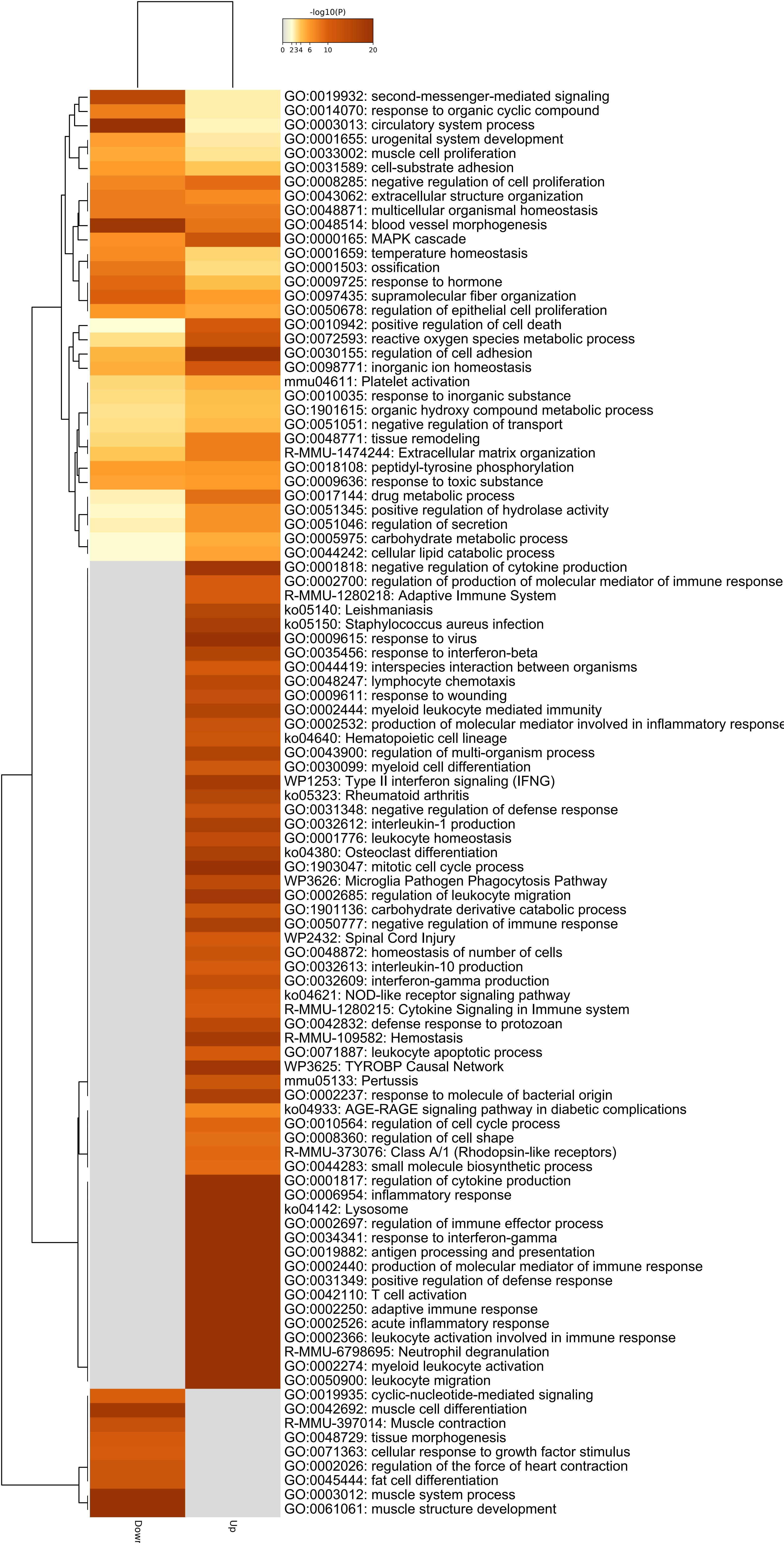


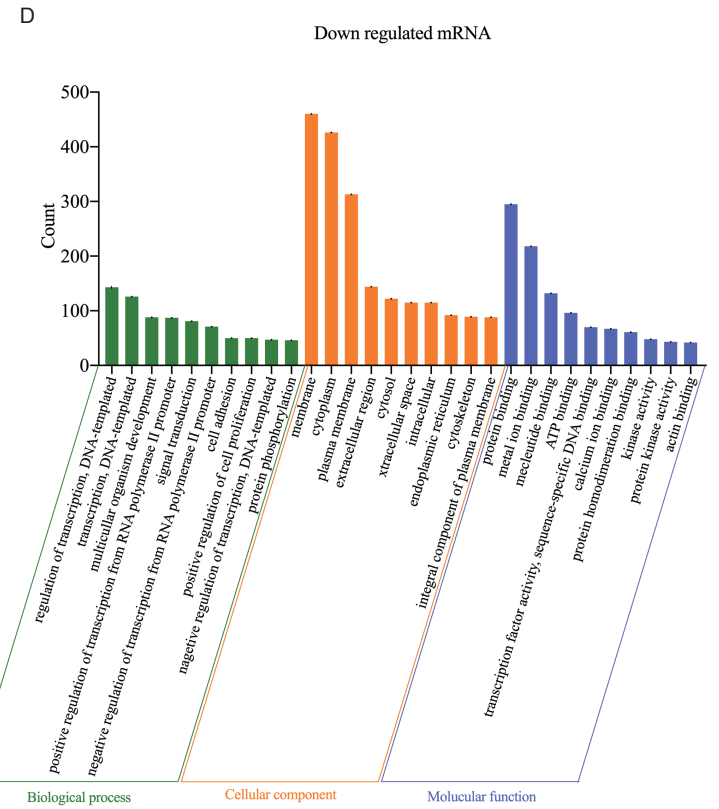
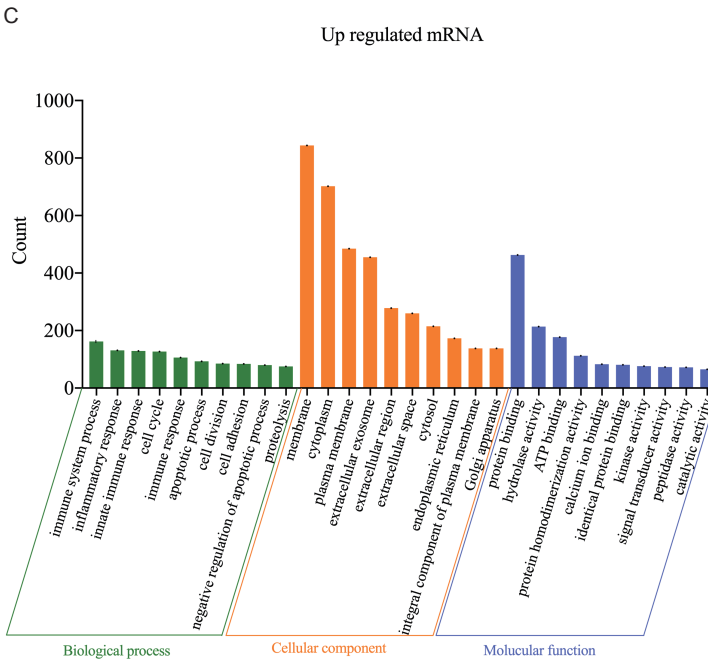
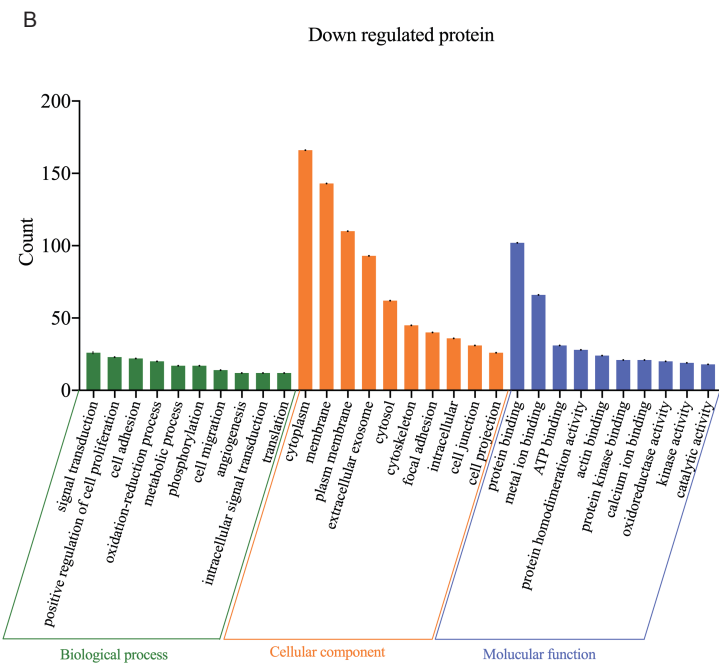
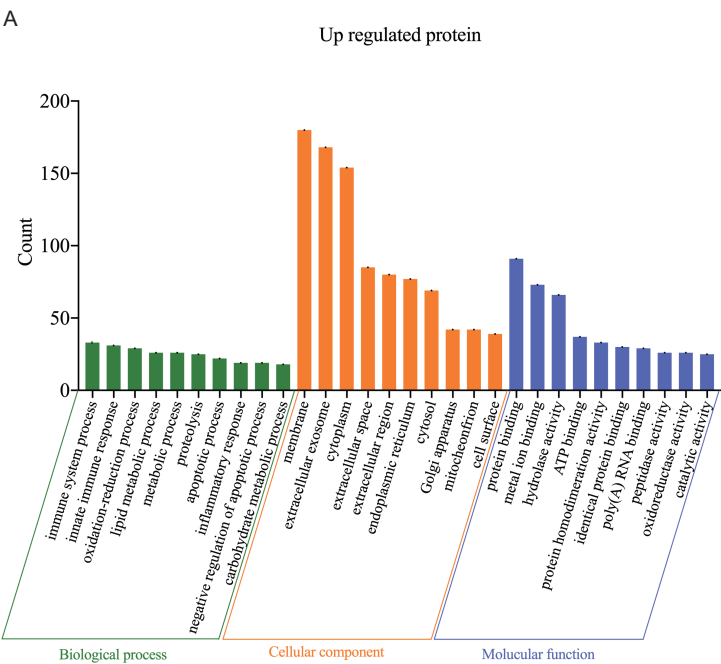


Il33*Il13**Hif1a**Esco2*

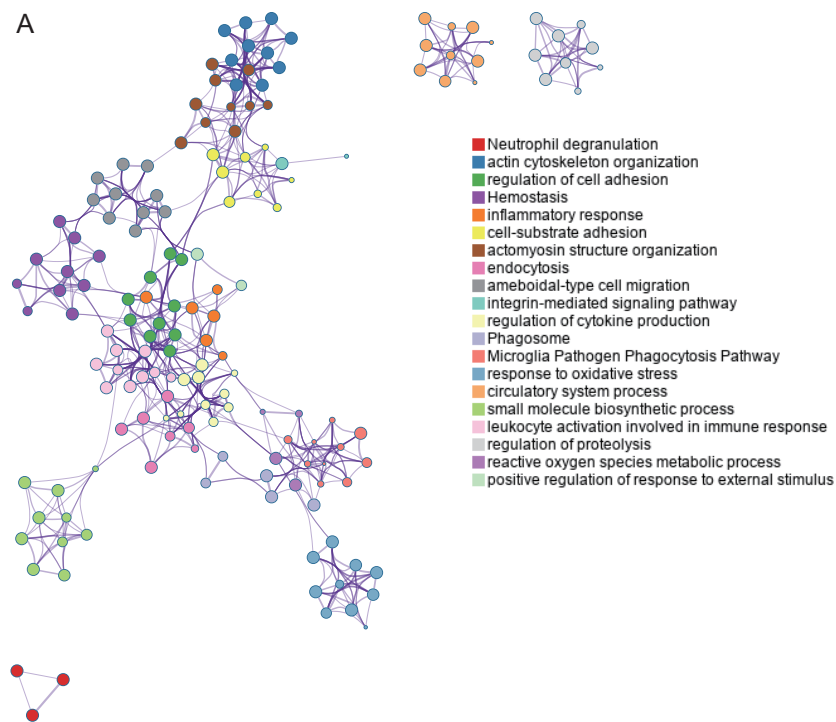
• PBS
• HDM

Tlr4*Muc5ac**Il6**Il5*

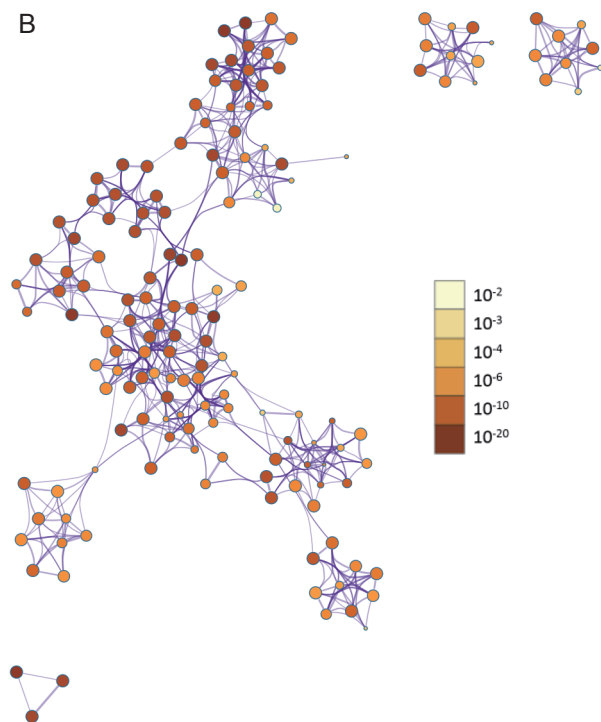




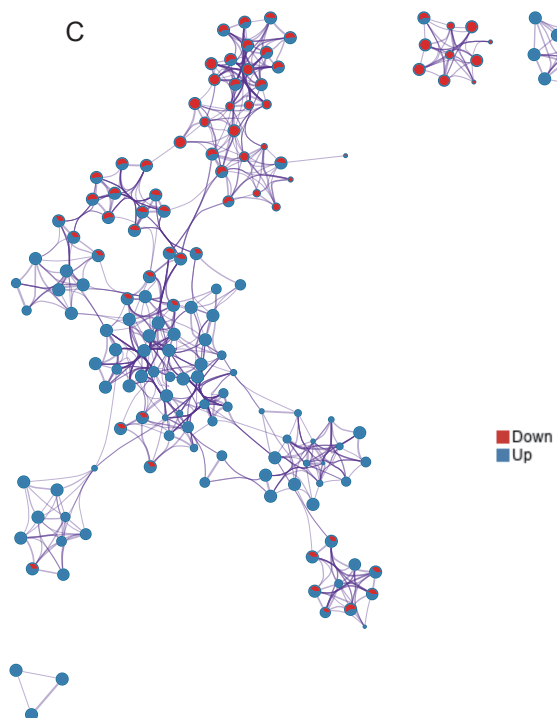
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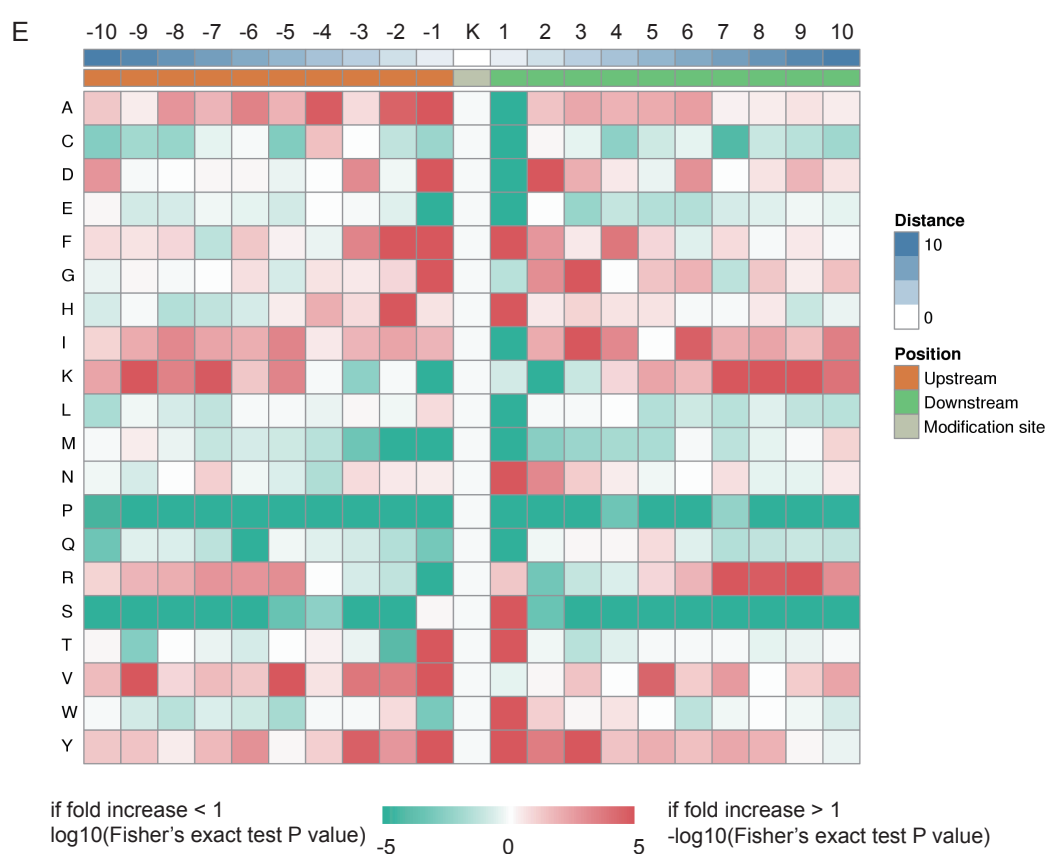
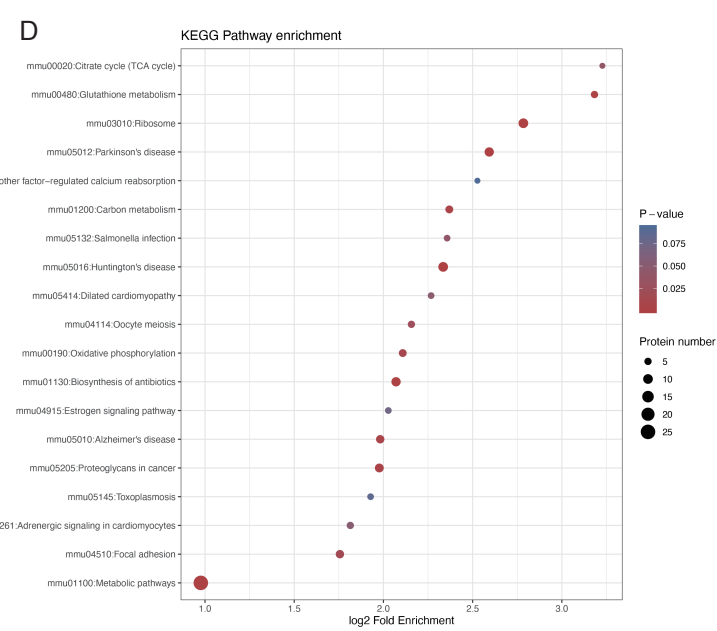
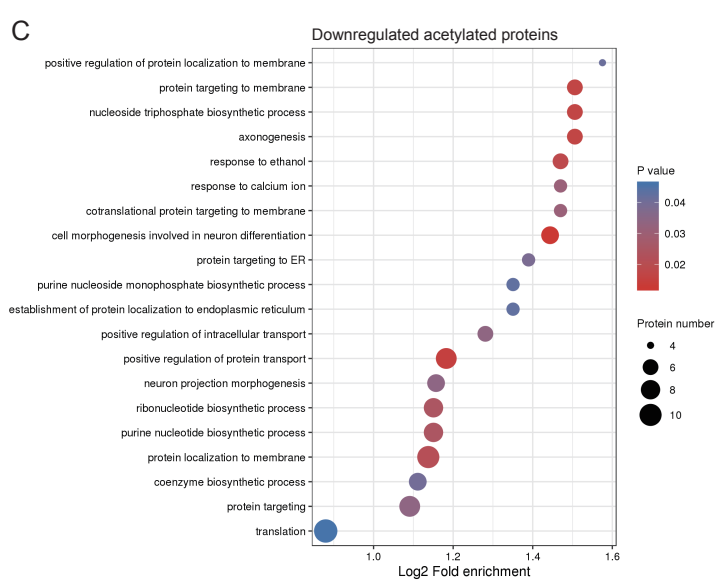
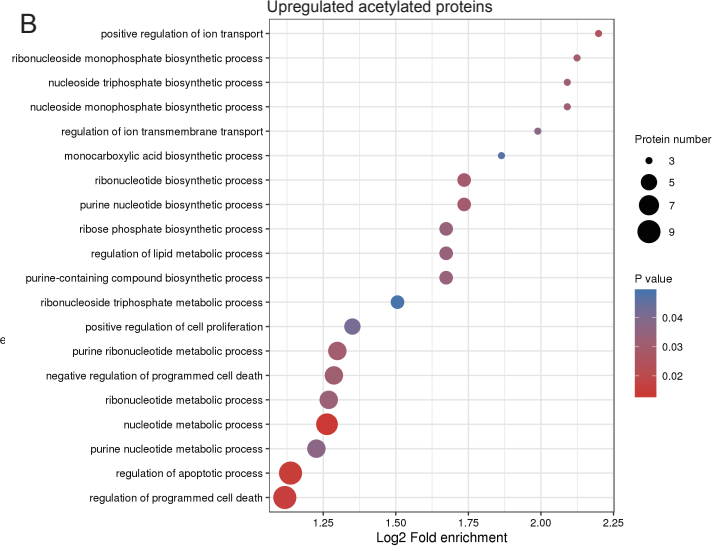
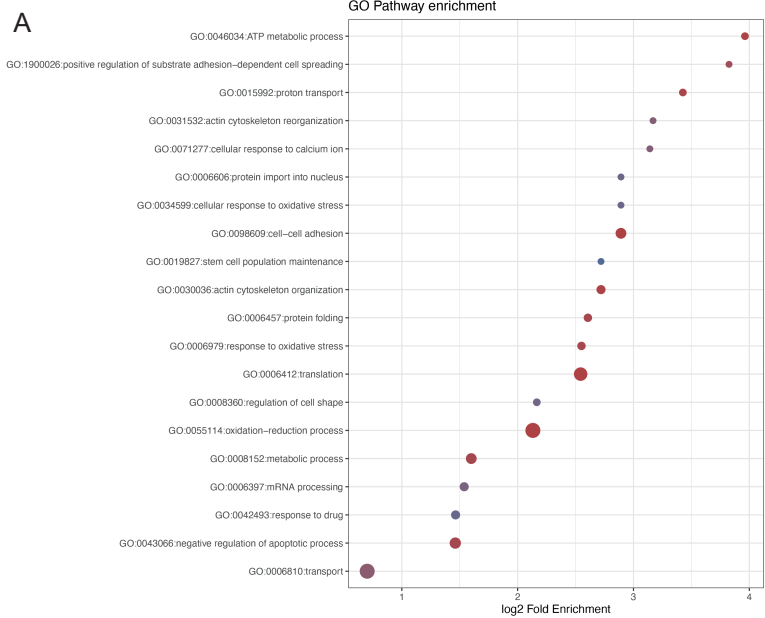


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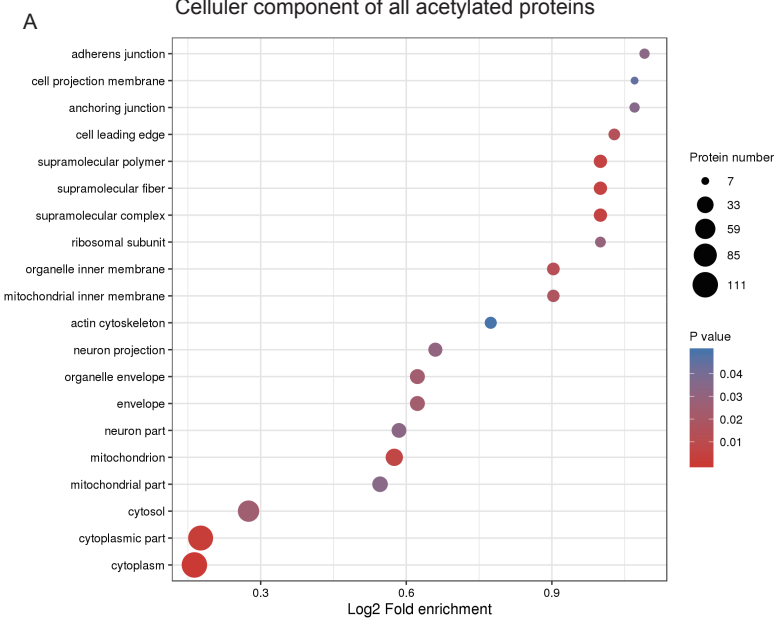


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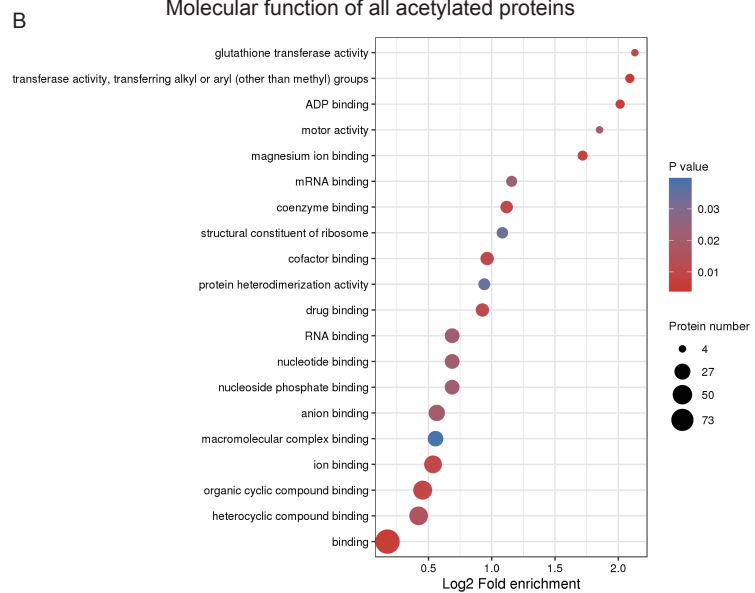




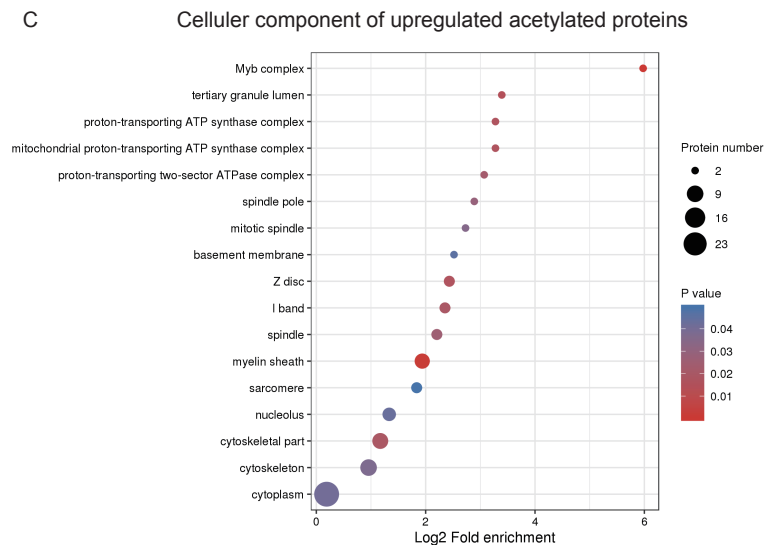
Cellular component of all acetylated proteins



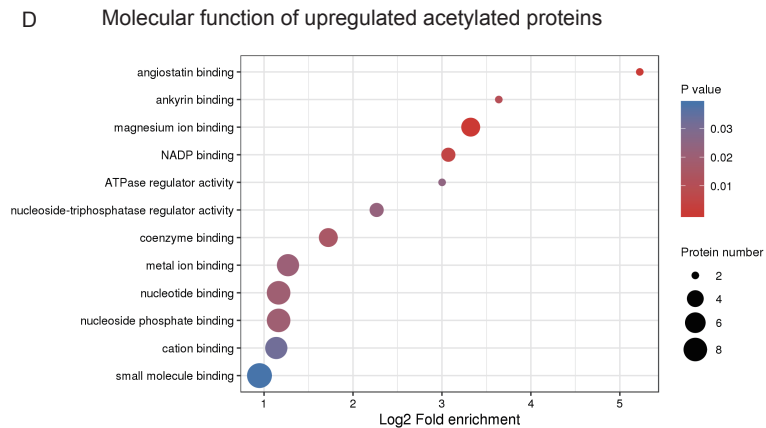
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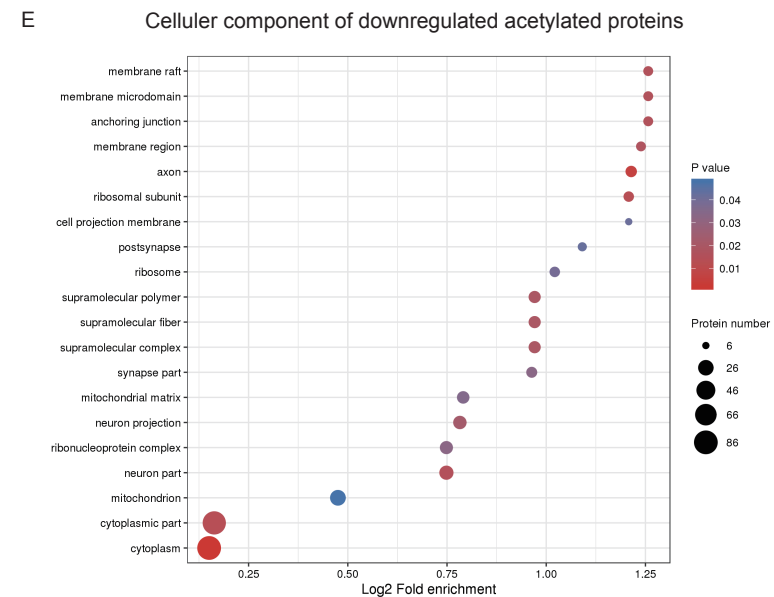
Cellular component of upregulated acetylated proteins



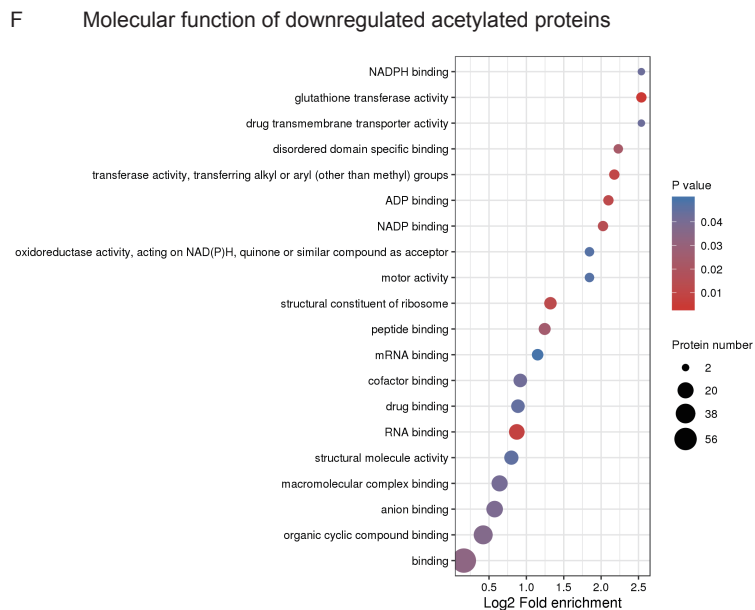
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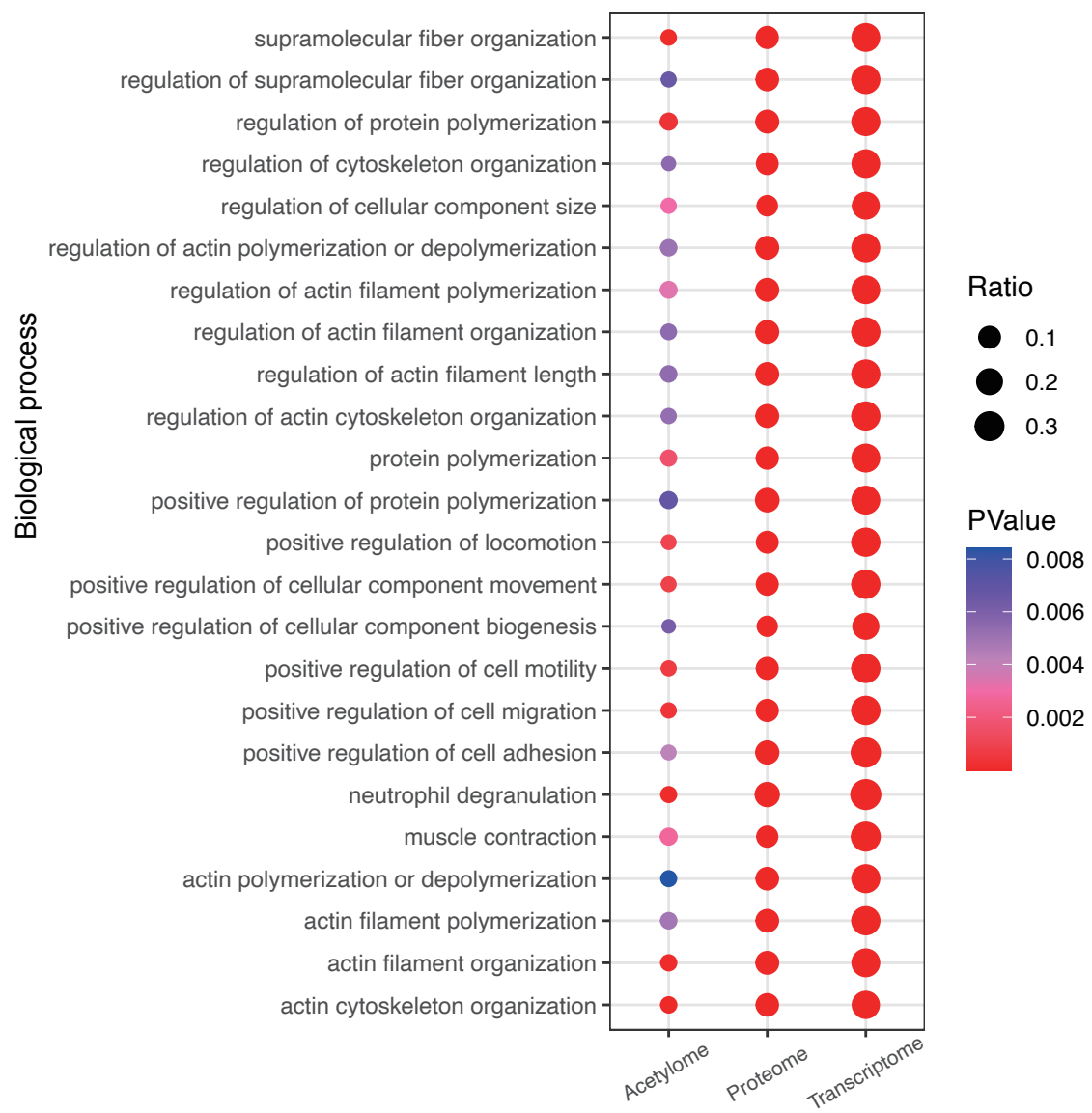


Table S3. Comparison of published transcriptome data.

Study	Year	Series accession	Strain	Environment	Modeling	Sacrifice
Jeanne et al. ³	2019	GSE137324	C57BL/6 mice	pathogen-free environment	HDM extract was resuspended in saline solution and administered intranasally at a dose of 50 mg to isoflurane-anesthetized mice 3 times per week for up to 18 consecutive weeks	3 days after the last HDM administration
Li et al. ⁴	2021	GSE102211	C57BL/6J mice	pathogen-free conditions	Each mouse was administered via the intratracheal route with 100 mg HDM extracts or PBS for three times on days 0, 7, and 14	3 days after the last HDM administration
Sabrina et al. ⁵	2020	GSE126610	BALB/c mice	/	Mice were sensitized with 50 mL endotoxin-free normal saline containing 50 mg protein on days 0, 1, and 2. Animals were challenged daily with HDM extract (5 mg protein/50 mL, i.n.) on days 14, 15, 16, and 17 to elicit allergic sensitization.	24 hours after the last HDM administration
Burleson et al. ⁶	2019	GSE124922	B6/129S4	/	On days 0 and 7, mice were sensitized intraperitoneally with 100 µg of HDM (representing 33 µg of protein) in 100 µL of PBS plus alum or an equivalent	2 days after the last HDM administration

					amount of PBS alone. On days 14, 19 and 21, mice were challenged intratracheally with 100 µg of HDM in 50 µL of PBS or PBS alone.	
Singhania et al. ⁷	2019	GSE119856	C57BL/6J mice	pathogen-free conditions	Female C57BL/6J mice were sensitized with 10 mg HDM and 2 mg Imject Alum in 200 µl PBS or Alum alone as control by i.p. injections on days 0 and 14, followed by i.t. challenge with 10 mg HDM in 20 µl of PBS or PBS on days 21 and 24.	24 hours after the last HDM treatment
Takashi et al. ⁸	2017	GSE100858	C57BL/6 mice	pathogen-free conditions	mice were sensitized intratracheally with 50 µg HDM in 25 µl PBS twice at a 7-d interval. 7 d after the last sensitization, mice were challenged with 5 µg HDM for four consecutive days	48 hours after the last HDM challenge
Kayla et al. ⁹	2014	GSE49047	C57BL/6 mice	/	Mice were immunized with intratracheal instillation of 100 mg of HDM extract in 50 ml of PBS or 50 ml of PBS alone 3 times per week for 3 weeks	24 hours after the last HDM challenge
Christopher et al. ¹⁰	2020	GSE116967	BALB/c mice	/	HDM at 25 µg of protein in 35 µL of sterile saline intranasally five times a week for 2 weeks	2 days after the last HDM administration

Ref num: see the reference of the main text for details.