A Novel Prognostic Model Based on N6-Methyladenosine Regulators Predicts the Prognosis of Acute Myeloid Leukemia

Li Wang (liwangls@yahoo.com)
First Affiliated Hospital of Chongqing Medical University
Guangyu Zhou
First Affiliated Hospital of Chongqing Medical University
Yu Zhu
First Affiliated Hospital of Chongqing Medical University
Han Xiao
First Affiliated Hospital of Chongqing Medical University
Duo Cai
First Affiliated Hospital of Chongqing Medical University

Article

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Abstract

Little is known about the role of N6-methyladenosine (m6A) RNA methylation in the pathogenesis of acute myeloid leukemia (AML). We identified m6A regulators as independent prognostic factors in AML and constructed a prognostic model based on m6A regulators. We performed differential analysis, functional enrichment analysis and protein-protein interaction relationships of the TCGA-LAML cohort. The relationship between m6A methylation and AML was demonstrated by copy number variation, methylation and co-expression analyses. Pathway activity and drug sensitivity analysis revealed possible pathogenic mechanisms of m6A in pan-cancer. Our study elucidates the important role of m6A regulators in the clinical prognosis of AML, and a prognostic model based on m6A regulators can accurately predict overall survival in AML patients.

Introduction

There are more than 100 chemical modifications in post-transcriptional modifications in eukaryotes, among which N6-methyladenosine (m6A) is the most abundant modification. [1] N6-methyladenosine remains three different types of regulators, including methyltransferases (“writers”), m6A-binding proteins (“readers”), and demethylases (“erasers”).[2] Methyltransferases complex containing methyltransferase-like3 (METTL3), methyltransferase-like14 (METTL14), Wilms' tumor 1-associating protein (WTAP), KIAA1429, ZC3H13, METTL16 and RBM15/RBM15B catalyze methyl transfer efficiently. [3–7] In the methyltransferases complex, METTL3 Primarily serve as the catalytic core, while METTLL14 serve as the RNA-binding platform. [8] Since the process of m6A is dynamic and reversible, it is removed by m^6A demethylases, including fat-mass and obesity-associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5). [9,10]. The m6A recruits m6A-binding protein called “readers”, which mediates its functions primarily, including the multiprotein eukaryotic initiation factor 3 complex and a group of proteins that contain YTH domains. In addition to being involved in RNA metabolism, the m6A reader is involved in different biological processes, including tumorigenesis, hematopoiesis, viral replication, immune responses, and adipogenesis [10–12].

The survival of patients with acute myelogenous leukemia (AML) remains poor, and current studies suggest that the pathogenesis of AML is affected by the joint action of multiple mechanisms. However, the mechanism and role of m6A in it and the impact on prognosis are still unclear. Therefore, it is necessary to further study the role of m6A in the pathogenesis of AML.[13] More and more studies have proved that m6A regulators plays an important role in the occurrence and progression of leukemia. A study found that METTL3 necessarily regulates the genes of acute myeloid leukemia, which takes part in the maintenance of leukemic state. [14] What is more, a study demonstrated that FTO, as an m6A demethylase, modulates the expression of genes such as ASB2 and RARA by reducing m6A levels in mRNA thereby enhancing leukemic oncogene-mediated cell transformation and leukemogenesis and inhibits all-trans-retinoic acid (ATRA)-induced AML cell differentiation.[15] Besides, the silencing of METTL14 facilitates terminal myeloid differentiation in normal hematopoietic stem/progenitor cells

(HSPCs) and AML cell, which is required for the maintenance of AML and self-renewal of leukemia stem/initiation cells (LSCs/LICs).[16] YTHDC1, regulating leukemogenesis through MCM4, is overexpressed in AML hence it is crucial for the proliferation of human AML cells.[17] However, the study of differential expression, function enrichment, protein-protein interaction network, CNV, methylation, pathway activity, drug sensitivity, survival and co-expression analysis of m6A regulators of AML is still lacking and an accurate prognostic model based on m6A regulators is vacant.

Here, for the first time, we established a risk scoring model based on m6A regulators to predict the survival of AML patients and analyzed the expression of 21 (METTL3, METTL14, WTAP, KIAA1429, RBM15, RBM15B, ZC3H13, METTL16, FTO, ALKBH5, YTHDF1, YTHDF2, YTHDF3, YTHDC1, HNRNPC, HNRNPG, HNRNPA2B1, IGF2BP1, IGF2BP2, IGF2BP3, and FMRP) widely reported and studied m6A related regulators based on TCGA, GTEx and GEO data sets. Moreover, we conducted the differential expression analysis, function enrichment analysis, protein-protein interaction network analysis, CNV analysis, methylation analysis, pathway activity analysis, drug sensitivity analysis, and co-expression analysis of m6A regulators of AML. In addition, the risk scoring model based on the most relevant m6A regulators significantly predicted the prognosis of AML patients, which may reveal the relationship between m6A methylation regulators and clinical outcomes in the process of AML (Fig. 1).

Materials & Methods

Data

We downloaded the expression data of acute myeloid leukemia patients in the TCGA database. The TCGA-LAML contained 151 patients' RNA sequencing data. Due to the lack of normal control group, we selected blood samples (n = 444) from the GTEx database to fill this gap. The TCGA-LAML and GTEx-blood expression data were all downloaded from the UCSC Xena (https://xenabrowser.net/). Moreover, owing that part of the clinical information were absent, the survival analysis covered a 136 patients' cohort of TCGA-LAML. And the GSE37642 (GPL570, n=140), which downloaded from NCBI GEO datasets (https://www.ncbi.nlm.nih.gov/gds), were chosen to validate the risk scoring prognostic model.

Survival Analysis

First, we used lasso regression by "glmnet" and "survival" R packages (R version 4.1) to screen out 7 genes significantly associated with prognosis and constructed a risk scoring model based on regression coefficients. The risk assessment model was validated using cox regression as reliable. Then, ROC and AUC through "timeROC" and "survival" R packages (R version 4.1) validated the value of our model. Next, we calculated the risk score of patients with TCGAs, which were divided into high risk and low risk by the cutoff value that obtained from "survminer" R packages, and used the "survival" R packages to obtain the Kaplan-Meier curve. Furthermore, we validated the risk scoring model with the GSE37642 cohort. The *p*-value less than 0.05 was regarded statistically significant. The risk scoring was as follow:

$$\text{Risk score} = \sum_{i=1}^{n} Coefi \times Xi$$

Differential expression analysis

We obtained the expression data of m6A, and used the R software (Version 3.6 and 4.1) to merge and standardized the data of TCGA-LAML and GTEx-blood. Then we used the “limma” R package to remove the batch effects. We defined TCGA-LAML as the tumor group and GTEx-blood as the normal group. Among the 21 regulators we studied, 19 regulators (METTL3, MTEEL14, WTAP, KIAA1429, RBM15, RBM15B, ZC3H13, METTL16, FTO, ALKBH5, YTHDF3, YTHDF2, YTHDF1, YTHDC1, HNPRNPC, HNRNPA2B1, IGF2BP1, IGF2BP2, and IGF2BP3) had expression data in both groups. Therefore, we performed differential expression analysis on these 19 genes by the “limma” R package. Finally, we used the “pheatmap” R package and the “corrplot” R package to visualize the results. The *p*-value <0.05 was regarded statistically significant.

Function Enrichment Analysis and Protein-Protein Interaction Network

We used the NetworkAnalyst (https://www.networkanalyst.ca/) to conduct a gene ontology molecular function enrichment (GO:MF) analysis. [18] The STRING datasets (https://string-db.org/) were used to conduct the protein-protein interaction network.[19]

CNV, Methylation, Pathway Activity analysis

We conducted copy number variation(CNV) analysis, methylation analysis and pathway activity analysis through GCSA online analysis tool (http://bioinfo.life.hust.edu.cn/GSCA/#/).[20] All of the 21 regulators we imported were valid. Firstly, the statistics of heterozygous and homozygous CNV of AML are displayed, and Pearson correlation is performed between gene expression and CNV of each gene we imported in AML to help to analyze the gene expression significantly affected by CNV. Secondly, the correlations between methylation and mRNA expression of m6A regulators were presented, as well as the relationship between methylation and survival difference. Thirdly, we conducted pathway activity to finger out the mechanism out m6A regulators. The pathway included TSC/mTOR, RTK, RAS/MAPK, PI3K/AKT, Hormone ER, Hormone AR, EMT, DNA Damage Response, Cell Cycle, Apoptosis pathways, which were all famous cancer related pathways. The *p*-value less than 0.05 was regarded statistically significant.

Drug Sensitivity Analysis

To identify the drug sensitivity of m6A related regulators, we used the GSCA online analysis tool (http://bioinfo.life.hust.edu.cn/GSCA/#/) to conduct a pan-cancer analysis. [20] On the one hand, Genomics of Therapeutics Response Portal (CTRP) collected the IC50 of 481 small molecules in 1001 cell lines, and Pearson correlation analysis was performed to gain the correlation between mRNA expression of m6A related genes and drug IC50. P-value was adjusted by FDR. On the other hand, GDSC (Genomics of Drug Sensitivity in Cancer) showed a plot for the top 16 ranked drugs that integrated by level of correlation coefficient and FDR.

Co-expression Analysis

Correlation analysis was then performed between the common mutated genes expression and the gene expression of m6a regulator. In the study, we chose 32 significantly related AML genes to conduct a co-expression analysis in order to uncover the state of m6A regulators' expression and AML common mutations. The "corrplot" R package (R version 4.1.2) was used to prove it.

Results

Survival Analysis

In this study, we used LASSO (Least Absolute Shrinkage and Selection Operator) regression to screen out 7 genes (ALKBH5, FTO, HNRNPA2B1, IGF2BP2, IGF2BP3, YTHDF1, YTHDF3) significantly associated with prognosis and constructed a risk scoring model based on regression coefficients (Figure 2A, Figure 2B). Then, the risk scoring model was validated using cox proportional-hazards model (Figure 2C). The forest plot showed the effect of the references, which were age, gender and risk score, on the outcome which defined as death. We concluded that gender, in the TCGA-LAML cohort, has no effect on the survival of patients ($p>0.05$). Both age and risk score were factors that affect survival ($p<0.001$). Furthermore, our risk scoring model has an ideal area under the curve (AUC), which is 0.752 in 1-year survival, 0.789 in 3-year survival, and 0.864 in 5-year survival (Figure 2D). The Kaplan-Meier curve based on the risk scoring model also showed statistical significance ($p<0.0001$). Among TCGA-LAML patients, the prognosis of the high-risk group was significantly worse than that of the low-risk group (Figure 2E), indicating the relationship between the regulators and the prognosis. In addition, the GSE37642 cohort validated the risk scoring model (Figure 2F), and in GSE37642, the high-risk and low-risk groups were also significantly separated, showing statistical significance ($p<0.01$).

Differential expression analysis of m6A regulators in AML

We obtained the expression data of m6A from the TCGA and GTEX databases, using TCGA-LAML as the tumor group and GTEx as the normal control group. Figure 3 shows that the differential expression level of m6A related regulators between tumor group and normal group. As we can see, the expression levels of METTL3, MTEEL14, WTAP, KIAA1429, RBM15, RBM15B, ZC3H13, METTL16, FTO, ALKBH5, YTHDF3,

YTHDF2, YTHDF1, YTHDC1, HNPRNPC, HNRNPA2B1, IGF2BP1, IGF2BP2, and IGF2BP3 were significantly different in AML and normal tissues ($p < 0.05$). Figure 4 showed that the differential expression heatmap of 19 regulators of m6A which indicated that the HNRNPA2B1, WTAP, HNPRNPC, IGF2BP3 and KIAA1429 were highly expressed in normal tissues compared with the tumor group. It also showed that the expression levels of IGF2BP2, RBM15, RBM15B, YTHDF3, YTHDF2, YTHDF1, YTHDC1, ZC3H13, MTEEL14, METTL16, METTL3, FTO, ALKBH5 were higher in AML samples than normal group. The overexpression or reduction of IGF2BP1 was unclear between the two groups.

Function Enrichment Analysis and Protein-Protein Interaction Network

The Gene Ontology molecular function enrichment (GO:MF) analysis indicated that the m6A-associated regulators were enriched in RNA binding, translation regulator activity, methyltransferase activity, transferase activity, RNA splicing, nucleotide binding, S_adenosylmethionine_dependent methyltransferase activity, oxidoreductase activity, and telomeric DNA binding, which showed that m6A regulators were involved in the metabolic process of RNA (Figure 5A). The enrichment of these functions may be the underlying mechanism of the pathogenesis of AML. Figure 5B showed the protein-protein interaction network of m6A regulators. As we can see, the proteins of m6A regulators interacted with each other, not only are they independent. It should be noted that they formed part of the cellular response network and regulated cell behavior to a certain extent.

CNV Analysis

Figure 6 presented the CNV analysis of m6A related regulators in AML. The pie plot summarized the CNV of m6A regulators' genes in the AML, in which pointed out HNRNPA2B1 had 8.9005236% of copy number deletion in total, including heterozygous and homozygous deletion (Supplement Table1). Similarly, IGF2BP3, ALKBH3, RBM15B, FMR1, RBMX, FTO and IGF2BP1 also showed copy number deletion. On the other hand, copy amplification also includes heterozygous amplification and homozygous amplification. Through Figure 6A, we can see that the heterozygous amplification of YTHDF3 was very significant accounted for a percentage of 12.565445 and ZC3H13, YTHDF2, YTHDF1, RBM15, METTL14, HNRNPC, YTHDC1, WTAP, METTL3, IGF2 IGF2BP2, FTO, RBMX, FMR1 also showed copy amplification. It is worth noting that in AML, METL16 and ALKBH5 only had copy deletion, but no copy amplification and RBM15, WTAP, YTHDF1, YTHDF3 only had copy amplification, but no copy deletion (Figure 6B and Figure 6C). Figure 6D showed the relationship between CNV and mRNA expression through correlation analysis which was not very obvious, because the Spearman correlation coefficients were all less than 0.03 (Supplement Table). Furthermore, Figure 6E provided that the LogRank tests were performed to test the difference in AML. We can see that the CNV of METTL3, IGF2BP2, ZC3H13, RBM15B, METTL14, FTO, IGF2BP1, YTHDC1, YTHDF2 genes and the overall survival of AML were statistically different (p <0.05).

Methylation Analysis

The results of correlation analysis between methylation level and mRNA expression level were shown in Figure 7A. We found that the methylation of IGF2BP2, FTO, ALKBH5, IGF2BP3 is negatively correlated with the expression level, and the correlation between the methylation of other regulators and the expression level is not very obvious. According to Figure 7B, there is a statistical significance between the methylation of ZC3H13, IGF2BP1, and HNRNPC and survival. Their hazard ratios are all less than 1, which means lower methylation has higher risk of death.

Pathway Activity Analysis

We conducted the difference of genes expression between pathway activity groups (activation and inhibition) that defined by pathway scores through GSCALite. Global percentage (Figure 8A) of cancers in which a gene has effect on the pathway among 32 cancers types, presents the percentage of cancer type that are activated or inhibited. We found that m6A related regulators can activate or inhibit apoptosis, cell cycle, DNA damage response, epithelial-mesenchymal transition (EMT), hormone AR, hormone ER, PI3K/AKT, RAS/MAPK, RTK, TSC/mTOR pathways. Figure 8B shows the m6A regulators that have function in at least 5 cancer types. Through the pan-cancer analysis, we can see that in the apoptosis pathway, FTO, METTL14 and YTHDC1 mainly play an inhibitory role and HNRNPA2B1, HNRNPC, IGF2BP3, RBM15 and WTAP mainly play a role in activation. In the cell cycle pathway, FTO strongly inhibit it, yet HNRNPA2B1, HNRNPC, IGF2BP1, IGF2BP3, KIAA1429, METTL3, RBM15, RBMX and YTHDF1 mostly activate it. In DNA damage response, HNRNPA2B1, HNRNPC, IGF2BP3, RBM15B and RBMX mainly play a role in activation. In epithelial-mesenchymal transition, HNRNPC plays a role in inhibition, and FTO and IGF2BP2 mainly play a role in activation. In hormone AR or ER pathways, HNRNPA2B1 and KIAA1429 activate the hormone AR, yet inhibit the hormone ER. Other m6A regulators are compatible with the regulation of estrogen and androgen receptors. In PI3K/AKT pathway, METTL14 activates it while IGF2BP2 inhibits it. In RAS/MAPK pathway, HNRNPA2B1, HNRNPC and RBMX play an inhibitory role and none of the m6A related regulators evidently activate the process of RAS/MAPK. In RTK pathway, HNRNPC plays an inhibitory role while FTO, METTL14 and YTHDF3 activate it. And we can see that YTHDF2 mainly activate the pathway of TSC/mTOR. In all, it should be noted that the involvement of m6A in the tumor process remains multi-pathway and multi mechanism.

Drug Sensitivity Analysis

As we can see, Figure 9A summarizes the correlation between gene expression and the sensitivity of Genomic of Drug Sensitivity in Cancer (GDSC) top 16 drugs. The expression of IGF2BP2 had a negative relationship with the sensitivity of trametinib and docetaxel and a positive relationship with the sensitivity of Navitoclax, KIN001-102, NPK76-II-72-1, I-BET-762 (FDR <0.05) (Supplement Table2). Moreover, Figure 9B and Supplement Table3 presents the correlation between m6A related gene expression and the

sensitivity of Genomics of Therapeutics Response Portal (CTRP) top 30 drugs. The higher the expression of IGF2BP2, the strong the drug sensitivity of LRRK2-IN-1, serdemetan, QW-BI-011, PRIMA-1, BRD-A94377914, belinostat, BIX-01294, PX-12, Panobinostat (FDR <0.05). It is important to point out that m6A related genes may become a new target for tumor therapy.

Co-expression Analysis

In this study, we analyzed the co-expression within m6A regulators and the co-expression between m6A and 32 common mutations in AML (Figure 10). First, in terms of the co-expression of m6A regulators, we found that METL14 has a moderately positive correlation with KIAA1429, ZC3H13, and YTHDC1. KIAA1429 has a moderately positive correlation with ZC3H13 and YTHDC1. METTL3 is negatively correlated with the expression of ALKBH5, and ALKBH5 is negatively correlated with the expression of YTHDC1. Then, in the co-expression between the common mutations of AML and m6A regulators, we can see that METTL3 is positively correlated with ASXL1 and SF3B1; METL14 has a positive correlation with NF1, PPM1D; KIAA1429 shows positive correlation with NF1, NRAS and STAG2, and negative correlation with CEBPA; there exists a correlation between RBM15B and IDH2; ZC3H13 has a correlation with NF1 and STAG2; METL16 has a correlation with NF1 and NPM1. There is a correlation between ALKBH5 and TP53, and ALKBH5 remains negatively correlated with SF3B1; YTHDF1 is negatively correlated with ZRSR2; YTHDF2 is correlated with NPM1; YTHDF3 is negatively correlated with IDH2, and is positively correlated with NRAS; YTHDC1 is positively correlated with NF1, STAG2; HNRNPC is positively correlated with CSF3R and negatively correlated and positively correlated with NPM1; HNRNPA2B1 is negatively correlated with BCORL1 and MPL; IGF2BP3 is negatively correlated with CALR and CEBPA and positively correlated with SETBP1.

Discussion

Our study shed new light on the role of m6A related regulators in the process and clinical outcomes of AML by using bioinformatics analysis method and public resource databases.

AML prognostic stratification is mainly based on NCCN guidelines. Nowadays, with the development of sequencing technology, more and more prognostic models have appeared, including those based on immune microenvironment correlation[21,22], surface-enhanced Raman spectroscopy[23], hypoxia[21] and other related prognostic models. In our study, we found that the expression level of ALKBH5, FTO, HNRNPA2B1, IGF2BP2, IGF2BP3, YTHDF1 and YTHDF3 significantly affected the overall survival of AML patients. The overall survival of patients in the high-risk group was significantly lower than that in the low-risk group. Therefore, the risk scoring model we established based on those 7 regulators' expression data could replenish the current AML prognostic assessment systems, especially in epigenetics.

RNA methylation sequencing has not been carried out on a large scale, let alone used in clinical patients. However, we can indirectly reflect m6A methylation levels through the expression of m6A-related genes,

which is a concise analysis method based on traditional RNA sequencing. We found that HNRNPA2B1, WTAP, HNPRNPC, IGF2BP3 and KIAA1429 were lowly expressed and IGF2BP2, RBM15, RBM15B, YTHDF3, YTHDF2, YTHDF1, YTHDC1 ZC3H13, MTEEL14, METTL16, METTL3, FTO, ALKBH5 were highly expressed in AML samples. Meanwhile, we also found that there was a correlation within the expression of m6A related genes, and between these genes and the common mutated genes in AML, which indicated the m6A regulators may be the biomarker of AML pathogenesis. Recently, a study found that high WTAP expression predicts poor prognosis in AML.[24] In addition, FTO inhibits all-trans-retinoic acid (ATRA)-induced AML cell differentiation by regulating expression of targets such as ASB2 and RARA by reducing m6A levels in mRNA transcripts.[25]

Copy number variations (CNVs) play an important role in human disease and biology.[26] A new study found that METTL3 with CNV is associated with immune infiltration and serves as a prognostic marker for bladder cancer.[27] A study found that copy number variation in the m6Aregulators gene affects both survival and disease-free survival in non-small cell lung cancer.[28] In our study, we identified that the CNV of METTL3, IGF2BP2, ZC3H13, RBM15B, METTL14, FTO, IGF2BP1, YTHDC1, YTHDF2 genes affected the overall survival of AML patients. Moreover, we also found that lower methylation of ZC3H13, IGF2BP1, and HNRNPC had higher risk of death, which may be a mechanism of poor disease prognosis.

Research on tumor-targeted drugs is in full swing, and there are many studies on m6A-related targets. We estimated that some drugs may be the potential targets for therapy on account of the correlation between m6A related gene expression and drug sensitivity. A recent study showed that inhibition of METTL3 in vivo results in impaired engraftment and prolonged survival in various AML mouse models.[29] Additionally, researchers pointed out that FTO inhibitors significantly inhibits the progression of human AML cell lines and primary cells in xeno-transplanted mice.[30] In our study, we first used the sensitivity of GDSC and CTRP to analysis the m6A regulators expression. We found that IGF2BP2, one of the core regulators of m6A “reader”, showed the correlation with various drugs, including navitoclax, serdemetan, belinostat etc. Navitoclax, as know as ABT264, is a BCL-2 inhibitor, which enhances the cytotoxicity of multiple chemotherapeutic agents in hematological tumors. [31] Our study provides new insight for the development of new tumor targeted-drugs, as well as a way to explain the drug resistance mechanisms. Besides, we found that m6A methylation played a role in various pathways that helped explain the close relationship between m6A and tumorigenesis. A study found that YTHDF2 localized as a suppressor of inflammatory pathways in HSCs and highlighted the importance of m6A in long-term HSC maintenance.[32]

Our findings on drug sensitivity and pathway activity are not only meaningful for AML research, but also have hints for pan-cancer. Nonetheless, our study has limitations. We merged the samples from two different databases (GTEx and TGCA), and there would be errors when we conducted differential analysis. However, due to the lack of normal controls for TCGA-LAML, we had to use this method, and previous studies also used the same method. [33] Additionally, we use “limma” R package to remove batch effects to minimize result error.

In conclusion, our study demonstrated that m6A regulators plays an important role in the occurrence and clinical prognosis of AML. The m6A-based risk scoring model can complement the prediction of the prognosis of leukemia patients. The m6A regulators complements the epigenetic explanation of the pathogenesis of AML and can become a new biomarker of AML, providing new targets for the research of tumor drugs.

- ## Future Perspective:

In future studies, more research is needed to find out the specific molecular mechanism of m6A and the pathogenesis of acute myeloid leukemia. Inhibitors or activators of m6A may become novel drugs for the treatment of acute myeloid leukemia.

- ## Summary Points:

Risk score model based on m6A regulators can independently predict the prognosis of AML patients.

The m6A regulators gene expression levels were significantly different between AML group and normal group.

The m6A regulators formed part of the cellular response network and regulated cell behavior to a certain extent.

The m6A regulators are functionally enriched and involved in various pathological processes.

The CNV of m6A regulators' genes affected the overall survival of AML patients.

In pan-cancer, the m6A regulators are associated with multiple pathways.

Some drugs are related to the m6A regulators which are potential targets for tumor treatment.

The m6A regulators correlate with the expression of common mutations in AML.

Declarations

- ## Data Availability:

The datasets analysed during the current study are available in the TCGA, GTEx (https://xenabrowser.net/) and GEO repositories (https://www.ncbi.nlm.nih.gov/gds).

- ## Author Contributions:

Guangyu Zhou analyzed the data, performed bioinformatics analysis and figure generation and wrote the manuscript; Yu Zhu analyzed the data; Han Xiao performed bioinformatics analysis; Duo Cai performed

figure generation; Li Wang provided expert advice and editing the manuscript.

- ## Acknowledgements:

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- ## Financial disclosure:

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- ## Information pertaining to writing assistance:

N/A

- ## Ethical disclosure:

TCGA, GEO, GTEx belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open-source data, so there are no ethical issues and other conflicts of interest.

- ## Method and experimental statement:

We confirm that all methods were carried out in accordance with relevant guidelines and regulations. We confirm that all experimental protocols were approved by licensing committee.

- ## Informed consent statement:

We confirm that informed consent was obtained from all subjects and/or their legal guardian(s).

- ## Data sharing statement:

The datasets presented in this study can be found in online repositories. The name of repositories and cohorts we used can be found in the article.

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Figures

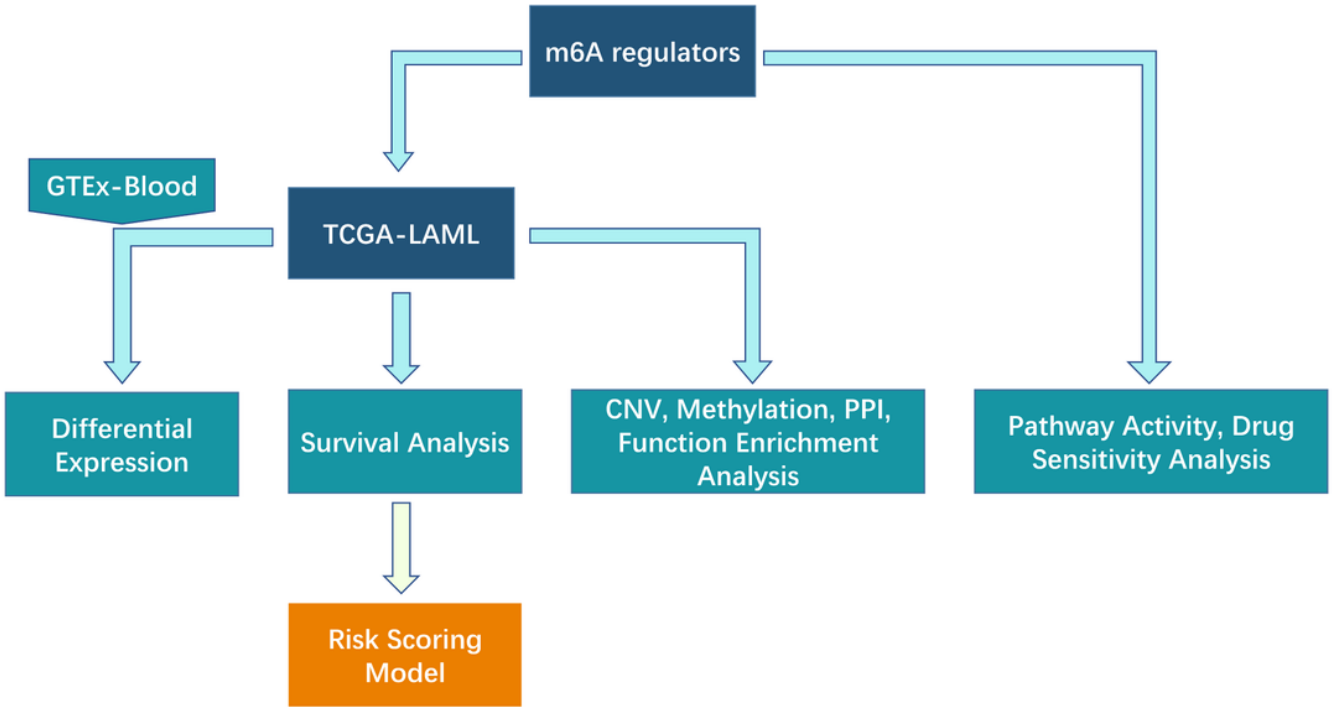


Figure 1

Flowchart of the study.

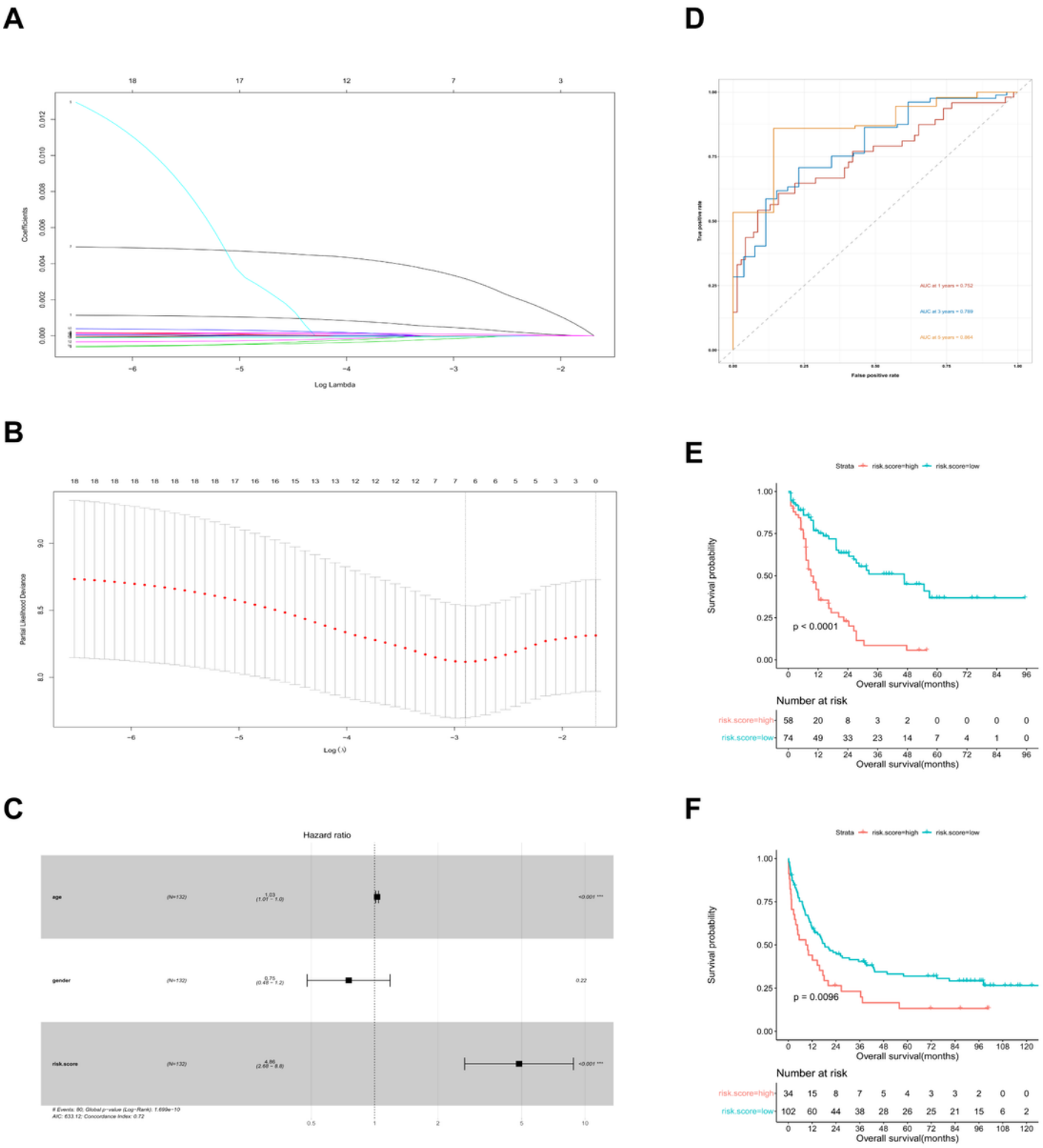


Figure 2

Survival analysis of m6A regulators for AML. **(A)** Log(Lambda) value of clinical factors in LASSO model; (**B**) The most proper Log(Lambda) value in LASSO model; (C) Forest plot of Cox regression analysis; (**D**) The receiver operating characteristic (ROC) curves of 1 years, 3 years and 5 years overall survival (OS) of risk scoring model for TCGA cohort; (**E**) The Kaplan-Meier curves of the TCGA patients in high and low

risk stratifications; (**F**) The Kaplan-Meier curves of the GSE37642 patients in high and low risk stratifications.

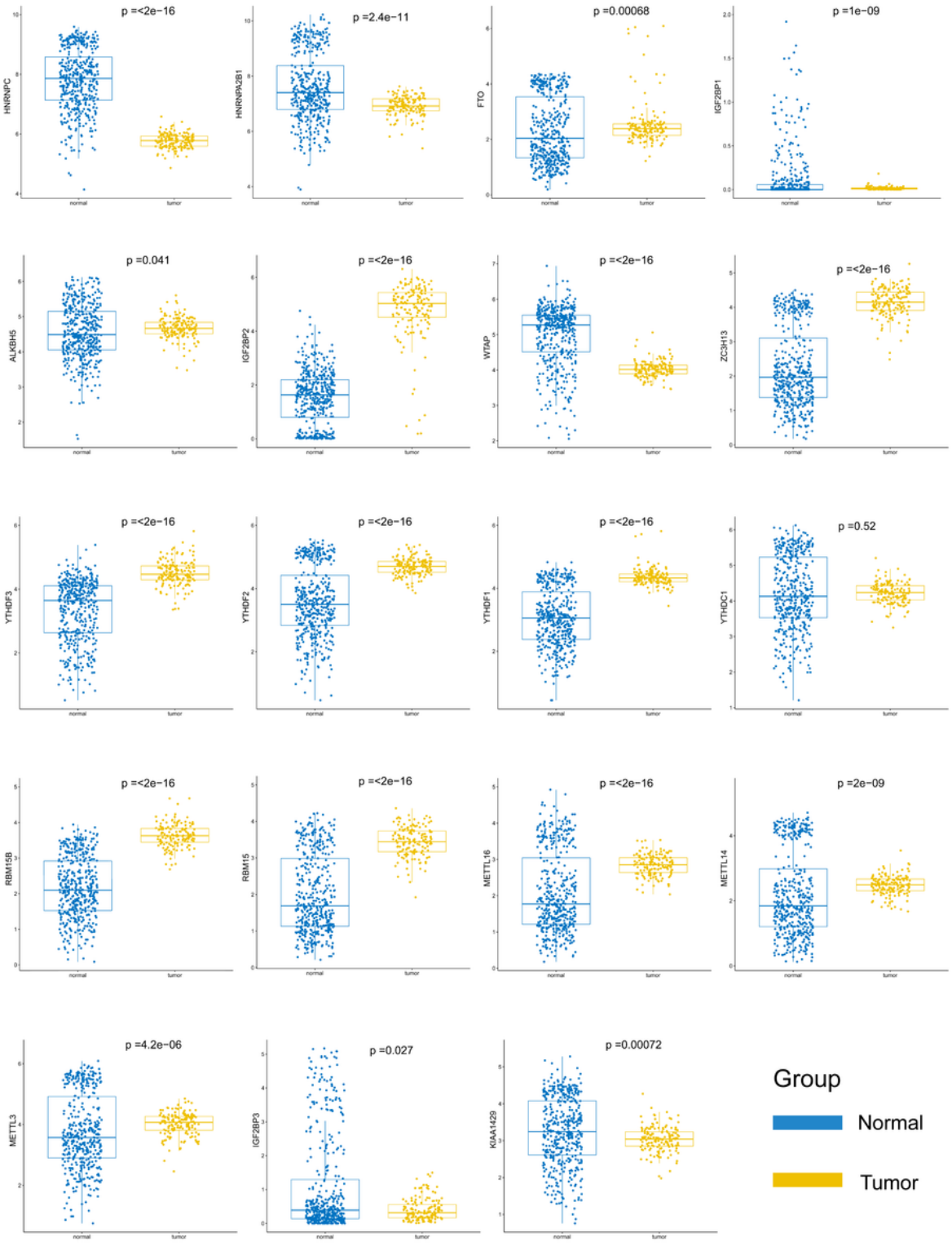


Figure 3

Boxplot of 19 m6A regulators differential expression between tumor and normal groups. The blue boxplots indicate the normal group. The yellow boxplots indicate the tumor group.

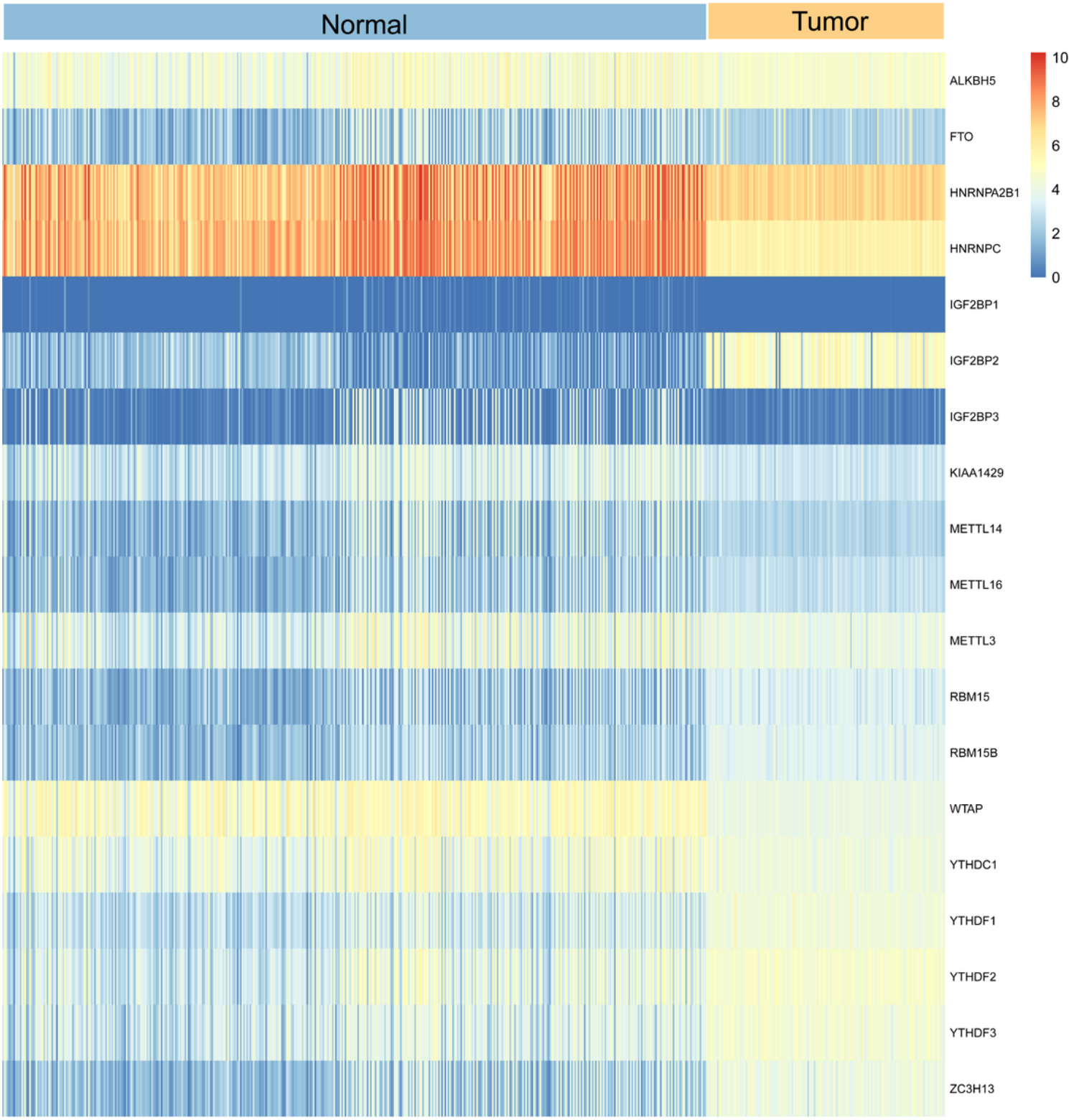


Figure 4

The heatmap of 19 m6A regulators differential expression between tumor and normal groups. The red boxes indicate the high expression and the blue boxes indicate the low expression.

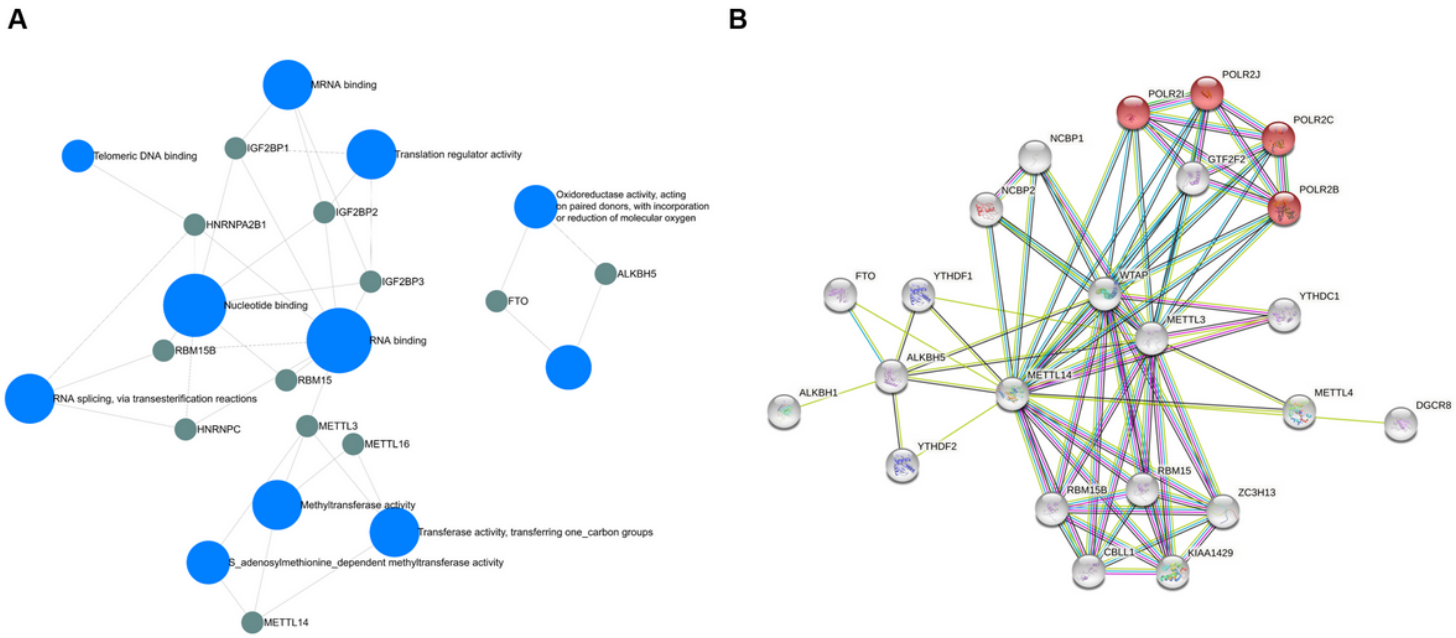


Figure 5

(A) The function enrichment network of m6A regulators; **(B)** The Protein-Protein Interaction (PPI) network of m6A regulators.

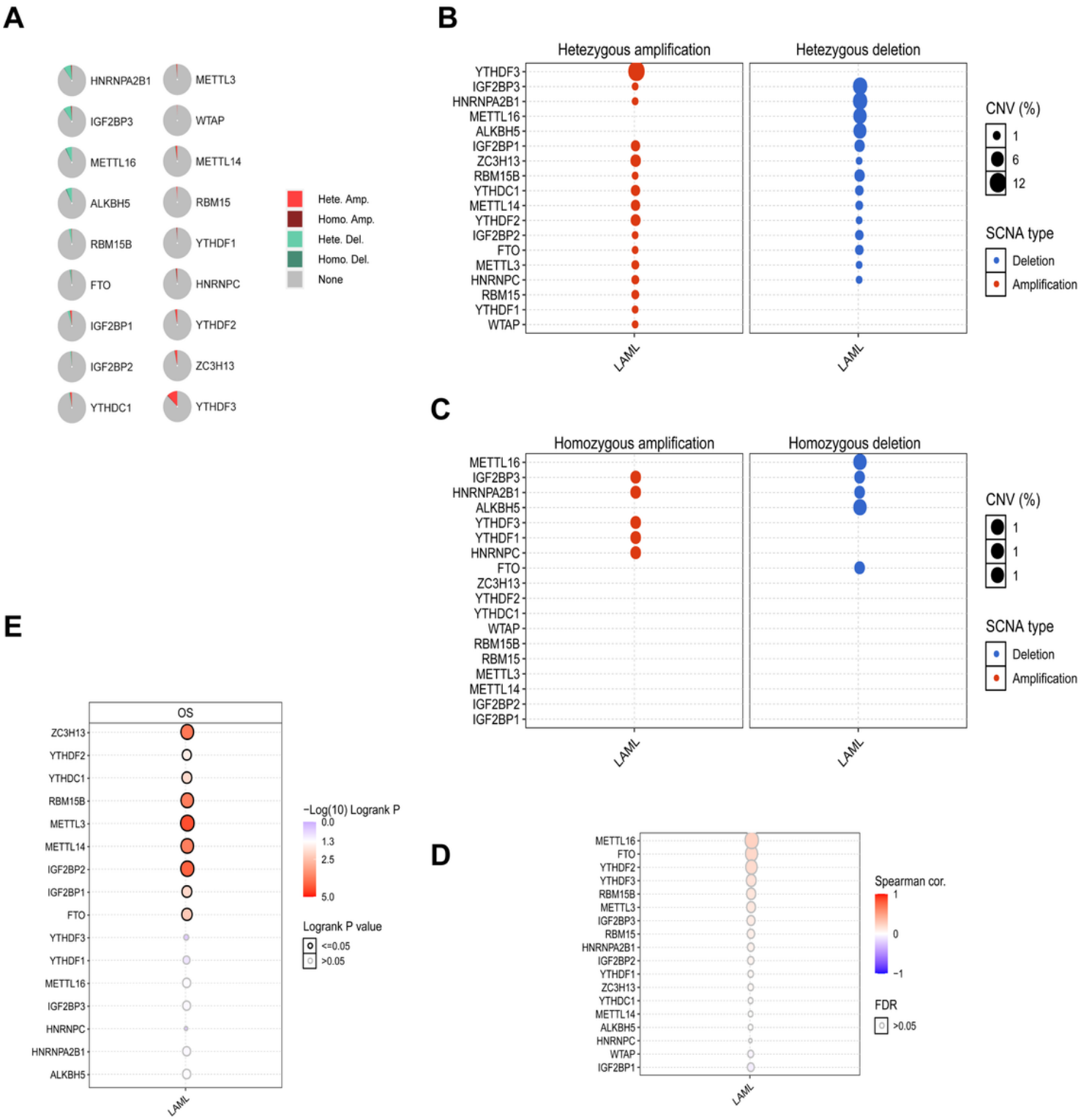


Figure 6

The copy number variations analysis. **(A)** The copy number variations (CNV) Percentage in AML. The red pies indicate the percentage of samples with copy number amplification, including heterozygous and homozygous amplification. The green pies indicate the percentage of samples with copy number deletion, including heterozygous and homozygous deletion; **(B)** Heterozygous CNV in AML. The red and blue bubbles indicate the hetezygous amplification and deletion. The size of the bubbles represents the

proportion of CNV; **(C)** Homozygous CNV in AML. The red and blue bubbles indicate the homozygous amplification and deletion; **(D)** Correlations of CNV with mRNA expression**.** The red bubbles indicate the spearman correlation high. The blue indicate the spearman correlation low. Grey outline border indicates the FDR > 0.05**; (E)** The LogRank tests between overall survival and CNV of AML. The bubble color from blue to red represents the significance of Logrank *P* value from low to high, the bubble size is positively correlated with the significance of Logrank *P* value. Black outline border indicates Logrank *P* value ≤ 0.05.

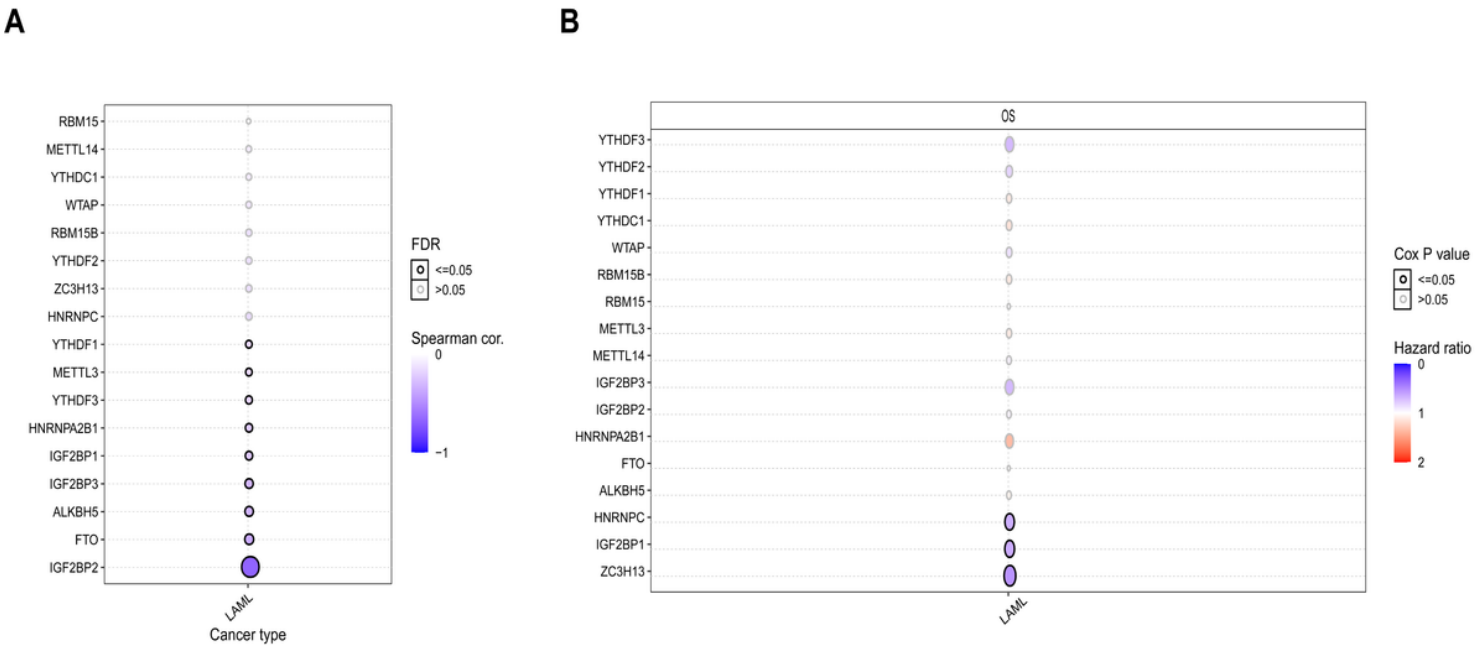


Figure 7

The methylation analysis. **(A)** Correlation between methylation and mRNA expression. The blue bubble represents a negative correlation, The darker the color, the higher the correlation. The bubble size is positively correlated with the importance of FDR. The black outline frame indicates FDR ≤ 0.05; **(B)** Survival difference between high and low methylation in each cancer. The hazard ratio and Cox *p* value are displayed by the color and size of the bubbles. The column is the gene symbol. The bubble color from blue to red represents the risk ratio from low to high, and the bubble size is positively correlated with the significance of the Cox *p* value. The black outline border indicates that the Cox *p* value is ≤ 0.05

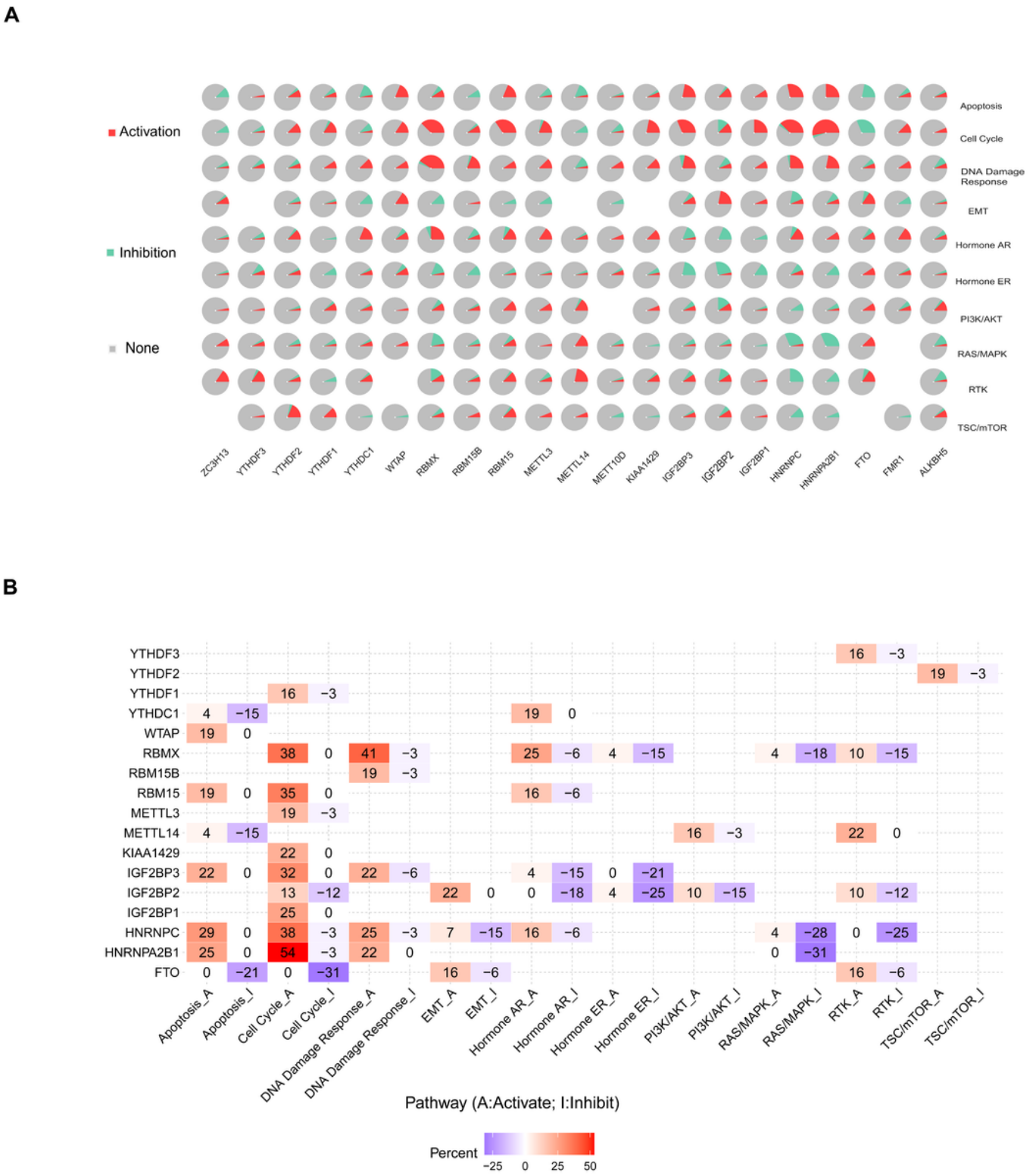
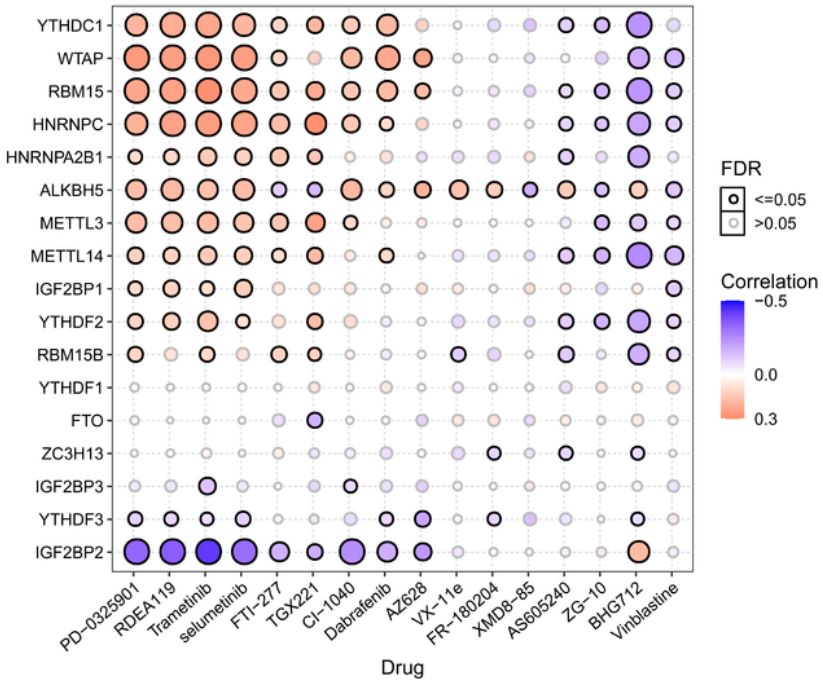


Figure 8

The pathway activity analysis. **(A)** Percentage of cancer critical pathways show the difference in gene expression. Red represents promotion and green represents inhibition; **(B)** Heatmap percent shows genes that are functional (repressed or activated) in at least 5 cancer types. Red represents the percentage of cancers where the pathway can be activated by a given gene, inhibition is shown in blue.

A



B

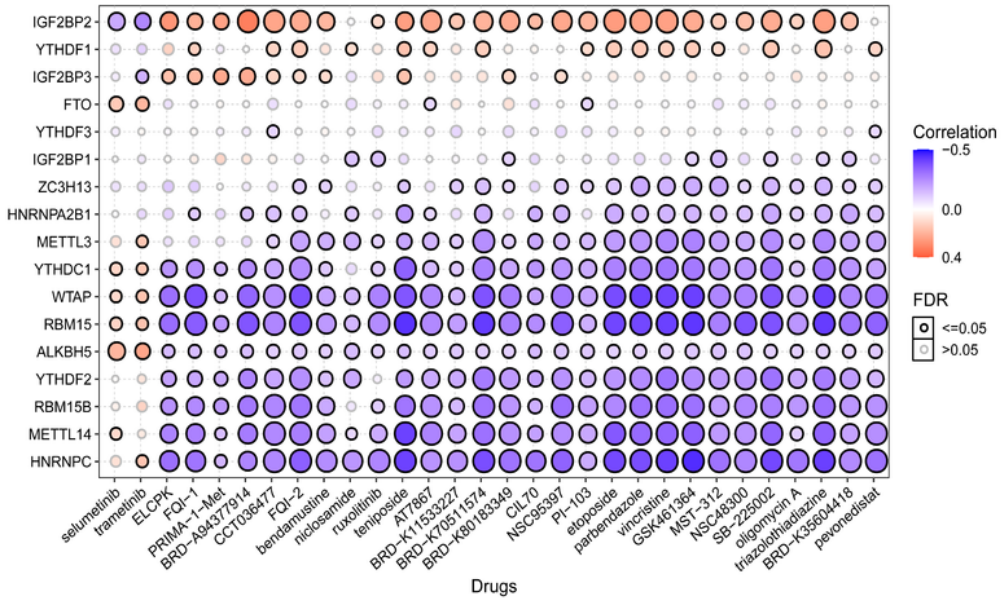


Figure 9

The correlation between m6A regulators mRNA expression and drug sensitivity analysis in GDSC and CTRP. **(A)** Correlation between GDSC drug sensitivity and mRNA expression; **(B)** Correlation between CTRP drug sensitivity and mRNA expression. Blue bubbles represent negative correlations, red bubbles represent positive correlations, the deeper of color, the higher of the correlation. Bubble size is positively correlate with the FDR significance. Black outline border indicates FDR ≤ 0.05.

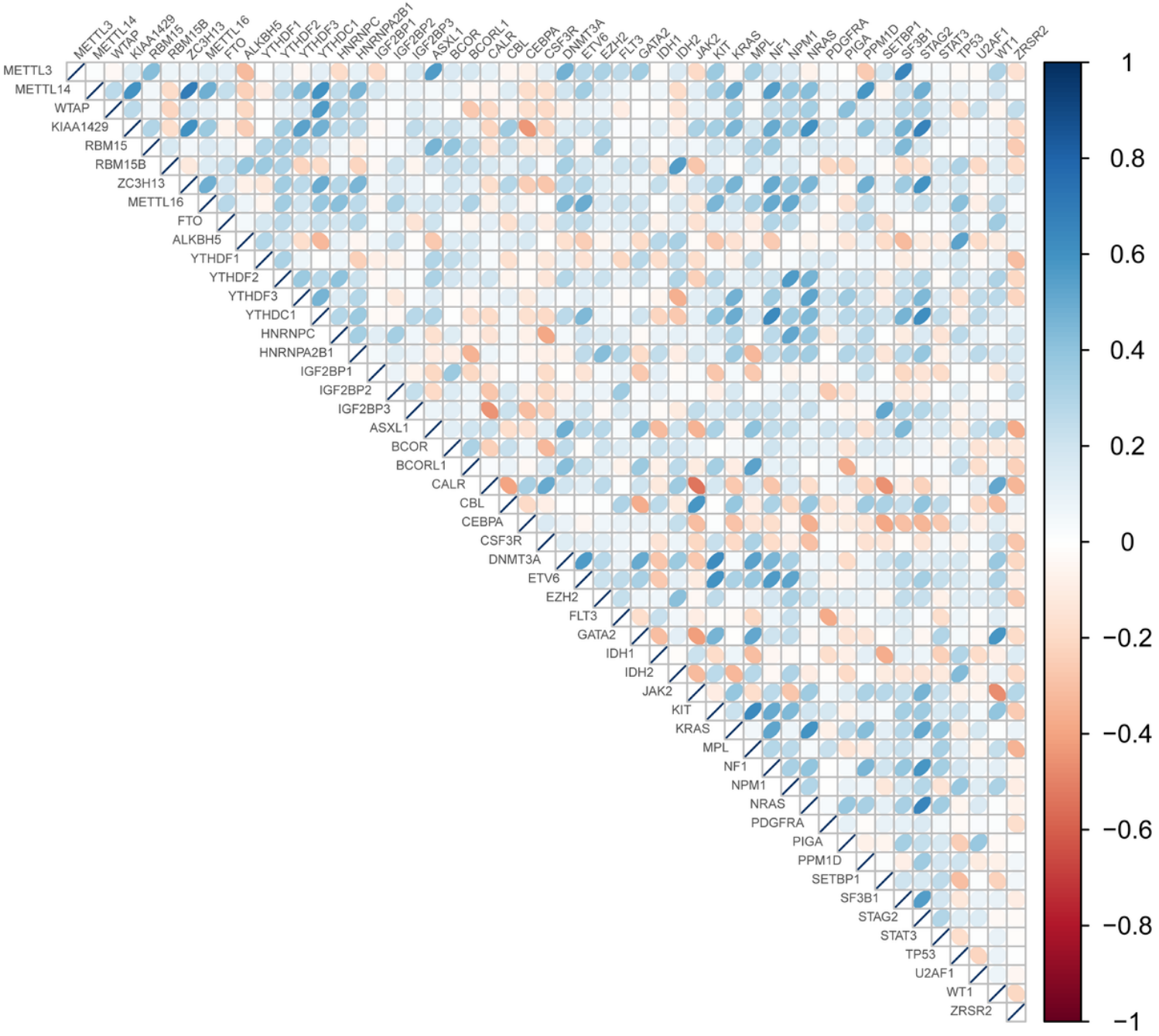


Figure 10

Gene co-expression analysis. The blue boxes indicate a positive relation between genes. The red boxes indicate a negative relation between genes. The deeper of color, the higher of the correlation.

Supplementary Files

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- SupplementTable.docx