Transcriptome-wide N6-methyladenosine methylome profiling of *Bombyx mori* reveals a potential mechanism of epigenetic regulation on diapause

Yan-Hua Chen
Jiangsu University of Science and Technology
Tao Jiang
Silkworm Eggs Administration Department of Jiangsu Province
Bing-Yan Fan
Jiangsu University of Science and Technology
Juan Zhu
Jiangsu University of Science and Technology
Mei-Xian Wang
Jiangsu University of Science and Technology
Xing-Jia Shen (shenxjsri@163.com)
Jiangsu University of Science and Technology
AYiNuEr YaSen
Jiangsu University of Science and Technology

Research Article

Keywords: Bombyx mori, m6A, Epigenetics, Diapause, MeRIP-Seq

Posted Date: April 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1561012/v1

Abstract

Background

Epigenetic regulation may be one of the potential determinants of diapause, which is a strategy for insects to cope with adverse environments and evolution. Epigenetic regulation is multi-dimensional and synergistic, how this combination responds to diapause induced environmental signals and how epigenetic regulation induced by environmental signals precisely regulates insect diapause ware still remain unknown. m^6A methylation is one of the most common internal modifications of mRNA, but its regulation on diapause is rarely reported.

Results

In this study, based on fluorescence immunoassay technology, the m^6A abundance of total RNA in the pupal stage of bivoltine silkworm (*Bombyx mori*) QiuFeng, QFHT (incubated at a normal temperature after HCl acid treatment to produce diapause eggs) and QFLT (incubated at 16.5℃,a low temperature after HCl acid treatment to produce non-diapause eggs) were detected to explore the effects of m6A methylation modification on the diapause traits of the silkworm. The results showed that there were 1563 m6A peaks in QFLT and 659 m^6A peaks in QFHT, and together 1,984 m6A peaks. The distribution and enrichment of m6A peak in gene functional elements were significantly different between the two groups. In each signaling pathway of QFLT, the gene transcripts with m^6A modification were more diverse, and the methylation level was higher than that of QFHT. The period of epigenetic response to diapause-induced environmental signals was much earlier than the period of diapause phenotype formation, and the epigenetic signals undergo multiple accumulation, transmission and transformation, which may be accompanied by the metamorphosis development and transgenerational regulation of *B. mori*.

Conclusions

m^6A methylation modification may be one of the molecular markers of diapause regulation or developmental decision, and its specific role in diapause regulation is of great importance for understanding of insect diapause mechanism.

Background

Epigenetics refers to the genetic phenomenon of phenotypic changes caused by non-DNA differences, including chromosomal remodeling, histone modification, DNA modification, RNA modification, etc., which is mainly regulated in the process of transcription or translation and affects gene functions and characteristics[1–4]. Regardless of DNA, RNA or histones, the essence of any epigenetic modification is to change the molecular structure and electrostatic charge balance through the modification group, which may lead to changes in secondary structure, stability and binding ability, and thus changes the cellular

regulatory network[5–7]. m^{6}A methylation is the most common modification of RNA, which has important significance for biological regulation mechanism [8]. The regulation of methylation modification of genes is under the co-regulation system of methylation-related enzymes methyltransferases (Writers), demethylases (Erasers), and methylated reading proteins (Readers)[9–11], which activates downstream regulatory pathways such as RNA degradation and miRNA processing and so on[12–14]. The co-regulation system plays an important role in regulating biological processes such as growth and development, neural activity and stress response[15–17].

The physiological characteristics of diapause of the silkworm, *Bombyx mori* are the special living habits and physiological reactions evolved to survive in unfavorable living space and time, which is also one of the evolutionary strategies. Through diapause the silkworms regulate growth and reproductive season to increase stress resistance, thereby maintain the population as a whole[18, 19]. Under the influence of environment, they secrete diapause hormones and lay diapause eggs [20, 21]. The diapause state of bivoltine strain *B.mori* can be terminated by HCl acid solution soaking or adjusting temperature and humidity during embryonic stage of parent eggs[22, 23]. Epigenetic modification of insects is a dynamic process, in which environmental changes cause special stress responses [24]. Methylation regulates diapause related genes through site selection and modification rate adjustment to achieve survival of the fittest. There have been breakthroughs in the research on diapause, but how the epigenetic modification induced by environmental signals regulates diapause in insects is still unknown[25–27]. Studies have shown that in Hempfly histone demethylase genes *Lsdl* and *Su* (var) 3–9 were up-regulated by 1.5 and 2 times respectively in diapaus larvae, suggesting that histone demethylation involves in the regulation of pupal diapause[28]. The DNA methylation levels of female *Nasonia calliflora* pupa were significantly different under different photoperiod conditions. RNA interference of the DNA methyltransferase gene *Dnmt*1a or feeding with 5-azacytosine nucleoside induces lag in female bees. Knockdown expression of *Dnmt*3 increases the diapause rate in offspring of *Nasonia* under long light exposure[29]. Recent studies on the m^{6}A epigenetic inheritance of *B. mori* showed that RNA m^{6}A methylation related genes Mettl3, Mettl4, Wtap/ Fi (2) D, Ythdc1 and Ythdf3 in four different voltinisms of *B.mori*, AK4 (univoltine), QiuFeng (bivoltine), NH (mulvoltine with diapause) and Nistari (non-diapause mulvoltine). And these expression patterns in ovary, head and egg of pupa and adult were different, showing a certain correlation of localization and tissue specificity[30]. m^{6}A methylation modification is one of the most extensive epigenetic modifications in RNAs, but the its regulatory mechanism on diapause is not fully understood.

In order to further study the function of m^{6}A methylation modification and its potential regulation on diapause traits of *B.mori*. The m^{6}A abundance of total RNA in pupal stage of QFHT and QFLT was detected respectively based on RNA methylated immunoprecipitation combined with high-throughput sequencing technology. The m^{6}A expression profiling data of the whole transcriptome of QFHT and QFLT were obtained. Comparative analysis of the two groups showed that 659 peaks specific to QFHT were enriched in 212 GO signaling pathways and 249 KEGG signaling pathways, and 1563 peaks specific to QFLT were enriched in 280 KEGG signaling pathways and 335 GO signaling pathways, totally 1984 peaks shared by QFLT and QFHT groups were enriched in 478 GO signaling pathways and 291 KEGG signaling

pathways. The results showed that the environmental induced signal affected the efficiency of m^6A methylation resulting in expression changes of a series of genes, and in turn affected the diapause characteristics of *B. mori*. These results provide a basis for further research on the role of epigenetics in diapause regulation of *B. mori*.

Materials And Methods

Animals

A bivoltine strain of *B.mori* Qiufeng was used in the experiment. According to the principle of diapause of bivoltine strain is regulated by environmental factors mainly temperature and light, diapauses-terminated silkworm egg batches (one batch produced by one female moth) were divided into two semi-batches, one of which was incubated at 16.5℃ in darkness (QFLT) for produce non-diapause eggs. To produce diapause eggs, the other sample was incubated at 25℃ under natural day/night cycle (QFHT) 15 days later for the purpose of hatching on the same day. After hatching, larvae of bith group were raised with fresh mulberry leaves under 25 °C with a relative humidity of 80% ± 5% under room natural light. On the third day of the pupae, the ovarian tissue was dissected and collected, and each sample was replicated in three groups.

RNA isolation and fragmentation

Total RNA from QFHT and QFLT tissue samples was extracted with three replicates for each group of samples and detected for concentration and integrity. The sample RNA was fragmented in 1×NEBNext Magnesium RNA Fragmentation buffer at 94°C for 1-5 minutes using the Kit NEBNexet RNA Fragmentation Module. The Fragmentation reaction stops in the 1×NEBNext Fragmentation Stop Solution. The sample was then diluted and analyzed.

RNA immunoprecipitation

The fragment RNA was divided into two parts, one of which was used as control (Input) for RNA-seq construction of transcriptome sequencing library to eliminate the background in the process of methylated fragments. The other RNA was immunoprecipitated with m6A specific antibody (IP) and eluted with magnetic beads to reduce background noise of non-specific binding.

MeRIP and RNA sequencing

Reverse transcription of RNA from IP and Input groups was conducted to construct specific libraries and conduct quality detection. Qualified libraries were subjected to high-throughput sequencing using the Illumina HiSeqTM-4000 sequencer. The amount of RNA in IP and Input groups was the same, which could be used for subsequent comparative analysis.

Colorimetric And MeRIP-qPCR

Total RNAs of QFHT and QFLT pupal 3rd day ovarian tissues were extracted according to the EpiQuik M^6A RNA Methylation Quantification Kit (Colorimetric, EpiGentek). The difference of m^6A modification abundance in total RNA of the two cultivars was detected. Two sets of samples were enriched according to the EpiQuik™ CUT&RUN m^6A RNA Enrichment (MeRIP) Kit (EpiGentek) for quantitative detection of sequenced differential genes.

Data analysis

Reads alignment and sequence analysis

The Fastp[31] software was used for quality control of raw reads, and low quality data were filtered to obtain clean reads, and then base quality analysis was performed. The short reads alignment tool Bowtie2[32] was used for sequence alignment analysis of high quality clean reads.

M^6A Modified Peak and Motif Analysis

Peak calling was conducted using MACS2[33] analysis software within the whole genome of Silkworm, and the threshold was set to $Q < 0.05$. The position of peak on the silkworm genome and the sequence information of the peak region were analyzed to screen out the peak-related genes for functional enrichment analysis of these genes.

TF-motif analysis was performed using MEME Suite (http://meme-suite.org/). We analyzed the motifs in two different length ranges, MEME (detected 8~30bp) and Dreme (detected ≤8bp), and found the motif with the highest frequency in the two ranges.

Intragroup consistent peak detection and annotation

The peaks between repeated samples within the group were filtered, and the common peaks with overlap > 50% were retained for subsequent analysis. According to the annotation of peaks, diapause related genes with m^6A-modification were annotated to compare the divergence of m^6A-modified genes in different treatment groups.

Pooling of peaks between groups and multi-sample cluster analysis

The DiffBind[34] was used to merge the peaks between groups. The union of peaks between treatment groups was obtained to calculate the abundance of each peak in each sample.

Differential analysis of the presence or absence of RNA methylation modification between groups

Transcripts from different treatment groups differed in the presence or absence of m6A methylation modifications. Compare and count common and unique peaks between groups and enrich for their associated genes

Analysis of differences in RNA methylation rates between groups

Based on data of MeRIP and Input to calculate the relative rate of methylation of each peak, the filter peak of rate difference in RNA methylation was analyzed using exomePeak[35] at FDR < 0.05 and | log2FC | > 1. GO and KEGG functional enrichment analysis was performed on genes related to differential peak.

Results

Transcriptome sequencing of m6A modified *Bombyx mori*

The ovarian tissues of day-3 pupae of bivoltine strain, QiuFeng were collected from QFHT and QFLT group respectively, and 3 replicates were designed for each group. And their transcriptomes were sequenced according to the Input and IP classification. On average, about 43000000 raw reads were obtained from each Input sample, and about 40000000 raw reads were obtained from each IP sample. After filtering out low-quality data, the Input has a retention rate of 99.9% and the IP reads retention rate is approximately 95% (Additional File 1 and File 2: Figure S1 and Table S1). To visually display the data quality, analysis of the composition and quality distribution of bases was performed. The more balanced and higher quality the base composition is, the more accurate subsequent analysis will be (Additional File 3: Date S1 and Data S2). High quality clean reads were aligned to the ribosomal database using the alignment tool Bowtie2 to remove reads from ribosomal RNA (Additional File 2: Table S2). Finally, statistically valid reads for the silkworm genome data, the results show that the average valid comparison rates of the three replicates of Input-QFHT and IP-QFHT samples are about 64.6% and 64.0%, respectively, and that of Input-QFLT and IP-QFLT samples are about 68.7% and 67.0% (Additional file 2: Table S2), separately. The unique and de-duplicated reads were compared to each chromosome (positive and negative chains) on the genome, and the density was statistically mapped, so that the relationship between chromosome length and the number of reads compared were seen more intuitively (Additional file 4: Data S1).

The number of peaks, total peak length, average peak length, the number of reads in the peak as a percentage of the total number of reads in all alignments (FRiP) and the peak genome alignment rate were analyzed for the number of peaks performed in a single sample by intra-group sequencing. There is a good consistency in the data between single samples in each group (Additional file 2: Table S4). Statistical analysis of the number of peaks at different depths shows that the proportion of peak number of IP-QFHT in total peak is slightly higher than that of IP-QFLT in sequencing depth of 5-50 regions (Additional File 5: Data S1). The unique peak related reads obtained were analyzed and the number of reads to different regions of the transcript was counted. The results show that the relative transcript numbers of IP-QFHT reads in the 5'UTR and 3'UTR regions were higher (Additional file 6: Data S1). Further statistics on the distribution and width distribution (indicating the length of protein binding sequences) of peak on chromosomes showed that the peak number of m6A modification of IP-QFLT on chromosomes was slightly higher than that of IP-QFHT. In addition, the peak number of IP-QFLT is also higher in the protein-bound sequence length range of 100-300(Additional file 7: Data S1 and Data S2). Finally, the distribution of fold enrichment and q value of peak was analyzed, and the results showed that IP-QFLT

was more significant both in enrichment multiple and significance degree, indicating that the peak expression of IP-QFLT was higher than that of IP-QFHT samples (Additional File 8: Data S1 and Data S2).

Filter peak among repeated samples within the group, and retain common peak which overlap > 50%. Statistical results show that the peak number of IP-QFLT is far more than that of IP-QFHT samples (Additional File 2: Table S5).

Distribution and enrichment of m6A modification

The peaks among three replicates in the same group with overlap>50% and co-occurring in at least two samples were filtered, and the statistical results showed that the number of peaks of IP-QFLT far exceeded that of IP-QFHT samples (Additional file 2: Table S5). In order to understand the difference of m6A modification in transcripts, the distribution number of peak on different gene functional elements such as 5'UTR, start codon, CDS, stop codon and 3'UTR in each group of samples was counted. Enrichment score of peak on functional elements of different genes was calculated for chi-square test with the formula : Enrichment score = n/(n× P)[5] (n: peak number on functional elements of each gene; N: total number of peak samples; P: proportion of genome length for each classification)[36]. The results showed that peak of QFLT and QFHT differed significantly in the distribution of functional elements and rich concentration. In terms of distribution, the proportions of QFLT and QFHT on functional components from high to low are CDS, start codon, stop codon, 5 'UTR and 3' UTR. The m6A modification in QFLT was more likely to distribute on CDS, and that in QFHT was more likely to distribute on the other gene functional elements except CDS (Figure 1A and 1B). In terms of enrichment, the highest enrichment rate of QFLT and QFHT was in the start codon area, followed by the stop codon area. But in the comparative analysis of the two groups of samples, the enrichment of QFLT in the CDS region was significantly higher than that of QFHT (Figure 1C and 1D). The m6A modification of QFLT and QFHT samples was highly similar in both distribution and enrichment of functional elements, indicating that m6A methylation modification was conservative in the silkworm. However, there were significant differences in the distribution and enrichment ratio of the same functional elements between the two groups, suggesting that m6A modification may have different effects on gene expression regulation in different diapause traits, and may have a potential regulation effect on diapause traits.

Peak combination between groups and multiple clusters

Principal component analysis (PCA) and correlation heat map method were used to conduct pair-way correlation analysis for all 6 samples from the two groups. The results showed that the peak data were significant different between the QFLT and QFHT groups, and the peak data difference within either group was relatively consistent, further indicating that the data can be used for subsequent analysis (Additional file 1: Figure S2 and S3). The peak filters of replicate samples between groups were merged and a Venn diagram was used to show the presence or absence of RNA methylation modifications between groups. Results showed that 659 peaks were unique to QFHT and 1563 peaks were unique to QFLT, with a total of 1,984 peaks (Figure 2A). According to the statistics on the number of peaks on related genes, more m6A modifications occurred in QFLT samples than in QFHT (Figure 2B). Motif analysis of MEME (detected

8~30 bp) and Dreme (detected ≤ 8 bp) in two different length ranges using the MEME Suite method showed that the motifs prefer “TCACT” sequences which is similar to the ubiquitous "RRACH" motifs in other species (Figure 2C), indicating that the motifs recognized by m^6A modifications in silkworm were highly conserved and supported the presence of methylation modification mechanisms during silkworm development.

Difference of RNA methylation modification level between two groups

There were differences in the genes with m^6A methylation modification between the two different treatment groups. Between groups, peaks without overlaps are defined as unique peaks, and peaks with overlaps as shared peaks. Venn diagram was used to show the relationship between groups with or without RNA m6A methylation modification. The analysis showed that IP-QFHT had 659 peaks mapped to 537 genes, and IP-QFLT had 1563 peaks mapped to 1042 genes. There were totally 1,884 peaks mapped to 1,275 genes in two groups (Figure 3A).

QFHT-specific peaks were enriched in 212 GO signaling pathways, of which GO_BP (biological process): 105, GO_CC (celluLar componen): 43, GO_MF (molecuLar function): 64 (Figure 3B); It mainly focuses on cellular process, metabolic process, individual development process, biological process regulation and etc. 249 KEGG signaling pathways (Figure. 3C) accounted for the largest proportion of environmental signal processing and signal transduction pathways, were enriched to 56 genes; followed by signaling molecules and interactions and membrane transport; In the cell process, the enrichment of cell growth and death was relatively more, followed by transport and catabolic pathways. In the body system, the enrichment from high to low were metabolic system, immune system and nervous system, respectively. The rest were concentrated in metabolic signaling pathways. In the process of genetic information, QFHT was more focused on translation, folding and degradation.

QFLT-specific peaks were enriched in 335 GO signaling pathways (Figure 3D), of which GO_BP: 105, GO_CC: 43, GO_MF: 64; mainly concentrated in cellular processes, metabolic process, individual process, biological process regulation and other pathways. Similar to QFHT, 280 KEGG signaling pathways (Figure 3E), in the environmental signal processing class, accounted for the largest proportion of signal transduction pathways followed by signal molecules and interaction signaling pathways, but the number of genes enriched in both was more than twice that in QFHT. In cell process, QFLT enrichment in cellular immune pathway was the most abundant compared with QFHT, followed by catabolism, and finally cell growth and death pathway. In the body system, the top three pathways of QFLT enrichment are metabolic, immune and nervous system, which are the same as QFHT, but the number of genes enriched is nearly three times that of QFHT. In the process of genetic information, QFLT focuses more on folding and degradation. These results indicated that the peak specific to QFLT and QFHT was significantly different and the m6A modified genes had a wider regulatory range. Compared with QFHT, the number of m6A modified gene transcripts occurred in QFLT was larger and involved in a broader depth of regulation.

The common peaks can be matched to 1275 genes, which can be enriched into 478 GO signaling pathways (Figure 3F), including GO_BP: 355, GO_CC: 33, GO_MF: 90. It mainly focuses on cellular process, metabolic process, individual process, biological process regulation and other pathways. There are 291 KEGG signaling pathways (Figure 3G), which mainly focus on metabolism, genetic information processing, environmental signal processing and cellular processes. The top 20 KEGG enrichment pathways mainly include PI3K-Akt signaling pathway, hippopotamus signaling pathway and MAPK signaling pathway, etc.

Analysis of differences in RNA methylation rates between two groups

MeRIP data and Input data were used to calculate the relative methylation rate of each peak. then the RNA methylation rate differences analysis filter peak using exomePeak at FDR < 0.05 and | log2FC | > 1. GO and KEGG functional enrichment analysis was performed on genes related to differential peak.

The results showed that compared with QFHT, there were 9 down-regulated peaks and 92 up-regulated peaks in QFLT, which could clearly show the significance of differential expression in the volcano map (Figure 4A). MeRIP-qPCR verification was performed for 10 genes with differentially expressed genes, and the results showed slightly different log2FC values from the sequenced, but the whole up-regulation or down-regulation trend was consistent (Additional file 2: Table S6). the 9 down-regulated peaks were matched to 4 genes, and no enrichment was found; 92 up-regulated peaks were mapped to 64 genes (Figure 4B). 19 genes were enriched in 43 KEGG signaling pathways (Figure 4C), and enriched in 41 GO signaling pathways (Figure 4D), which were GO_BP:23, GO_CC:4, GO_MF:14. Enrichment analysis indicated that these differentially methylated genes were mainly related to signal transduction. This result suggests that in QFLT (diapause egg producer) m^6A modification involves in signal transduction in the pupal ovary through the occurrence of more m^6A modifications, thereby affecting the phenotype of diapause in progeny.

Discussion

Insects can predict impending adverse conditions based on their cyclically changing environment every year, and use this information to rationally regulate their own development and reproduction[37]. Diapause-inducing stimuli are sensed by parental individuals during a sensitive period, which is generally genetically determined and species-specific[38]. The induced signals of diapause in *B. mori* include temperature, light, humidity and some chemical substances, among which temperature has the greatest influence on diapause, especially in the late stage of embryonic development, followed by photoperiod[39–41]. Bivoltine silkwormstrains, mothes from the eggs incubated at 25℃ and long sunshine produce diapause eggs; mothes from the eggs incubated at 15℃ and short daytime treatment produce non-diapause eggs; moth from the eggs incubated at 20℃ produced diapause and non-diapause mixed eggs. The environmental stimuli received during the maternal embryonic stage act on the target organ ovary through complex signal transduction and gene regulation during the pupal stage, thereby determine diapauses or non-diapause for their offspringsr [42].

In this study, MeRIP-seq was used to sequence the epitranscriptome of the ovaries of the silkworm on day 3 in the pupal stage of different groups, QFHT (producing diapauses eggs) and QFLT (producing non-diapause eggs). Analysis of epigenetically modified differential genes between the two groups provides a way to see insight for understanding the relationship between m^6A epigenetic modification and silkworm diapause. Sequencing analysis showed that compared with QFHT, more m^6A modification events occurred in QFLT, which reflected not only the number of modified genes, but also the frequency of the same gene modification. These results were consistent with the transcriptional characteristics and protein expression levels of METTL3 in pupal ovaries, and highly correlated with the m^6A abundance in the ovaries of two multivoltine silkworm strains with different diapause characteristics. The functional enrichment and significance analysis of differentially modified genes between two groups showed that differentially modification of m^6A methylation in QFLT mostly occurred in signaling, signal transduction, cell communication, purine nucleic acid metabolism, purine nucleic acid biosynthesis, kinase activity, ligase activity, nucleic acid binding. The regulatory functions of these pathways are closely related to stress response, cell differentiation, occurrence and removal of protein modifications, phosphorylation, and phospholipid metabolism. Diapause may be a more stable genetic phenotype during the evolution of the silkworm, while non-diapause is the more often result of adaptation to the environment.

In addition, the categories of differential modified genes found in this sequencing were similar to the previous analysis results of ovarian transcriptome sequencing at the third day of pupal stage[43]. These results suggested that m^6A modification mediated a number of molecular mechanisms related to the non-diapause fate of progenies in the ovaries of QFLT pupal stage, further confirming the judgment that the third day of silkworm pupal stage is highly associated with diapauses. Based on this research, the expression changes and biological functions of some diapause associated differential modification genes can be further clarified, and also provide a basis for further exploration of the role of RNA m6A modification in the occurrence and release of diapause in silkworm.

Abbreviations

3'-UTRs: 3'-untranslated regions; 5'-UTRs: 5'-untranslated regions; CDS: Coding sequence; GO: Gene ontology; IP: Immunoprecipitation; m6A: N6 -methyladenosine; MeRIP-Seq: Methylated RNA immunoprecipitation with next-generation sequencing; QFHT: bivoltine Bombyx mori, QiuFeng, incubated at a normal temperature after HCl acid treatment to produce diapause eggs; QFLT: QiuFeng, incubated at 16.5℃,a low temperature after HCl acid treatment to produce non-diapause eggs; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Declarations

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 32072791/C170902) and the Postgraduate Research & Practice Innovation Program of Jiangsu

Province (No. KYCX21_3508).

Availability of data and materials

All analysis results data generated during this study are included in this published article and its Additional files 1, 2, 3, 4, 5, 6, 7and 8. Requests for the raw data should be made to the corresponding authors.

Authors' contributions

CYH, JT and SXJ led the experiments and designed the analytical strategy, CYH and JT performed the experiments, CYH, JT, AYNE, FBY, ZJ, WMX and SXJ analyzed the data, CYH, JT and SXJ wrote the manuscript. All authors contributed to the production of the final manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Acknowledgements

Not applicable.

Ethics approval and consent to participate

All research involving animals were conducted according to the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised in June 2004) and approved by Key Laboratory of Sericulture Genetic Improvement, Ministry of Agriculture and Rural Affairs, Institute of Sericulture, Chinese Academy of Agricultural Sciences, Zhenjiang Jiangsu.

References

1. 1. Nan X, Ng H, Johnson C, Laherty C, Turner B, Eisenman R, Bird AJN: **Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex (see comments)**. 1998, **393**(6683):P.386–389.
2. 2. Bachman KJCC: **Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene**. 2003.
3. 3. Russo, V., Bernabò, N., DiGiacinto, O., Martelli, A., Mauro, Histochemistry BJJo *et al*: **H3K9 Trimethylation Precedes DNA Methylation during Sheep Oogenesis: HDAC1, SUV39H1, G9a, HP1, and Dnmts Are Involved in These Epigenetic Events**. 2013.

4. 4. Sarraf SA, Stancheva IJMC: **Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly**. 2004, **15**(4):595–605.
5. 5. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec MJN: **Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq.** 2012, **485**(7397):201.
6. 6. Dai, Qing, Zheng, Guanqun, Liu, Nian, Pan, Tao, Parisien, Nature MJ: **N-6-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions**. 2015.
7. 7. Ito K, Barnes PJ, Adcock IMJMimm: **Histone acetylation and deacetylation**. 2000, **44**(7):309–319.
8. 8. Lee M, Kim B, Kim VJC: **Emerging roles of RNA modification: m(6)A and U-tail**. 2014, **158**(5):980–987.
9. 9. P Ś, Jinek MJe: **Structural insights into the molecular mechanism of the m(6)A writer complex**. 2016, **5**:e18434-.
10. 10. **ALKBH5 Is a Mammalian RNA Demethylase that Impacts RNA Metabolism and Mouse Fertility %J Rna Biology**. 2013, **49**(1):18–29.
11. 11. Dominik T, Cyril D, Markus B, Julien B, Research AFH-TJNA: **Solution structure of the YTH domain in complex with N6-methyladenosine RNA: a reader of methylated RNA**. 2014, **42**(22).
12. 12. Yan R, Dai W, Wu R, Huang H, Shu M: **Therapeutic targeting m6A-guided miR-146a-5p signaling contributes to the melittin-induced selective suppression of bladder cancer**. *Cancer letters* 2022, **534**:215615.
13. 13. Zhang G, Xu Y, Wang X, Zhu Y, Wang L, Zhang W, Wang Y, Gao Y, Wu X, Cheng Y *et al*: **Dynamic FMR1 granule phase switch instructed by m6A modification contributes to maternal RNA decay**. *Nature Communications* 2022, **13**(1):859.
14. 14. Schwartz S, Agarwala S, Mumbach M, Jovanovic M, Mertins P, Shishkin A, Tabach Y, Mikkelsen T, Satija R, Ruvkun GJC: **High-Resolution Mapping Reveals a Conserved, Widespread, Dynamic mRNA Methylation Program in Yeast Meiosis - ScienceDirect**. 2013, **155**(6):1409–1421.
15. 15. Li HB, Tong J, Shu Z, Batista PJ, Flavell RAJN: **m6A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathways**. 2017, **548**(7667):338–342.
16. 16. Taketo K, Konno M, Asai A, Koseki J, Ogawa KJIJoO: **The epitranscriptome m6A writer METTL3 promotes chemo- and radioresistance in pancreatic cancer cells**. 2018, **52**(2):621–629.
17. 17. Hou G, Zhao X, Li L, Yang Q, Liu X, Huang C, Lu R, Chen R, Wang Y, Jiang BJNAR: **SUMOylation of YTHDF2 promotes mRNA degradation and cancer progression by increasing its binding affinity with m6A-modified mRNAs**. 2021.
18. 18. Hahn DA, Denlinger DLJARoE: **Energetics of insect diapause**. 2011, **56**(1):103–121.
19. 19. Danks HVJB: **Insect Dormancy: An Ecological Perspective**. 1987.
20. 20. Yamashita O, Suzuki KJMHiA: **Roles of morphogenetic hormones in embryonic diapause**. 1991.
21. 21. Denlinger DL, Yocum GD, Rinehart JP: **Hormonal Control of Diapause**; 2012.

22. 22. Sirigineedi S, Ponnuvel KM, Vijayaprakash NBJBAoB, Technology: **Changes in Diapause Related Gene Expression Pattern during Early Embryonic Development in HCl-treated Eggs of Bivoltine Silkworm Bombyx mori (Lepidoptera: Bombycidae)**. 2013, **56**(1):1–10.
23. 23. Sato Y, Oguchi M, Menjo N, Imai K, Saito H, Ikeda M, Isobe M, Yamashita OJPotNAoSotUSoA: **Precursor polyprotein for multiple neuropeptides secreted from the suboesophageal ganglion of the silkworm Bombyx mori: characterization of the cDNA encoding the diapause hormone precursor and identification of additional peptides**. 1993, **90**(8):3251–3255.
24. 24. Reynolds JAJAiip: **Epigenetic Influences on Diapause**. 2017.
25. 25. Kos̆Tál V: **Review Eco-physiological phases of insect diapause**. 2013.
26. 26. Wadsworth CB, Dopman EBJJoEB: **Transcriptome profiling reveals mechanisms for the evolution of insect seasonality**. 2015.
27. 27. Bing L, Pei H, Zhang SZ, Toufeeq S, Wang J, Zhao K, Xu X, Xu JP, Biochemistry SJHJAoI, Physiology: **DNA methyltransferase BmDnmt1 and BmDnmt2 in silkworm (Bombyx mori) and the regulation of silkworm embryonic development**. 2019, **100**.
28. 28. Reynolds JA, Clark J, Diakoff SJ, Denlinger DLJIB, Biology M: **Transcriptional evidence for small RNA regulation of pupal diapause in the flesh fly, Sarcophaga bullata**. 2013, **43**(10):982–989.
29. 29. Pegoraro M, Bafna A, Davies NJ, Shuker DM, Tauber EJGR: **DNA methylation changes induced by long and short photoperiods in Nasonia**. 2016, **26**(2):203.
30. 30. Jiang T, Li J, Qian P, Xue P, Reproduction XSJM, Development: **The role of N6‐methyladenosine modification on diapause in silkworm (Bombyx mori) strains that exhibit different voltinism**. 2019, **86**(8).
31. 31. Chen S, Zhou Y, Chen Y, Gu J: **fastp : an ultra-fast all-in-one FASTQ preprocessor**. 2018.
32. 32. Langmead B, Salzberg SLJNM: **Fast gapped-read alignment with Bowtie 2**. 2012, **9**(4):357–359.
33. 33. Yong, Zhang, Tao, Liu, Clifford, Meyer, Jérôme, Eeckhoute, Biology DJG: **Model-based Analysis of ChIP-Seq (MACS)**. 2008.
34. 34. Stark R, Brown G: **DiffBind differential binding analysis of ChIP-Seq peak data**. 2011.
35. 35. Chen Y, Cui X, [illegible], Rao MK, [illegible], [illegible], Jia M, Huang Y:**Unveiling the dynamics in RNA epigenetic regulations**. In: *IEEE International Conference on Bioinformatics & Biomedicine: 2014*; 2014.
36. 36. Luo GZ, Macqueen A, Zheng G, Duan H, Dore LC, Lu Z, Liu J, Chen K, Jia G, Bergelson JJNC: **Unique features of the m6A methylome in Arabidopsis thaliana**. 2013, **5**:5630.
37. 37. Tougeron KJEEeA: **Diapause research in insects: historical review and recent work perspectives**. 2019, **167**(1):27–36.
38. 38. Ecology WJARo, Systematics: **Insect Seasonality: Why?** 1988.
39. 39. [illegible], [illegible], [illegible], [illegible] [illegible]. 2017, **43**(6):8.
40. 40. Jackson, Botany SJAo: **Photoperiodism. The biological calendar**. 2011, **108**(7):vi-vi.
41. 41. Hahna DA, Denlingerb DLJJOIP: **Meeting the energetic demands of insect diapause: nutrient storage and utilization**. 2007, **53**(8):760–773.

42. 42. Hodek IJEJoE: **Diapause development, diapause termination and the end of diapause**. 1996, **93**(3):475–487.

43. 43. Chen YR, Jiang T, Zhu J, Xie YC, Tan ZC, Chen YH, Tang SM, Hao BF, Wang SP, Huang JSJCBPPDGP: **Transcriptome sequencing reveals potential mechanisms of diapause preparation in bivoltine silkworm Bombyx mori (Lepidoptera: Bombycidae)**. 2017:68–78.

Figures

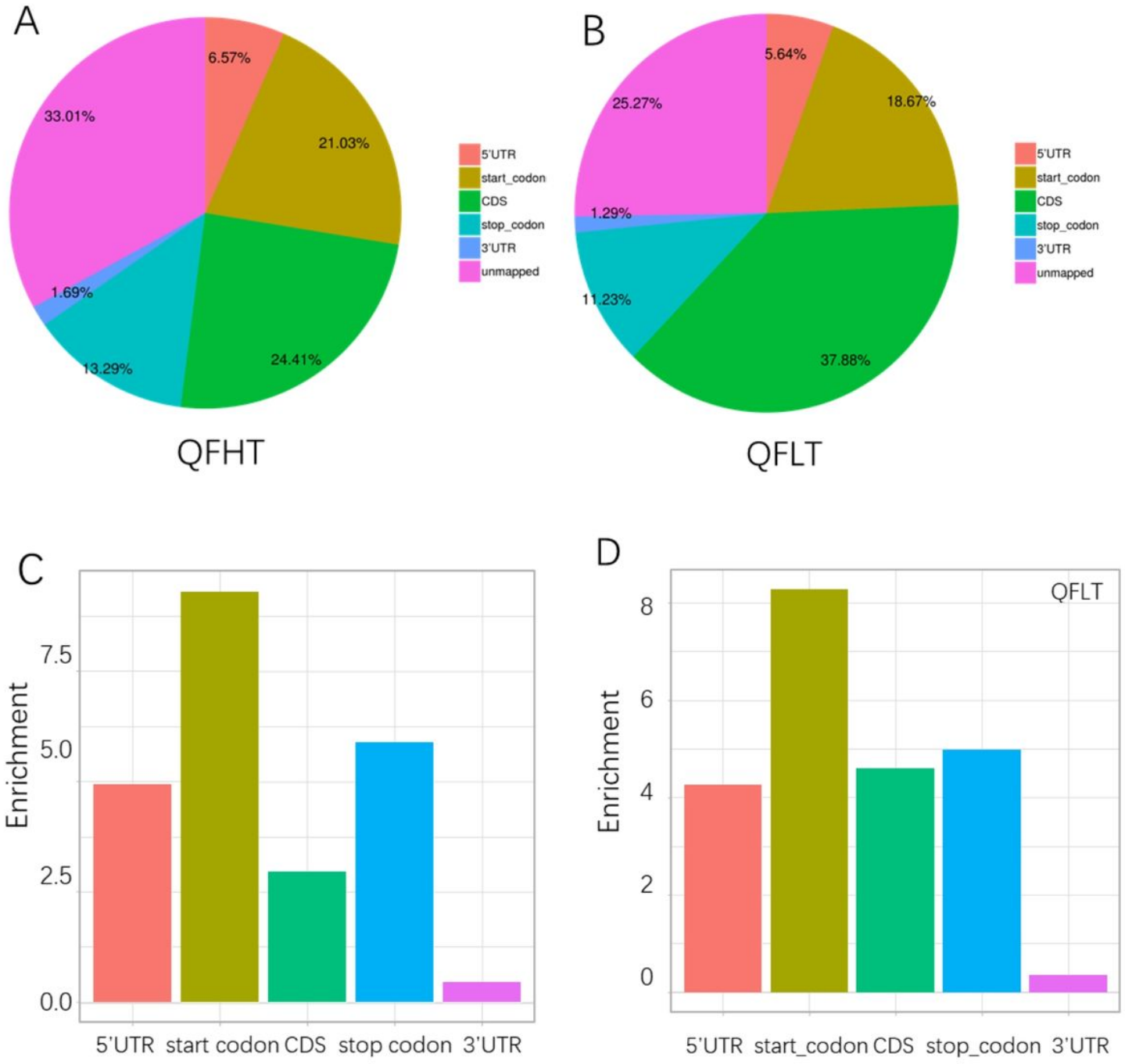

Figure 1

Distribution and enrichment of M6A modification. **A and B.** Distribution of IP-QFHT and IP-QFLT sample peaks in gene functional elements. **C and D.** Enrichment of IP-QFHT and IP-QFLT sample peaks in gene functional elements.

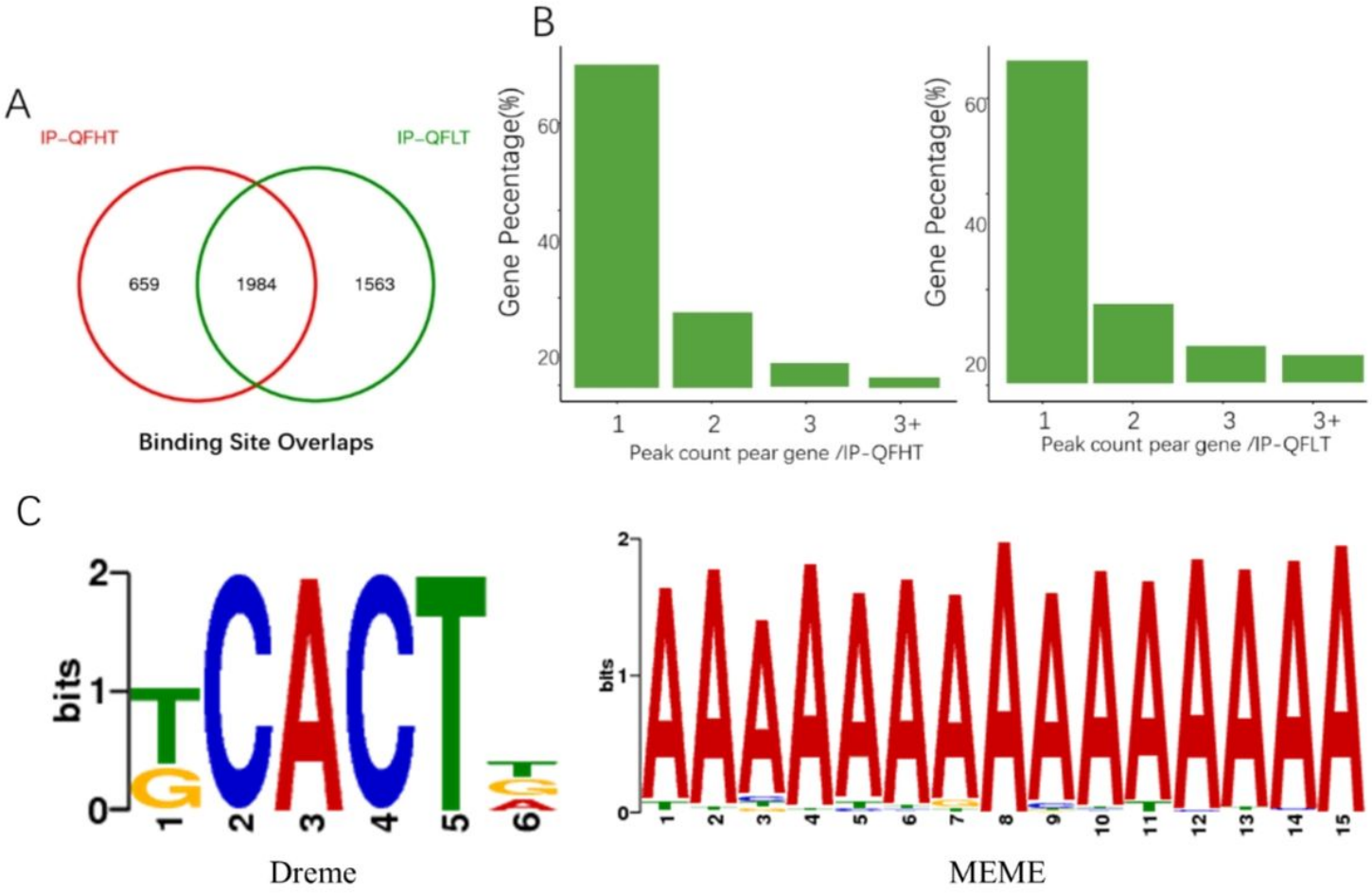

Figure 2

Peak combination between groups and multiple clusters. **A.** Venn diagram of the number of common and unique peaks in the comparison group IP-QFLT-vs-IP-QFHT. **B.** the number of peaks on peak-related genes. **C.** m6A recognition motif analysis.

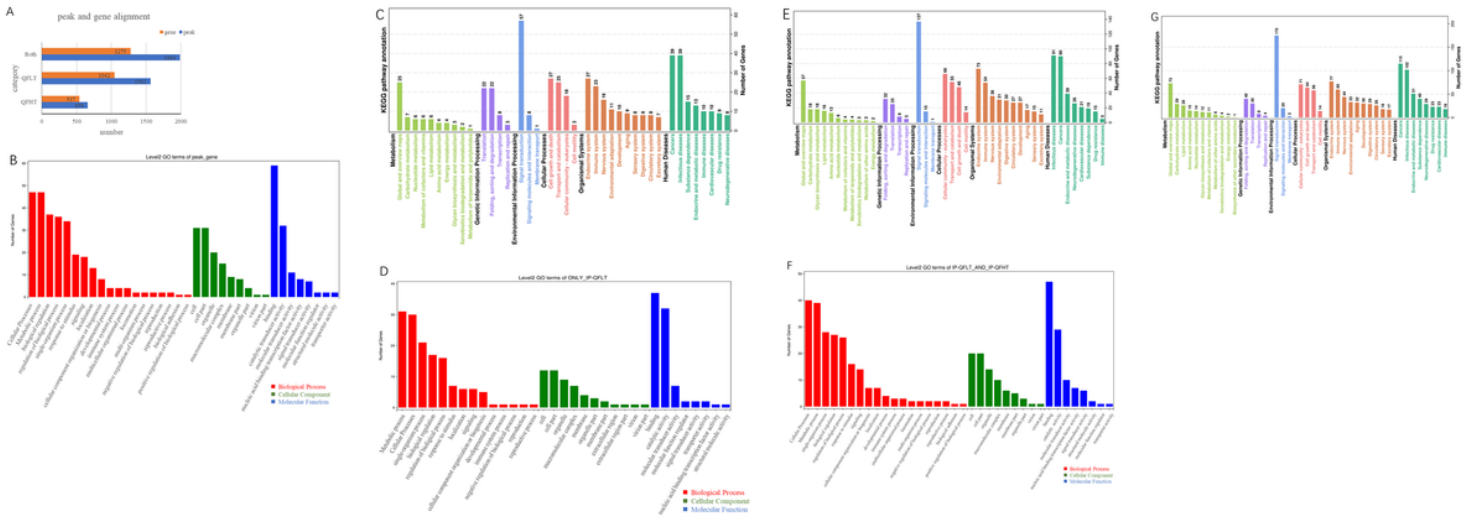

Figure 3

Differential analysis of the presence or absence of RNA methylation modification between groups. **A.** Alignment analysis of peak of IP-QFHT, IP-QFLT and common in the silkworm genome. **B and C.** GO and KEGG signaling pathway enrichment analysis of IP-QFHT specific peaks. **D and E.** GO and KEGG signaling pathway enrichment analysis of IP-QFLT specific peaks. **F and G.** GO and KEGG signaling pathway enrichment analysis of common to IP-QFHT and IP-QFLT.

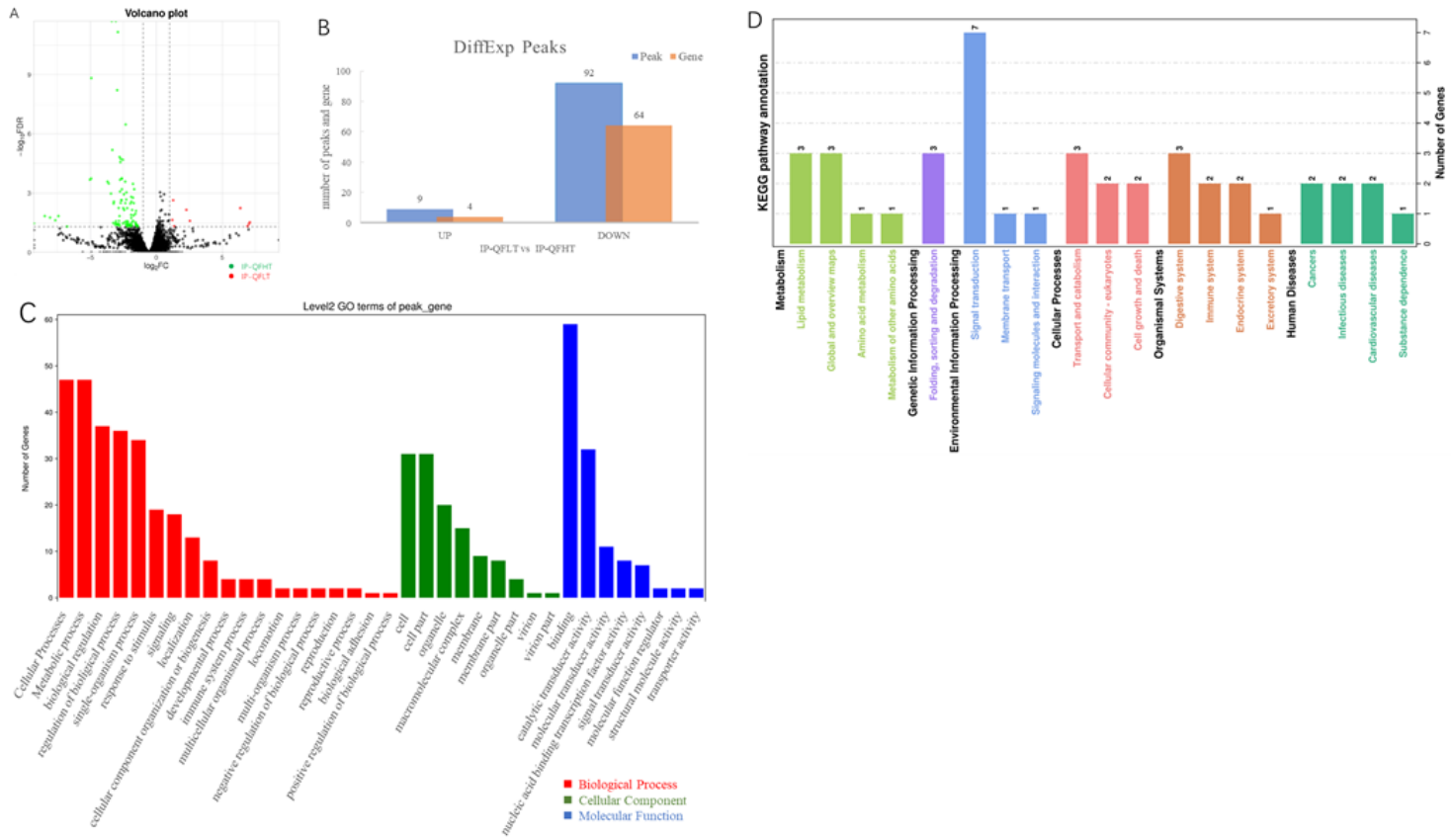

Figure 4

Difference analysis of peak methylation rate between groups. **A.** differentially expressed genes in IP-QFHT and IP-QFLT. **B.** number of differentially expressed peaks and corresponding genes. **C and D.** GO and KEGG enrichment analysis of m^6A differential modification rates of peak in IP-QFHT and IP-QFLT.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1figures.docx
- Additionalfile2Tables.docx
- Additionalfile3DataS1.xls
- Additionalfile3DataS1.xls
- Additionalfile3DataS2.pdf
- Additionalfile4DataS1.pdf
- Additionalfile5DataS1.pdf
- Additionalfile6DataS1.pdf
- Additionalfile7DataS1.pdf
- Additionalfile7DataS2.pdf
- Additionalfile8DataS1.pdf
- Additionalfile8DataS2.pdf