# Analysis of N6-methyladenosine (m6A) methyltransferase reveals METTL14 and ZC3H13 as tumor suppressor genes in breast cancer

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**Research**

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## Abstract

# Background

Recently, an increasing number of studies have revealed that N6-methyladenosine (m6A) functions as a significant post-transcriptional modification which plays a critical role in the occurrence and progression of enriched tumors by regulating mRNA and non-coding RNA biogenesis. However, the biological function of m6A in breast cancer remains largely unclear.

# Materials and Methods

In this study, we used a serious of bioinformatic databases and tools to jointly analyze the expression of m6A methylation transferases (METTL13, METTL14, WTAP, RBM15, RBM15B and ZC3H13) and investigate the prognostic value of METTL14 and ZC3H13 in breast cancer. Besides, we analyzed the downstream carcinogenic molecular mechanisms related to METTL14 and ZC3H13, and their relationship with immune infiltration in breast tumor tissues.

# Results

The results showed that METTL14 and ZC3H13 were the down-regulated m6A “writers” in breast cancer. Survival outcomes analysis suggested that abnormally low expression of METTL14 and ZC3H13 could predict unfavorable prognosis in four breast cancer subtypes. Moreover, the down-regulation of them was associated with ER-, PR- and basal-like, TNBC patients, as well as tumor progression (increased Scarff, Bloom and Richardson grade status and Nottingham Prognostic Index classification). Co-expression analysis revealed that METTL14 and ZC3H13 had a strong positive correlation with APC, an antagonist of the Wnt signaling pathway, indicating they might cooperate in regulating proliferation, invasion and metastasis of tumor cells. METTL14, ZC3H13 and APC expression levels had significant positive correlation with infiltrating levels of CD4 + T cells, CD8 + T cells, neutrophils, macrophages and Dendritic cells, and negatively with Treg cells in breast cancer.

# Conclusions

This study demonstrated that down-regulation of METTL14 and ZC3H13 which act as two tumor suppressor genes was found in breast cancer and predicted poor prognosis. Their abnormal expression promoted breast cancer invasion by affecting pathways related to tumor progression and mediating immunosuppression.

## Background

The morbidity and mortality of breast cancer rank first and second in global female tumors, respectively. Globally, the incidence of breast cancer has been increasing year by year, and the age of onset has gradually become younger[1]. The incidence of breast cancer is relatively low in China, but the number of newly diagnosed breast cancer cases has been increasing continuously in recent years. It has already been the malignant tumor with the highest incidence among women in some large or medium-sized cities, which seriously threatens women's health and life, and brings huge economic and health problems to society of China. Although the prognosis of breast cancer continues to improve with the progress of treatment, breast cancer is still the main cause of female death at present[2].

The occurrence and development of tumors are driven by the disorder of genetic, epigenetic and environmental factors, and epigenetic factors play an important role as a bridge between genetic and environmental factors[3, 4]. RNA modifications are also important types of post-transcriptional epigenetic modification, N6-methyladenosine (m6A) is the most predominant modification of mRNA. In mammalian cells, there is an average of 1–2 m6A sites per 1000 nucleotides[5, 6]. At present, many proteins involved in m6A have been identified, which are classified into m6A methylation transferases (“writer” proteins), m6A demethylases (“eraser” proteins), and m6A reading and binding proteins (“reader” proteins)[7]. RNA is methylated at the sixth nitrogen atom of adenylate by the action of “writer” proteins, and methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14) form a hetero complex, which complete this modification process together with Wilms tumor 1 associated protein (WTAP) and other factors, such as Putative RNA-binding protein 15 (RBM15) and Zinc Finger CCCH-Type Containing 13 (ZC3H13). By contrast, the m6A modified regions can be demethylated under the action of 2 “eraser” proteins, fat mass and obesity-associated protein (FTO) and alkB homolog 5 (ALKBH5), making m6A a reversible modification. These m6A modified sites can be recognized by “readers” proteins to regulate RNA metabolism, including translation, splicing, translocation, degradation, and processing[7].

The abnormal expression of m6A “writer” proteins results in abnormal levels of m6A modification in tumor cells, which further abnormalizes the metabolism of tumor-related genes mRNA, thereby promoting the development of a variety of cancers[8, 9]. The upregulated METTL3 in gastric cancer was positively correlated with the poor prognosis of patients, and METTL3 promoted epithelial–mesenchymal transition of gastric cancer, leading to tumor invasion and metastasis[10]. WTAP expression was up-regulated in hepatocellular carcinoma, WTAP-mediated m6A modification led to epigenetic silencing of the tumor suppressor gene ETS1, and promoted the progression of hepatocellular carcinoma by regulating the cell cycle[11]. The expression of METTL14 was down-regulated in colorectal cancer, which indicated a poor prognosis of patients, and promoted the proliferation and invasion of tumor cells by inhibiting the degradation of the oncogene XIST[12]. However, the abnormal expression of m6A “writer” proteins in breast cancer is still remain largely unknown, and the gene targets and molecules mechanisms involved downstream also need to be further elucidated.

In this study, we used Oncomine and The Cancer Genome Atlas (TCGA) databases to jointly analyze the expression of m6A “writer” in breast cancer including METTL3, METTL14, WTAP, RBM15, RBM15B and ZC3H13, indicating that the mRNA expression levels of METTL14 and ZC3H13 were lower in tumor

samples. We further combined the data of TIMER, cBioPortal, Kaplan-Meier Plotter and other databases to analyze the prognostic value of METTL14 and ZC3H13 in breast cancer, their co-expressed genes and related signaling pathways, as well as the relationship between their expression levels and infiltrating immune cells in tumor tissues. In total, we highlighted the important role of METTL14 and ZC3H13 in breast cancer, provided promising markers for predicting prognosis of patients, and explained the potential molecular mechanism of the two as tumor suppressor genes.

# Materials And Methods

### Gene expression data mining

ONCOMINE gene expression array datasets[13] (https://www.oncomine.org), an online cancer microarray database, was utilized to analyze the transcription levels of different m6A RNA methylation writer genes: METTL3, METTL14, WTAP, RBM15, RBM15B and ZC3H13 in different cancers. The cut-off of P value was 0.05 and fold change were 1.5, respectively. Then, METTL3, METTL14, RBM15B, ZC3H13 and APC gene expression levels in various cancer types were further analyzed and verified via The Tumor IMmune Estimation Resource 2.0 (TIMER 2.0) algorithm database[14] (http://timer.cistrome.org/) based on data from The Cancer Genome Atlas (TCGA). The heatmaps of the selected gene mRNA expression were conducted and visualized by the UCSC Xena[15, 16] (https://xena.ucsc.edu/public), an interactive online visualization of seminal cancer genomics dataset, based on the data from TCGA.

### Cancer genomics analysis

The Breast Invasive Carcinoma (TCGA, PanCancer Atlas) was selected for further analyses of METTL14 and ZC3H13 by cBioPortal [17, 18] (https://www.cbioportal.org/). The genomic profiles containing mutations, putative copy-number alterations, mRNA expression and protein expression were visualized. Then, the correlations between mRNA expression levels of METTL14, ZC3H13 and putative copy-number alterations, mutations were analyzed.

### Survival outcomes analysis

The prognostic values of METTL14, ZC3H13, APC and selected hub genes mRNA expression in breast cancer were assessed by the online tools and database, Kaplan-Meier Plotter[19] (http://kmplot.com/analysis/index.php?p=service&cancer=breast). To analyze the overall survival (OS) and progression-free survival (RFS) of BRCA patients, patient samples were divided into two groups by the best cut-off value by the tool automatically and calculated via the Kaplan-Meier analysis and Logrank-P test. Besides, the prognostic roles of METTL14, ZC3H13 and APC in enriched datasets from Gene Expression Omnibus database (GEO) were further analyzed and verified by PrognoScan database[20] (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html).

### The associations of gene expression and clinical parameters

The METTL14 and ZC3H13 mRNA expression data and the clinical data of Breast cancer patients in TCGA were extracted from the UCSC Xena[15, 16]. After deleting incomplete cases, the high and low expression groups were divided by the median value of the mRNA expression level, and then for further analysis. Besides, the associations of METTL14 and ZC3H13 gene expression and clinical parameters were also evaluated and verified using the Breast Cancer Gene-Expression Miner v4.0 (bc-GenExMiner v4.0) online dataset[21] (http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1), in accordance with the RNA-seq data and clinical data from TCGA and GEO datasets.

### Gene correlation analysis

The Top 200 co-expressed genes of METTL14 and ZC3H13 were analyzed and obtained from the the Breast Invasive Carcinoma (TCGA, PanCancer Atlas) on cBioPortal by Spearman's Correlation method[17, 18]. Then, the co-expressed genes of the two genes were cross-referenced to obtain a cohort of 76 common co-expressed genes. The correlation between APC and METTL14, ZC3H13 were also analyzed on GEPIA[22] (http://gepia.cancer-pku.cn/detail.php) and

bc-GenExMiner v4.0[21] by Spearman's and Pearson’s Correlation methods, respectively.

### Pathway and Gene Ontology enrichment

Functional enrichment analysis including Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed based on the WebGestalt website[23] ( http://www.webgestalt.org/option.php))to understand the function of the extracted 76 common co-expressed genes. GO analysis can be divided into three categories: biological processes (BP), cellular components (CC) and molecular functions (MF). P-value < .05 was considered significant via Fisher's exact test.

### Protein- protein interaction network and sub-module construction

The STRING database [24]was utilized to construct the PPI network of 76 common co-expressed genes with an interaction score above 4.0 and hiding the unconnected genes. Then, ClueGO APP on Cytoscape 3.7.2 [25]was employed to assess the function of the given PPI network, and the most important module and hub genes of the PPI network were selected and constructed by the MCODE APP according the rules as follow: degree cut-off = 2, node score cut-off = 0.2, Max depth = 100, and k-Score = 2.

### Infiltrating immune cells analysis

The correlation of mRNA expression level of METTL14, ZC3H13, APC with the abundance of 7 types of infiltrating immune cells ( CD4+T cells, CD8+T cells, Treg cells, B cells, neutrophils, macrophages and Dendritic cells), and the association between immune infiltrates and somatic CNV of METTL14 and ZC3H13 in breast cancer patients were calculated using The Tumor IMmune Estimation Resource 2.0 (TIMER 2.0) algorithm database [26, 27] (http://timer.cistrome.org/), noting that the abundance of Treg

cells were analyzed by the CIBERSORT method. Tumor purity is an important factor affecting the analysis of immune infiltration in tumor samples by genomic methods.

### Tissue samples and Immunohistochemical staining

The breast cancer tissue microarrays (F601101) comprising 50 breast tumor tissues and 10 adjacent normal tissues were purchase from Zhong Ke Guang Huang Biotech (http://bioaitech.com). IHC was performed on the tissue microarrays using the UltraSensitive$^{TM}$S-P Methods. Briefly, after the tissue samples were first dewaxed, H2O2 was used to block the activity of endogenous peroxidase. The primary antibodies for METTL14 and ZC3H13 were incubated with a diluted solution, and then a secondary antibody was added and developed by diaminobenzoquinone (DAB). At last, hematoxylin was used to counterstain the microarrays. Staining index analyzed by two independent investigators were used to compare the expression of METTL14 and ZC3H13 between tumor samples and normal control. Staining intensity was graded as follows: 0 (-, no staining); 1 (+, weak staining, light yellow); 2 (++, moderate staining, yellow brown); 3 (+++, strong staining, brown). Tumor cells proportion was scored according to: 0 (no positive tumor cells); 1 (<10% positive tumor cells); 2 (10%-25% positive tumor cells); 3 (26%-49% positive tumor cells); 4 (≥50% positive tumor cells). The results were given by randomly observing at least 5-10 HPFs and taking the average. Staining index was the product of staining intensity grade and the score of positive tumor cells.

### Statistical analysis

Students' *t* test was employed to compare mRNA expression in Oncomine and TIMER 2.0, also to analyze the staining index. ANOVA was used to identify the significance of METTL14 and ZC3H13 mRNA levels in different putative copy-number alterations, mutations groups. The Kaplan-Meier curve and Log-rank P test in Kaplan-Meier (KM) plotter, and Univariable COX in PrognoScan were used to analyze the survival outcomes. The Chi-squared test were used to investigate the significance of the correlation of METTL14 and ZC3H13 expression with clinical parameters in different breast cancer groups. Gene expression significant difference between subgroups is assessed by Welch's test on bc-GenExMiner v4.0. The correlation of gene expression or infiltrating immune cells was evaluated using the Spearman's and Pearson's correlation method. Fisher's exact test was adopted to measure the gene enrichment. P-values < 0.05 were considered statistically significant.

## Results

### Down-regulation of METTL14 and ZC3H13 mRNA expression in Breast cancer

First off, the expression profiles of N6-methyladenosine (m6A) RNA methylation associated methyltransferases were analyze via Oncomine database. The expression of METTL3, METTL14, RBM15B and ZC3H13, but not of WTAP and RBM15, were significantly decreased in breast cancer (Figure 1A). More details, Oncomine analysis of cancer vs normal samples in enriched breast cancer patient datasets showed that their expression was reduced in invasive breast carcinoma stroma, invasive ductal

breast carcinoma stroma, invasive mixed breast carcinoma and ductal breast carcinoma in situ, respectively (Figure 1B-F). Furthermore, mining of TIMER database based on TCGA BRCA samples demonstrated that only the mRNA expression levels of METTL14 and ZC3H13 were also lower in tumor samples (Figure 1G, H), and there was not any difference of METTL3 and RBM15B (Figure S1).

### Rare genomic alteration of METTL14 and ZC3H13 in Breast cancer

We evaluated the genomic alteration of METTL14 and ZC3H13 by the cBioPortal online database for BRCA (TCGA, PanCancer Atlas). Overall, about 36 (4%) samples, a very small ratio, had genetic changes found in breast cancer, with 0.9% in METTL14 and 2.7% in ZC3H13, respectively (Figure 2A). Moreover, there were 0.5% samples with amplification and 0.2% with mutation in METTL14, and 1.4% with deep deletion, 0.2% with amplification and 1.1% with mutation in ZC3H13 (Figure 2B). The mutation ratio of ZC3H13 was relatively higher, including 11 missense and 6 truncating points, whereas only 4 missense mutation points were found in METTL14 (Figure 2C). In addition, for the relationship between expression and genomic alteration, the expression of METTL14 and ZC3H13 was much higher in samples with amplification, and lower in those with deletion compared with normal samples (Figure 2D). However, we did not find any differences between mutant and wild type samples (Figure 2E).

### Low METTL14 and ZC3H13 expression demonstrate poor prognosis in Breast cancer

In order to explore the roles of METTL14 and ZC3H13 in the survival of breast cancer patients, the Kaplan-Meier Plotter was used to calculate the correlation between the mRNA expression levels and the survival. The Kaplan-Meier curve and Log-rank P test confirmed that the low expression of METTL14 and ZC3H13 was negatively correlated with the overall survival (OS) and progression-free survival (RFS) (Figure 3A-D). Generally, breast cancer can be divided into four types according to the molecular characteristics, including luminal type A, luminal type B, HER2 enriched type and triple negative type, which could influence the adjuvant treatments and the prognosis of patients [28]. Survival analysis showed that the decreased expression levels of METTL14 and ZC3H13 were related to RFS in all of four types (Figure 3E, F). Besides, we also made a validation of their prognostic roles using Prognoscan database and got a consistent trend in various survival types (Table 1). Thus, the two genes play a role in tumor-suppressing, and can be used as potential prognostic markers of breast cancer.

### The association of METTL14 and ZC3H13 expression with Clinical and pathological features

After excluding cases with incomplete clinical data of TCGA BRCA from UCSC Xena, 637 cases were included to analyzed the association of METTL14 and ZC3H13 expression with clinical and pathological features through chi-square test. The results showed that the expression of METTL14 and ZC3H13 was significantly associated with ER statues, PR statues, PAM50 subtype. Besides, the proportion of patients with N2-N3 stage is higher in the low expression group of ZC3H13 (Table 2). Further, the profiles of 4712 breast cancer patients cohorts in bc-GenExMiner 4.0 were examined for validation. METTL14 and ZC3H13 mRNA expression was significantly decreased in ER-, PR- and basal-like, TNBC groups. However, HER2- patients had somewhat increase in METTL14 and ZC3H13 mRNA expression. In addition,

compared with P53 wild type samples, the genes mRNA expression was remarkably decreased in P53 mutated samples. As for the Scarff, Bloom and Richardson (SBR) grade status and Nottingham Prognostic Index (NPI) classification, the mRNA expression of the two genes gradually decreased with increasing grade and Index (Figure 4 and Figure S2). Generally, with higher rate of SBR or NPI, the lower of the survival rate was associated. There was no significant relationship between nodal statues.

## KEGG and GO enrichment of co-expressed genes

The cBioPortal database was applied to select top 200 positively co-expressed genes of METTL14 and ZC3H13 based on the data from Breast invasive carcinoma (TCGA, PanCancer Atlas). The co-expressed genes obtained from the two gene were used intersection to build a set of 76 common co-expressed genes (Table 3 and Figure 5A). Then, we used WebGestalt for functional and pathway enrichment analysis of the 76 co-expressed genes. The results of GO analysis indicated that these genes mainly play a vital role in the regulation of chromatin organization, cellular response to DNA damage stimulus, cell cycle, histone modification and regulation of microtubule, consistent with their molecular function and cellular component. It has been confirmed that these processes and functions are involved in tumor development and drug sensitivity (Figure 5B, C, D). Meanwhile, the pathways enriched by KEGG were also related to tumors, including Hepatocellular carcinoma, Hippo signaling pathway, MAPK signaling pathway and pluripotency of stem cells (Figure 5E).

## METTL14 and ZC3H13 associated PPI network construction

The PPI network was construction based on the 76 co-expressed genes using the STRING database, and the crucial modules were built by Cytoscape (Figure 6A). The function of this PPI network mainly involved in transcription factor binding, chromatin organization and cellular response to abiotic stimulus (Figure 6B). There were 10 genes in 3 crucial modules, including GOLGB1, TRIP11, GCC2, ASH1L, WDFYS, KIAA1109, RNF111, KLHL11, HECTD1 and UBR1 (Figure 6C). The clustered heatmap of these 10 genes and METTL14, ZC3H13 was analyzed and visualize by UCSC Xena, indicating their related expression pattern (Figure 6D, Figure S3). Furthermore, the prognostic roles of the 10 genes were performed using KM plotter. Accordingly, WDFYS, KLHL11, GOLGB1, KIAA1109, UBR1, ASH1L, GCC2 and TRIP11 exhibited poor RFS in lower expression groups (Figure 6E).

## Co-expression of APC and METTL14, ZC3H13

Based on the enrichment results of KEGG pathway, we noticed a familiar tumor suppressor gene, APC, which associated in several carcinoma related pathways: Hippo signaling pathway, MAPK signaling pathway and pluripotency of stem cells. It is confirmed that APC acts as an antagonist of the Wnt signaling pathway in several cancer types, and the low expression of APC promotes tumor progression. Besides, it is also involved in other processes, like cell migration and adhesion, transcriptional activation, and apoptosis[29, 30]. The data and results from different databases and datasets showed that both METTL14 and ZC3H13 had high correlation coefficients with APC ($R>0.6$, $P<0.05$) (Figure 7A-F). Then, the expression profile of APC was made a thorough inquiry using TIMER database, indicating that its mRNA

expression was significantly decreased in enriched cancer types, such as breast cancer, Colorectal cancer, lung squamous cell carcinoma, lung adenocarcinoma, etc (Figure 7G). Subsequently, the prognostic value of APC in breast cancer was also performed using KM plotter, the results confirmed that the low expression of APC mRNA was associated with the worse RFS of different breast cancer types (Figure 7H).

### Correlation of METTL14 and ZC3H13 expression with 7 types of immune cells

Studies have confirmed that the excessive activation of the Wnt signaling pathway in tumors can lead to tumor immunosuppression and immune escape, further promoting tumor invasion and metastasis[31, 32]. Based on the correlation between METTL14, ZC3H13 and APC expression pattern, we speculated that METTL14 and ZC3H13 would also be indirectly involved in mediating the immune response of tumors. So, we analyzed the correlation between the genes expression and 7 types of infiltrating immune cells (CD4+T cells, CD8+T cells, Treg cells, B cells, neutrophils, macrophages and Dendritic cells) by TIMER database. METTL14, ZC3H13 and APC were all significantly positively correlated with CD4+T cells, CD8+T cells, neutrophils, macrophages and Dendritic cells in BRCA, negatively with Treg cells ($P<0.05$) (Figure 8A). In different breast cancer subtypes, the correlations were not all the same. Importantly, APC showed a better correlation with these immune cells than METTL14 and ZC3H13 (Figure 8C, D, E). Besides, we also calculated the association between immune infiltrates and somatic CNV of METTL14 and ZC3H13 in breast cancer, and these immune cells showed diverse enrichment trends in different CNV types across different types of breast cancer (Figure S4).

### Validation of METTL14 and ZC3H13 expression in Breast cancer tissues

To verify the results of bioinformatics analysis, immunohistochemical (IHC) staining was performed on tissue microarray slides containing 50 breast cancer tissues and 10 adjacent normal tissues. The staining patterns of METTL14 and ZC3H13 in tumor and normal tissues were shown in Figure 8A & B, METTL14 mainly localized in the nucleus but not in other parts of cells, and ZCH3H13 was observed not only in the nucleus but also in the cytoplasm in cells. Considering that m6A modification took place in the nucleus, we compared the expression level of the two proteins in the nucleus. The levels of the expression were quantitated by the Staining index based on tumor cell proportion and staining intensity. The result further confirmed that both METTL14 and ZC3H13 were significantly down-regulated in the nucleus among tumor tissues relative to normal control, which was consistent with the results of bioinformatics analysis (Figure 8C-F).

## Discussion

The pathological and biological functions of enriched chemical modifications of proteins and DNA have been well verified and extensively studied. Recently, m6A modification, as a new layer of RNA epigenetic regulation, has also been increasingly studied in human diseases including cancer [33]. With the development of m6A sequence technology, m6A modification sites (RRACH, R = A or G; H = A, C, or U) which are mainly enriched near stop codons, in 3'-untranslated regions (3'-UTRs) and within long internal exons have been identified in a great many of coding or non-coding RNAs [34]. With the in-depth study of

the process of m6A, researchers have identified a series of proteins related to this modification, including “writers” (METTL3, METTL14, WTAP, RBM15, RBM15B and ZC3H13), “erasers” (FTO and ALKBH5) and “readers” (IGF2BP1/2/3, YTHDF1/2/3, YTHDC2, HNRNPA2B1 and etc) [7]. By regulating the level of m6A modification, the abnormal expression of these proteins in tumors affects the fate and metabolism of tumor-related mRNAs, including translation, splicing, translocation, degradation, and processing, and plays an important role in the occurrence and development of cancer [35]. For the m6A “writers”, several previous studies have demonstrated that dysregulation of METTL3 and METTL14 could contributed to human carcinogenesis. METTL3 was up-regulated in acute myeloid leukemia cells, enhanced translation of c-MYC, BCL2 and PTEN mRNAs, blocks cell differentiation and apoptosis and promotes leukemia progression [36, 37]. METTL3 was also over-expressed in pancreatic cancer, bladder cancer, glioma and gastric cancer, and promotes proliferation, invasion and drug resistance of cancer cells [38–41]. By contrast, METTL14 was down-regulated in colorectal cancer and hepatocellular carcinoma, and could inhibit cancer cells growth and metastasis by regulating m6A-dependent primary microRNA processing[12, 42].

In agreement with previous reports, our study also identified METTL14 as a tumor suppressor gene in breast cancer, as well as ZC3H13, while other m6A “writers” (METTL3, RBM15 and RBM15B) were not dysregulated in tumor tissues compared with normal control base on the data from TCGA BRCA and Oncomine databases. We also compared their expressions in tissue samples, and the obtained expressions was consistent with the bioinformatics analysis. What is more, survival analysis showed that low METTL14 and ZC3H13 expression could demonstrate poor prognosis in Breast cancer for OS and RFS. Low expression of METTL14 and ZC3H13 was related to breast cancer progression (increased SBR grade status and NPI classification), and was significantly decreased in ER-, PR- and basal-like, TNBC patients. The above results suggested that the down-regulated METTL14 and ZC3H13 led to a decrease in m6A modification levels in breast cancer, which promoted cancer cells invasion and metastasis. Interestingly, it has been demonstrated that m6A “eraser” FTO was significantly upregulated in breast cancer, which could promote breast cancer cell proliferation, colony formation and reduce apoptosis[43]. In addition, it is confirmed that ALKBH5 was also upregulated in breast carcinoma, promoted demethylation of m6A, and then improved the stemness of breast cancer cells [44]. In summary, these studies combined with our results have confirmed the reduction of m6A modification levels in breast cancer.

M6A “writers” and “erasers” determine the m6A modification on a specific mRNA. It is also important that m6A “readers” can specifically recognize and bind the modification sites to sort the mRNA and transmit information, thereby establishing an effective m6A associated network[7]. To further elucidate the downstream molecular mechanism involved in METTL14 and ZC3H13 by regulating m6A in breast cancer, we selected a set of 76 common positively co-expressed genes of METTL14 and ZC3H13. The pathways enriched by KEGG of these genes were associated with tumors, such as Hepatocellular carcinoma, Hippo signaling pathway, MAPK signaling pathway and pluripotency of stem cells. Importantly, we noticed a familiar tumor suppressor gene, APC, among these genes. It was confirmed that APC could inhibit the Wnt signaling pathway, a carcinogenic signaling pathway in several cancers, and

the low expression of APC promoted tumor progression [45]. Afterwards, we analyzed and confirmed that there were multiple m6A sites on the APC mRNA via the Whistle database (Table S1). In addition, by analyzing RNA binding proteins that can bind to APC mRNA by Starbase datsbase, we found that m6A "reader" Insulin Like Growth Factor 2 MRNA Binding Protein 1/2/3 (IGF2BP1/2/3) could bind to several regions of APC mRNA (Figure S5). IGF2BP1/2/3 can recognize and bind the conserved GG(m6A)C sequence on mRNA under normal and stress conditions and enhance the stability of mRNA. In HEK293T cells, IGF2BP1/2/3 mainly recognize and bind to the m6A sites near the stop codon of transcription, leading to the accumulation of carcinogenic products such as MYC [46]. In colorectal cancer, IGF2BP2 can recognize m6A sites in the CDS region of SOX2, enhancing its stability and preventing its degradation, leading to the occurrence and development of colorectal cancer [47]. Based on these documents and results, we proposed a scientific hypothesis: METTL14 and ZC3H13 can promote the m6A modification process and level of APC mRNA, after which IGF2BP1/2/3 recognizes the m6A sites and improves the stability of APC mRNA. However, since the expressions of METTL14 and ZC3H13 in breast cancer were significantly reduced, this process is reversed, resulting in a decrease in the stability of APC mRNA and inhibiting the protein level of APC. Thus, Decrease in APC protein level leads to abnormal activation of Wnt signaling pathway in breast cancer.

Abnormal activation of the Wnt pathway is thought to positively regulate various properties of tumor cells, including proliferation, survival, and metastasis [48]. In addition, Wnt signaling also plays an important role in the regulation of tumor immune microenvironment, which can regulate the infiltration and activity of various types of T cells in tumors. Usually excessive activation of Wnt signaling pathway in tumors can lead to tumor immunosuppression and immune escape [49, 50]. In colorectal cancer, inhibiting the activation of the Wnt signaling pathway can reduce the infiltration of Treg cells in the tumor and up-regulate the content of CD8 + T cells in the tumor, thereby enhancing the response to immunotherapy [51]. Base on the correlation of APC and METTL14, ZC3H13 in breast cancer, we speculated that METTL14 and ZC3H13 were indirectly involved in mediating tumor immune responses. Analysis of the correlation between gene expression and 7 infiltrating immune cells via the TIMER database showed that METTL14, ZC3H13 and APC were all significantly positively correlated with CD4 + T cells, CD8 + T cells, neutrophils, macrophages and Dendritic cells in BRCA, negatively with Treg cells. This indicated that low expression of METTL14 and ZC3H13 promoted immunosuppression in the breast cancer, which is also an important downstream mechanism for their role in the progression and metastasis of breast cancer.

# Conclusion

In conclusion, our study revealed that METTL14 and ZC3H13, as two tumor suppressor genes, were down-regulated in breast cancer, and the low expression of METTL14 and ZC3H13 was negatively correlated with the OS and RFS. Their abnormal expression promoted the progression of breast cancer by affecting pathways related to tumor progression. One of these downstream molecular mechanisms, they could affect the m6A modification level of APC mRNA, then activate the Wnt signaling pathway. However,

further experimental studies are necessary to provide a solid confirmation of our scientific hypothesis and results.

# Abbreviations

m6A: N6-methyladenosine; METTL3:methyltransferase-like 3; METTL14:methyltransferase-like 14; WTAP:Wilms tumor 1 associated protein; ZC3H13:Zinc Finger CCCH-Type Containing 13; FTO:obesity-associated protein; ALKBH5:alkB homolog 5; APC:Adenomatous polyposis coli; TCGA:The Cancer Genome Atlas; BRCA:Breast invasive carcinoma; GO:Gene Ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; BP:biological processes; CC:cellular components; MF:molecular functions; SBR grade:Scarff, Bloom and Richardson grade; NPI:Nottingham Prognostic Index; HR:hazard ratio; CI:Confidence interval. ER:estrogen receptor; PR:progesterone receptor; HER2:human epidermal growth factor receptor 2.

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets analyzed in this study were described in Materials and methods.

### Competing interests

The authors declare that they have no conflict of interest.


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### Authors' contributions

PJG and JWZ contributed to the conception of the study. PJG and YCS contributed to experimental technology and experimental design. PJG, YY, XH, and WJS performed the data analyses. PJG, SRH, and YFZ wrote the manuscript. LW and JWZ supervise the study.


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# Tables

Table 1. Survival analysis of METTL14, ZC3H13 and APC mRNA in breast cancer patients (the PrognoScan).

| Gene | Dataset | Endpoint | Number | ln(HR-high / HR-low) | COX P-value | ln(HR) | HR [95% CI-low CI-up] |
|---|---|---|---|---|---|---|---|
| METTL14 | GSE1456-GPL97 | Relapse Free Survival | 159 | -0.901843 | 0.015023 | -0.807295 | 0.45 [0.23 - 0.86] |
| | GSE3494-GPL97 | Disease Specific Survival | 236 | -1.03975 | 0.014308 | -1.25269 | 0.29 [0.10 - 0.78] |
| | | Disease Specific Survival | 236 | -0.845651 | 0.021584 | -1.011 | 0.36 [0.15 - 0.86] |
| ZC3H13 | GSE9195 | Relapse Free Survival | 77 | -2.4538 | 0.015768 | -1.77061 | 0.17 [0.04 - 0.72] |
| | GSE1456-GPL96 | Relapse Free Survival | 159 | -1.14417 | 0.04399 | -0.971572 | 0.38 [0.15 - 0.97] |
| | GSE1456-GPL97 | Overall Survival | 159 | -1.15878 | 0.014582 | -0.818691 | 0.44 [0.23 - 0.85] |
| | | Relapse Free Survival | 159 | -1.27869 | 0.009604 | -0.858074 | 0.42 [0.22 - 0.81] |
| | | Disease Specific Survival | 159 | -1.61681 | 0.00257 | -1.19064 | 0.30 [0.14 - 0.66] |
| APC | GSE9195 | Distant Metastasis Free Survival | 77 | -1.80092 | 0.008811 | -4.577 | 0.01 [0.00 - 0.32] |
| | | Relapse Free Survival | 77 | -15.5222 | 0.03119 | -3.02081 | 0.05 [0.00 - 0.76] |
| | GSE1379 | Relapse Free Survival | 60 | -2.36113 | 0.001616 | -2.01845 | 0.13 [0.04 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | - 0.47] |
| | GSE9893 | Overall Survival | 155 | -0.975572 | 0.015758 | -0.351693 | 0.70 [0.53 - 0.94] |
| | GSE1456-GPL96 | Disease Specific Survival | 159 | -1.12282 | 0.03057 | -0.634452 | 0.53 [0.30 - 0.94] |
| | | Overall Survival | 159 | -1.13145 | 0.00432 | -0.695455 | 0.50 [0.31 - 0.80] |
| | | | | | | | |

Table 2. Association between METTL14, ZC3H13 mRNA and clinicopathological characteristics in breast cancer patients.

| characteristic | Total | METTL14 | | P Value | ZC3H13 | | P Value |
|---|---|---|---|---|---|---|---|
| | | Low expression | High expression | | Low expression | High expression | |
| ER Statues | | | | | | | |
| Negative | 149 | 113 | 36 | <0.001 | 99 | 50.00 | <0.001 |
| Positive | 488 | 206 | 282 | | 220 | 268.00 | |
| PR Statues | | | | | | | |
| Negative | 208 | 142 | 66 | <0.001 | 128 | 80.00 | <0.001 |
| Positive | 429 | 177 | 252 | | 191 | 238.00 | |
| HER2 Statues | | | | | | | |
| Negative | 548 | 268 | 280 | 0.147 | 267 | 281.00 | 0.089 |
| Positive | 89 | 51 | 38 | | 52 | 37.00 | |
| PAM50 Subtype | | | | | | | |
| Her2 | 51 | 37 | 14 | <0.001 | 38 | 13.00 | <0.001 |
| Luminal A | 316 | 120 | 196 | | 114 | 202.00 | |
| Luminal B | 140 | 62 | 78 | | 83 | 57.00 | |
| Basal/Normal | 130 | 100 | 30 | | 84 | 46.00 | |
| T Stage | | | | | | | |
| T1-T2 | 547 | 272 | 275 | 0.228 | 271 | 276.00 | 0.239 |
| T3-T4 | 90 | 47 | 43 | | 48 | 42.00 | |
| N Stage | | | | | | | |
| N0-N1 | 530 | 267 | 263 | 0.960 | 252 | 278.00 | 0.009 |
| N2-N3 | 107 | 52 | 55 | | 67 | 40.00 | |
| M Stage | | | | | | | |
| M0 | 624 | 311 | 313 | 0.760 | 312 | 312.00 | 0.963 |
| M1 | 13 | 8 | 5 | | 7 | 6.00 | |
| Stage | | | | | | | |
| Stage I-II | 490 | 248 | 242 | 0.323 | 232 | 258.00 | 0.393 |
| Stage III-IV | 147 | 71 | 76 | | 87 | 60.00 | |

Table 3. 76 common co-expressed genes of METTL14 and ZC3H13

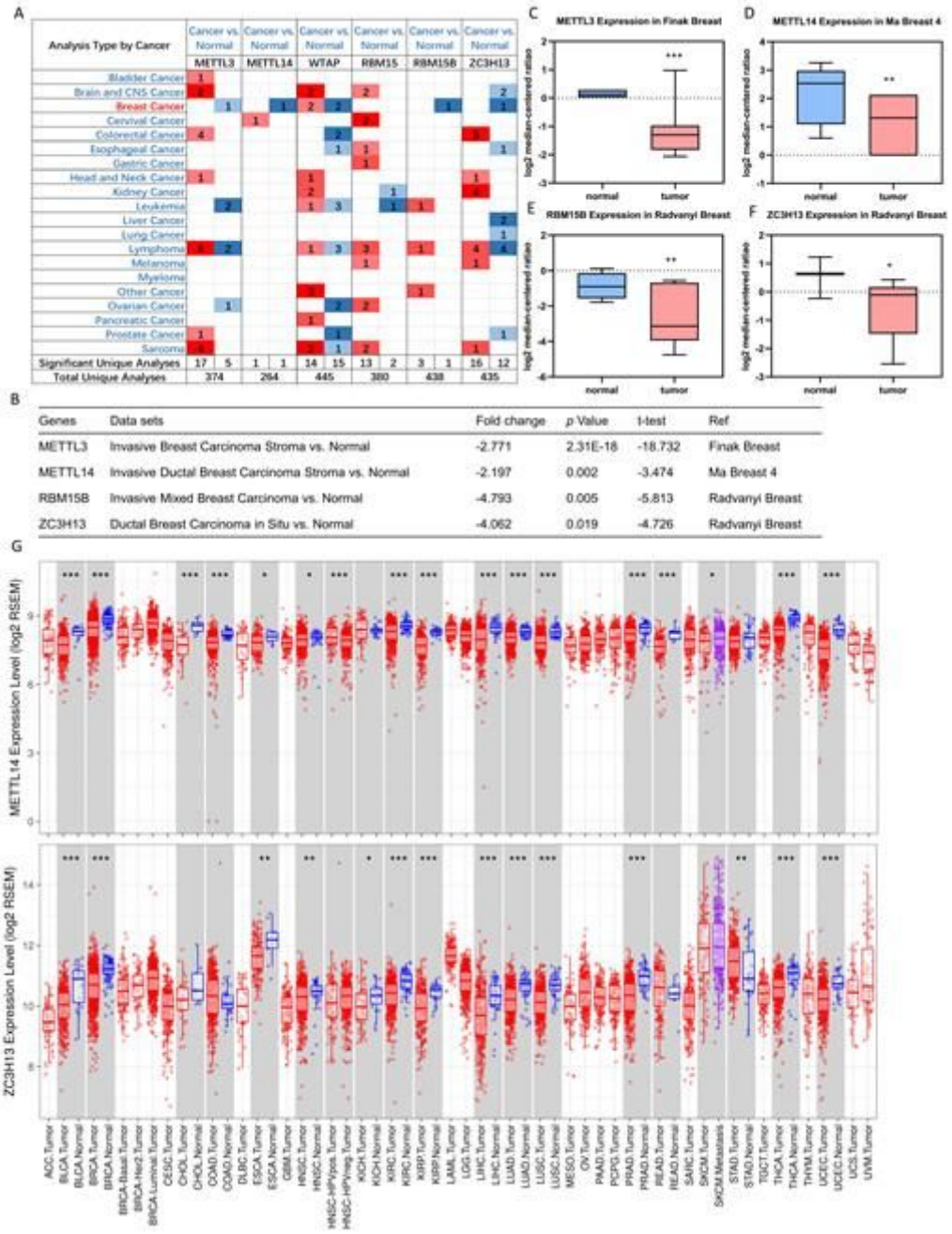
| Correlated Gene | METTL14 | | | ZC3H13 | | |
|---|---|---|---|---|---|---|
| | Spearman's Correlation | p-Value | q-Value | Spearman's Correlation | p-Value | q-Value |
| AFF4 | 0.664 | 3.4E-127 | 5E-124 | 0.579 | 3.68E-90 | 7E-88 |
| ANKRD17 | 0.564 | 1.15E-84 | 9.5E-83 | 0.545 | 6.4E-78 | 5.72E-76 |
| APC | 0.649 | 3.9E-120 | 3E-117 | 0.606 | 1.3E-100 | 4.49E-98 |
| ARID2 | 0.612 | 2.6E-103 | 7E-101 | 0.559 | 7.17E-83 | 8.62E-81 |
| ARID4A | 0.570 | 8.17E-87 | 7.7E-85 | 0.574 | 3.01E-88 | 5.11E-86 |
| ASH1L | 0.568 | 4.19E-86 | 3.7E-84 | 0.588 | 1.24E-93 | 2.95E-91 |
| ATE1 | 0.620 | 8.9E-107 | 3E-104 | 0.606 | 1.4E-100 | 4.86E-98 |
| ATF2 | 0.573 | 9.04E-88 | 9.1E-86 | 0.592 | 6.76E-95 | 1.82E-92 |
| ATG2B | 0.599 | 5.58E-98 | 1.1E-95 | 0.575 | 1.39E-88 | 2.41E-86 |
| ATRX | 0.635 | 4E-113 | 2E-110 | 0.628 | 4.3E-110 | 2.8E-107 |
| BDP1 | 0.601 | 1.16E-98 | 2.3E-96 | 0.563 | 4.57E-84 | 6.03E-82 |
| BOD1L1 | 0.658 | 2.9E-124 | 3E-121 | 0.690 | 1.4E-141 | 2.2E-138 |
| CCNT1 | 0.621 | 4.9E-107 | 2E-104 | 0.609 | 4.2E-102 | 1.6E-99 |
| CFAP97 | 0.678 | 5.6E-135 | 1E-131 | 0.547 | 1.21E-78 | 1.13E-76 |
| CHD6 | 0.598 | 2.56E-97 | 4.7E-95 | 0.550 | 1.11E-79 | 1.12E-77 |
| CLCN3 | 0.694 | 1.1E-143 | 3E-140 | 0.549 | 3.2E-79 | 3.06E-77 |
| CLOCK | 0.615 | 2E-104 | 6E-102 | 0.583 | 1.26E-91 | 2.64E-89 |
| CREBRF | 0.640 | 9.1E-116 | 6E-113 | 0.591 | 1.78E-94 | 4.66E-92 |

| | | | | | | |
|---|---|---|---|---|---|---|
| DDI2 | 0.585 | 2.55E-92 | 3.5E-90 | 0.602 | 4.9E-99 | 1.54E-96 |
| DENND4C | 0.594 | 7.93E-96 | 1.3E-93 | 0.571 | 4.75E-87 | 7.66E-85 |
| DMXL1 | 0.649 | 4.8E-120 | 4E-117 | 0.584 | 9.32E-92 | 2E-89 |
| DNAJB14 | 0.737 | 2.9E-171 | 1E-167 | 0.593 | 1.27E-95 | 3.51E-93 |
| DPP8 | 0.612 | 3E-103 | 8E-101 | 0.628 | 3.3E-110 | 2.2E-107 |
| ERCC6L2 | 0.583 | 1.14E-91 | 1.5E-89 | 0.613 | 1.9E-103 | 8.6E-101 |
| EXOC6B | 0.583 | 1.83E-91 | 2.3E-89 | 0.580 | 2.74E-90 | 5.27E-88 |
| GCC2 | 0.578 | 7.71E-90 | 8.7E-88 | 0.549 | 2.6E-79 | 2.52E-77 |
| GIGYF2 | 0.606 | 1.4E-100 | 3.2E-98 | 0.587 | 4.51E-93 | 1.02E-90 |
| GOLGB1 | 0.577 | 1.93E-89 | 2.2E-87 | 0.544 | 1.22E-77 | 1.06E-75 |
| GTF2A1 | 0.564 | 1.01E-84 | 8.4E-83 | 0.598 | 2.09E-97 | 6.01E-95 |
| HECTD1 | 0.595 | 2.31E-96 | 4E-94 | 0.601 | 1.93E-98 | 5.99E-96 |
| IL6ST | 0.671 | 6.3E-131 | 9E-128 | 0.548 | 5.56E-79 | 5.22E-77 |
| KDM3B | 0.623 | 8.7E-108 | 3E-105 | 0.560 | 3.71E-83 | 4.54E-81 |
| KIAA1109 | 0.767 | 2.1E-193 | 2E-189 | 0.621 | 7.5E-107 | 4.1E-104 |
| KLHL11 | 0.663 | 5.3E-127 | 7E-124 | 0.576 | 9.15E-89 | 1.63E-86 |
| KLHL28 | 0.565 | 5.26E-85 | 4.4E-83 | 0.544 | 9.61E-78 | 8.48E-76 |
| LATS1 | 0.571 | 3.81E-87 | 3.7E-85 | 0.623 | 1E-107 | 5.8E-105 |
| LCOR | 0.635 | 3.5E-113 | 2E-110 | 0.645 | 5.4E-118 | 5.2E-115 |
| LMBRD2 | 0.631 | 1E-111 | 5E-109 | 0.574 | 2.93E-88 | 5.01E-86 |

| | | | | | | |
|---|---|---|---|---|---|---|
| LNPEP | 0.621 | 5.2E-107 | 2E-104 | 0.551 | 5.13E-80 | 5.31E-78 |
| MBD5 | 0.575 | 1.52E-88 | 1.6E-86 | 0.566 | 2.58E-85 | 3.6E-83 |
| MINDY2 | 0.587 | 2.79E-93 | 4E-91 | 0.616 | 4.6E-105 | 2.3E-102 |
| N4BP2 | 0.622 | 1.6E-107 | 6E-105 | 0.608 | 1.6E-101 | 5.9E-99 |
| NF1 | 0.630 | 4.8E-111 | 2E-108 | 0.622 | 1.5E-107 | 8.5E-105 |
| PHC3 | 0.590 | 3.56E-94 | 5.4E-92 | 0.542 | 5.21E-77 | 4.42E-75 |
| PIK3C2A | 0.602 | 5.1E-99 | 1.1E-96 | 0.583 | 1.01E-91 | 2.15E-89 |
| PREPL | 0.605 | 3.5E-100 | 8E-98 | 0.542 | 4.05E-77 | 3.48E-75 |
| RAPGEF6 | 0.661 | 6.1E-126 | 7E-123 | 0.563 | 4.69E-84 | 6.15E-82 |
| REST | 0.624 | 3.5E-108 | 1E-105 | 0.631 | 2.3E-111 | 1.7E-108 |
| RNF111 | 0.630 | 2.9E-111 | 1E-108 | 0.600 | 2.7E-98 | 8.25E-96 |
| RSC1A1 | 0.583 | 1.85E-91 | 2.3E-89 | 0.570 | 1.46E-86 | 2.23E-84 |
| SCAF11 | 0.602 | 7.2E-99 | 1.5E-96 | 0.587 | 4.41E-93 | 1.01E-90 |
| SLX4IP | 0.615 | 1.3E-104 | 4E-102 | 0.551 | 5.26E-80 | 5.42E-78 |
| SMARCAD1 | 0.692 | 9.5E-143 | 2E-139 | 0.560 | 4.15E-83 | 5.04E-81 |
| SPG11 | 0.615 | 1.6E-104 | 5E-102 | 0.549 | 2.38E-79 | 2.32E-77 |
| TAOK1 | 0.596 | 1.16E-96 | 2E-94 | 0.612 | 3.7E-103 | 1.6E-100 |
| TCP11L2 | 0.569 | 1.76E-86 | 1.6E-84 | 0.561 | 1.63E-83 | 2.06E-81 |
| TOGARAM1 | 0.629 | 1.1E-110 | 5E-108 | 0.569 | 3.55E-86 | 5.3E-84 |
| TRIM44 | 0.570 | 9.44E- | 8.9E- | 0.559 | 1.27E- | 1.46E- |

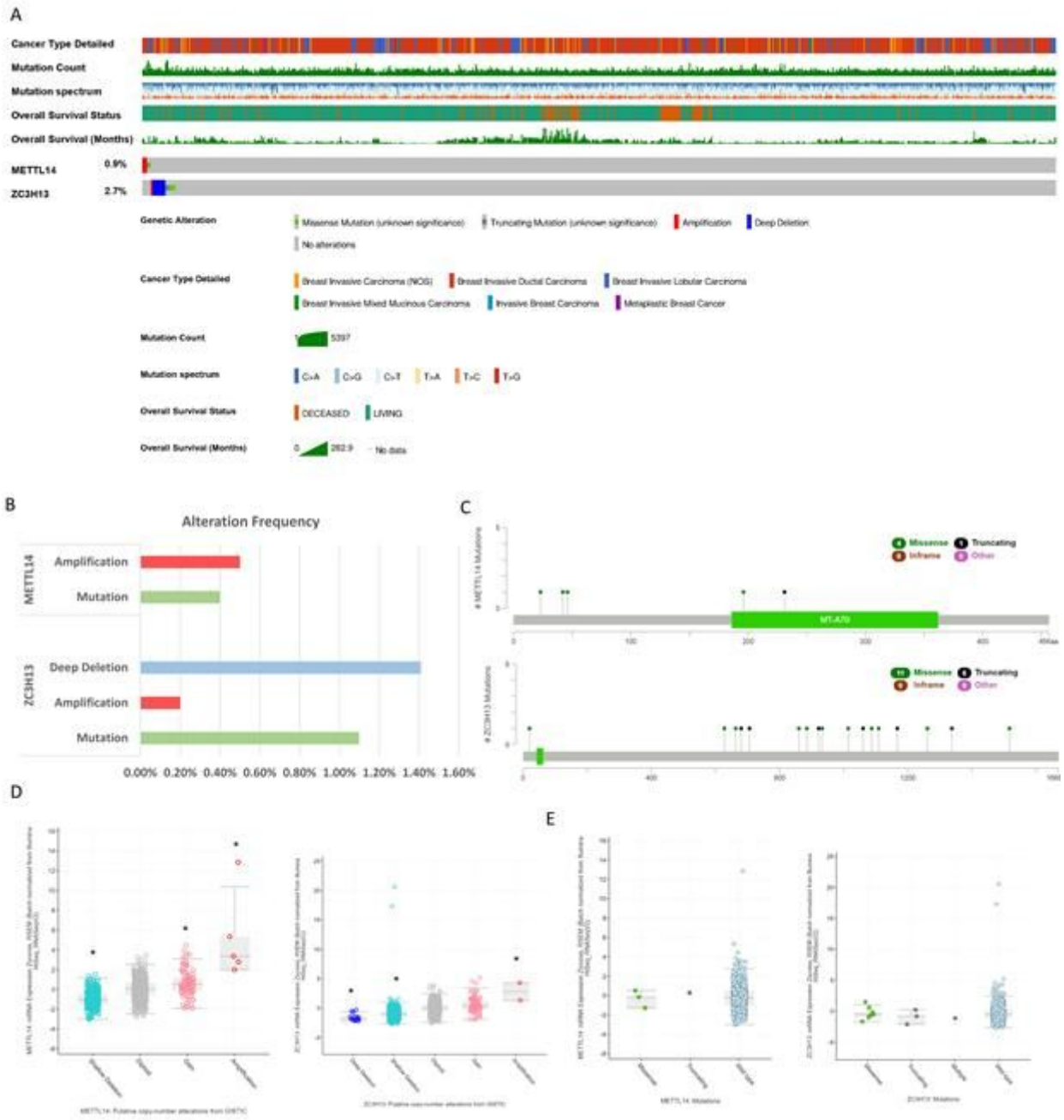
| | | | | | | |
|---|---|---|---|---|---|---|
| | | 87 | 85 | | 82 | 80 |
| TRIP11 | 0.591 | 1.4E-94 | 2.1E-92 | 0.547 | 1.6E-78 | 1.48E-76 |
| TTBK2 | 0.594 | 8.36E-96 | 1.4E-93 | 0.643 | 4.9E-117 | 4.5E-114 |
| TTC37 | 0.634 | 4.9E-113 | 3E-110 | 0.549 | 3.21E-79 | 3.06E-77 |
| UBR1 | 0.618 | 7.9E-106 | 2E-103 | 0.557 | 6.42E-82 | 7.33E-80 |
| UHMK1 | 0.619 | 2.3E-106 | 8E-104 | 0.566 | 3.95E-85 | 5.42E-83 |
| USP8 | 0.604 | 7.9E-100 | 1.8E-97 | 0.552 | 2.81E-80 | 2.95E-78 |
| UTP14C | 0.576 | 5.42E-89 | 5.8E-87 | 0.817 | 4.5E-239 | 4.6E-235 |
| WDFY3 | 0.701 | 4.5E-148 | 1E-144 | 0.619 | 2.9E-106 | 1.5E-103 |
| ZBTB37 | 0.575 | 2.21E-88 | 2.3E-86 | 0.551 | 6.72E-80 | 6.85E-78 |
| ZKSCAN1 | 0.591 | 1.2E-94 | 1.9E-92 | 0.580 | 1.85E-90 | 3.59E-88 |
| ZKSCAN8 | 0.569 | 1.77E-86 | 1.6E-84 | 0.616 | 9.8E-105 | 4.6E-102 |
| ZNF510 | 0.602 | 4.1E-99 | 8.6E-97 | 0.570 | 7.73E-87 | 1.22E-84 |
| ZNF585B | 0.577 | 2.94E-89 | 3.2E-87 | 0.560 | 3.24E-83 | 4.01E-81 |
| ZNF619 | 0.624 | 1.7E-108 | 7E-106 | 0.564 | 1.29E-84 | 1.75E-82 |
| ZNF678 | 0.583 | 1.68E-91 | 2.1E-89 | 0.579 | 5.67E-90 | 1.07E-87 |
| ZNF791 | 0.571 | 6.52E-87 | 6.2E-85 | 0.587 | 3.26E-93 | 7.55E-91 |
| ZNF81 | 0.588 | 1.34E-93 | 2E-91 | 0.653 | 8.6E-122 | 8.7E-119 |
| ZNF827 | 0.602 | 4E-99 | 8.6E-97 | 0.570 | 9.62E-87 | 1.49E-84 |

# Figures



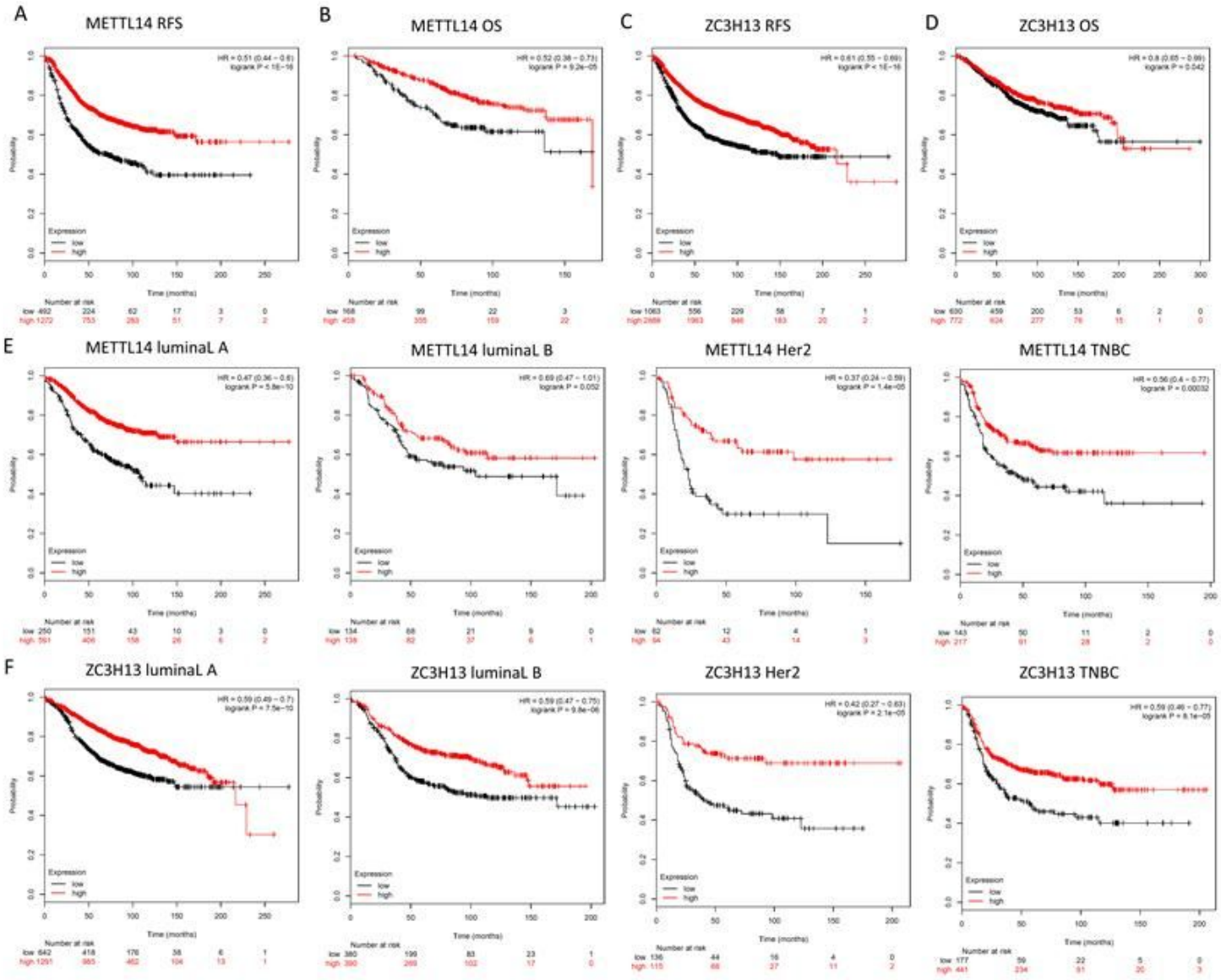

## Figure 1

Down-regulation of METTL14 and ZC3H13 mRNA expression in Breast cancer. (A) Oncomine database indicates the numbers of datasets with statistically significant overexpressed mRNA (red) or downexpressed (blue) of METTL3, METTL14, WTAP, RBM15, RBM15B and ZC3H13 (cancer tissues vs normal tissues). (B, C, D, E, F) The expression of METTL3, METTL14, RBM15B and ZC3H13 in breast cancer tissues vs normal control in the datasets of Oncomine databas. (G) The expression of METTL14 and ZC3H13 in cancer tissues and normal control in TCGA database generated by TIMER database. * * *P < 0.001, * * P < 0.01, * P < 0.05.



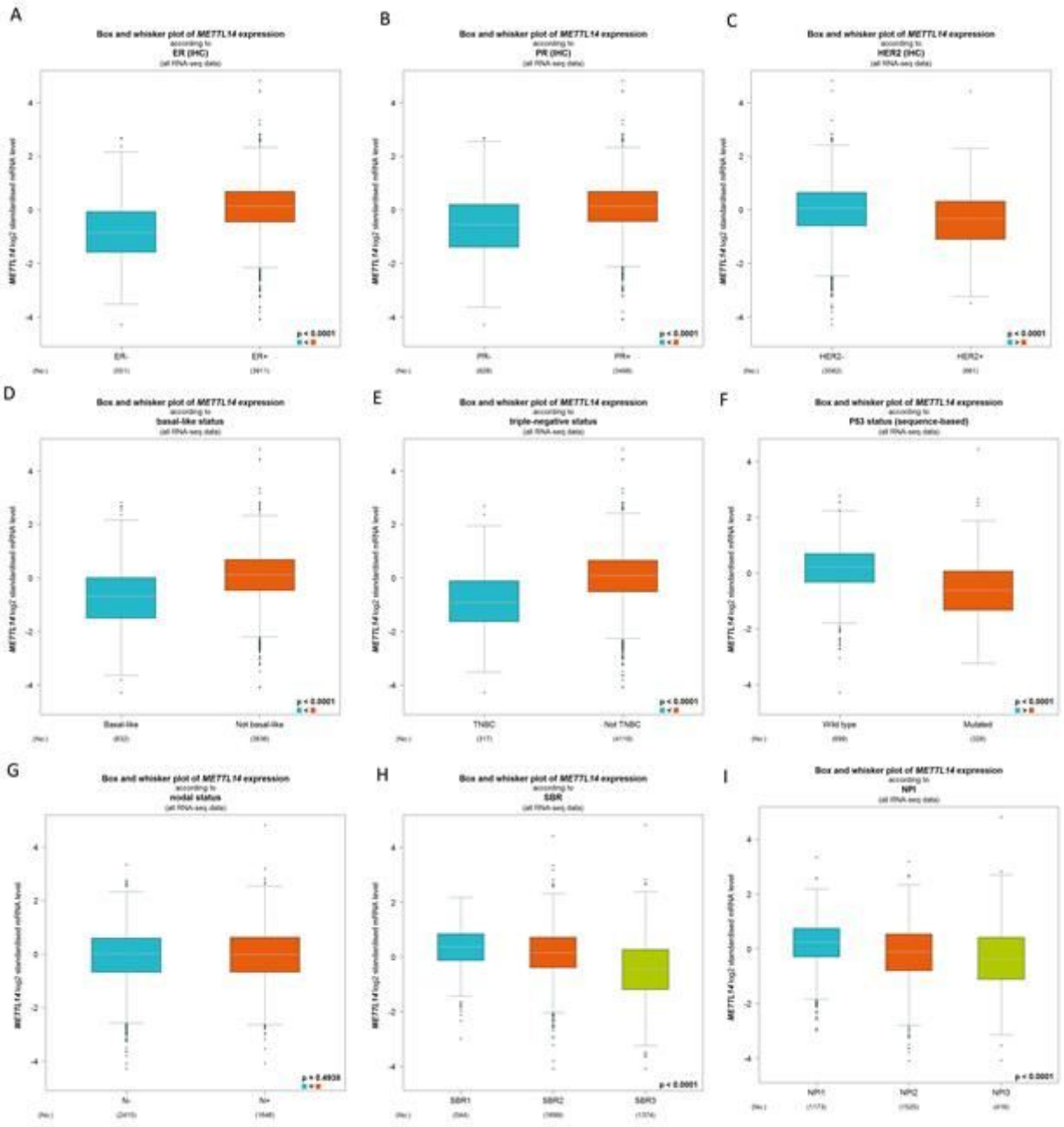

## Figure 2

Rare genomic alteration of METTL14 and ZC3H13 in Breast cancer. (A, B) The genetic alteration rate of METTL14 and ZC3H13 in Breast cancer via the cBioPortal online database (TCGA, PanCancer Atlas). (C) Schematic representation of METTL14 and ZC3H13 mutations (TCGA, PanCancer Atlas) using the cBioportal. (D) The relationship between expression and putative copy-number alteration from GISTIC of METTL14 and ZC3H13. (E) The relationship between mutation of METTL14 and ZC3H13. * * *P < 0.001, * * P < 0.01, * P < 0.05.



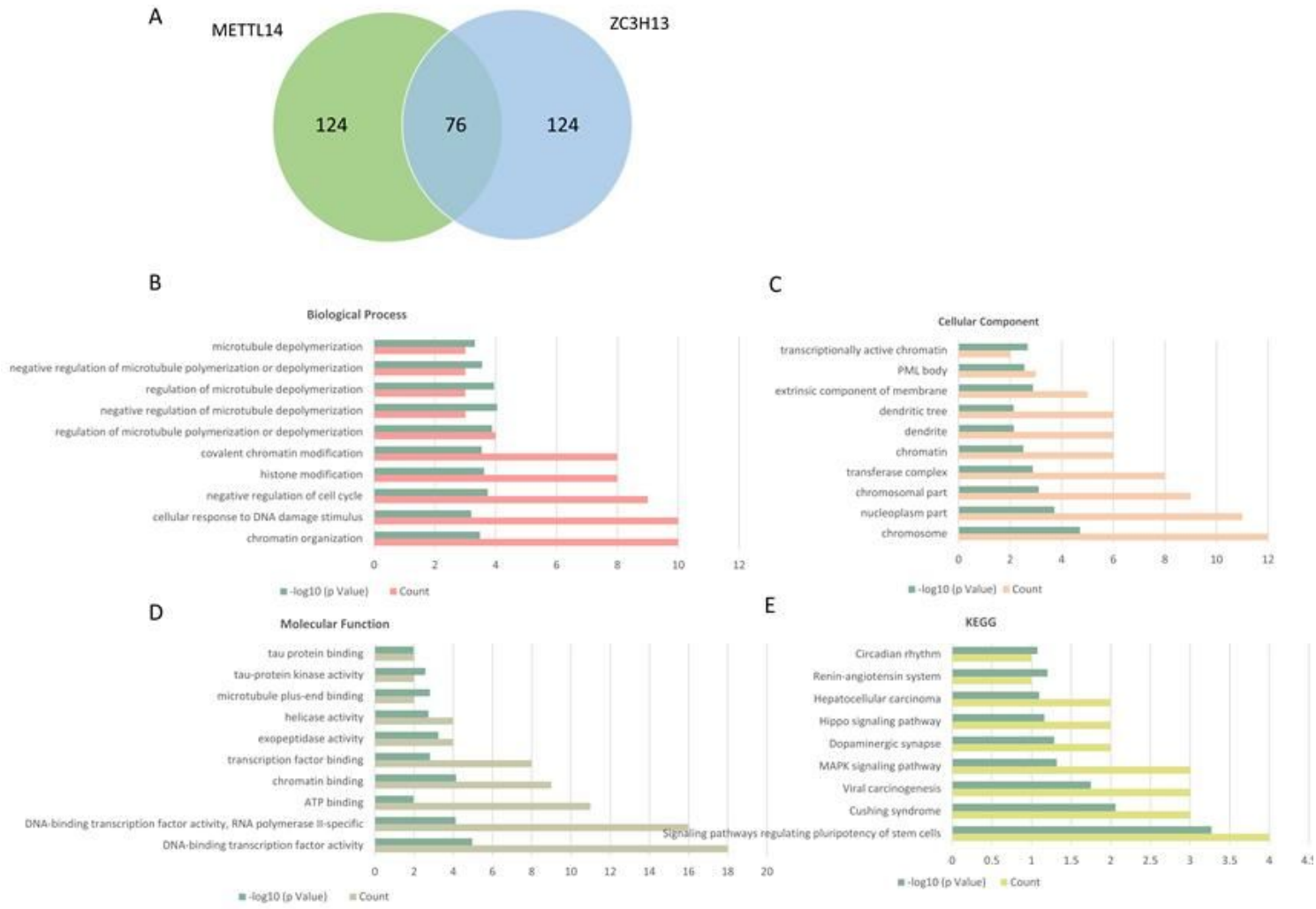

## Figure 3

Low METTL14 and ZC3H13 expression demonstrate poor prognosis in Breast cancer. (A, B, C, D) Prognostic significances of METTL14 and ZC3H13 gene expression in breast cancer patients based on the KM plotter database. RFS, relapse-free survival; OS, overall survival. (E, F) The RFS curves of METTL14 and ZC3H13 gene expression in four types breast cancer patients, including luminal type A, luminal type B, HER2 enriched type and triple negative type (TNBC).



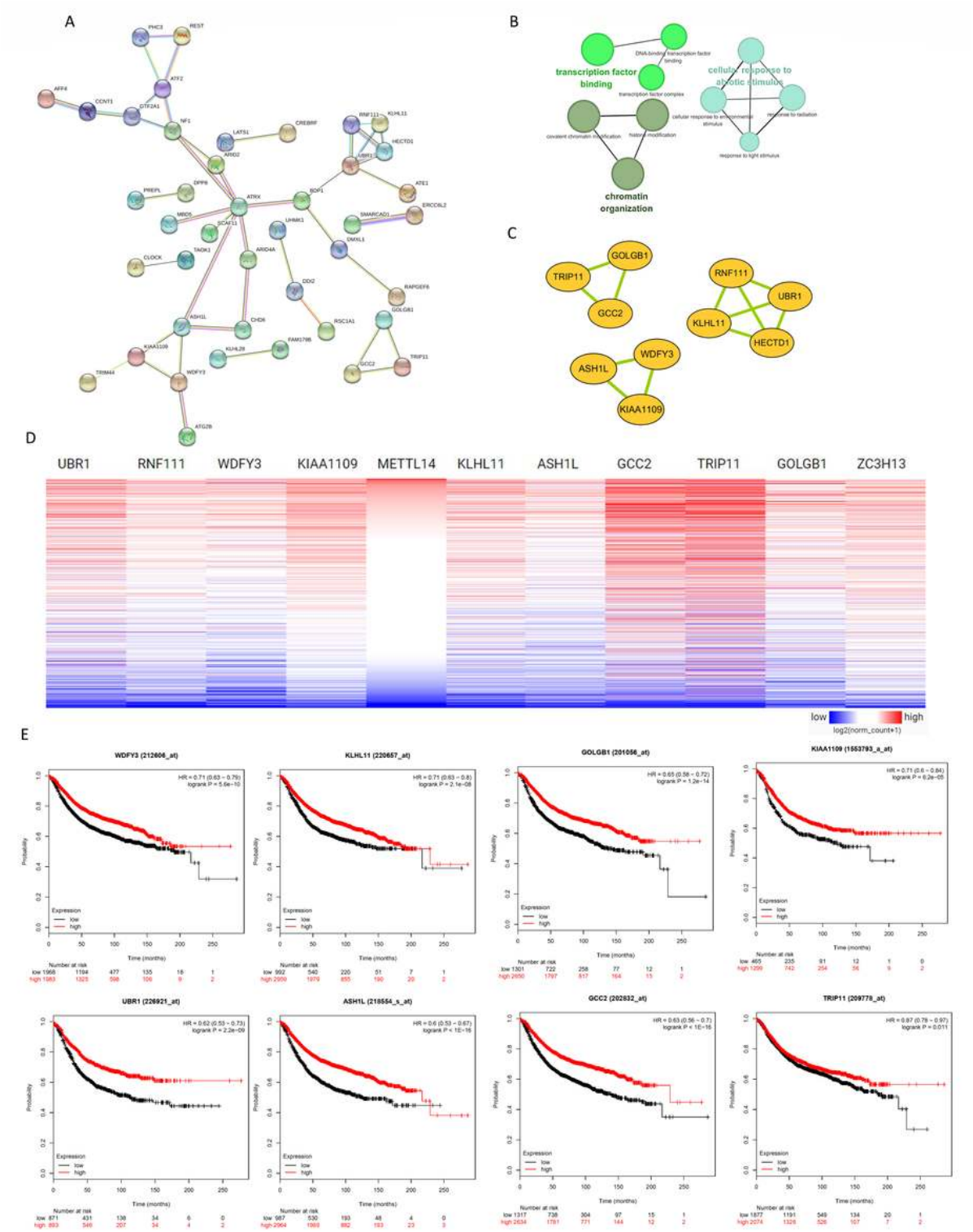

## Figure 4

The association of METTL14 expression with Clinical and pathological features. Notable global differences between the groups were analyzed by Welch's t test. (A) ER status, estrogen receptor. (B) PR status, progesterone receptor. (C) HER2 status, human epidermal growth factor receptor 2. (D) Basal-like status. (E) Triple-negative status. (F) P53 status. (G) Noda status. (H) SBR status. (I) NPI status.




## Figure 5

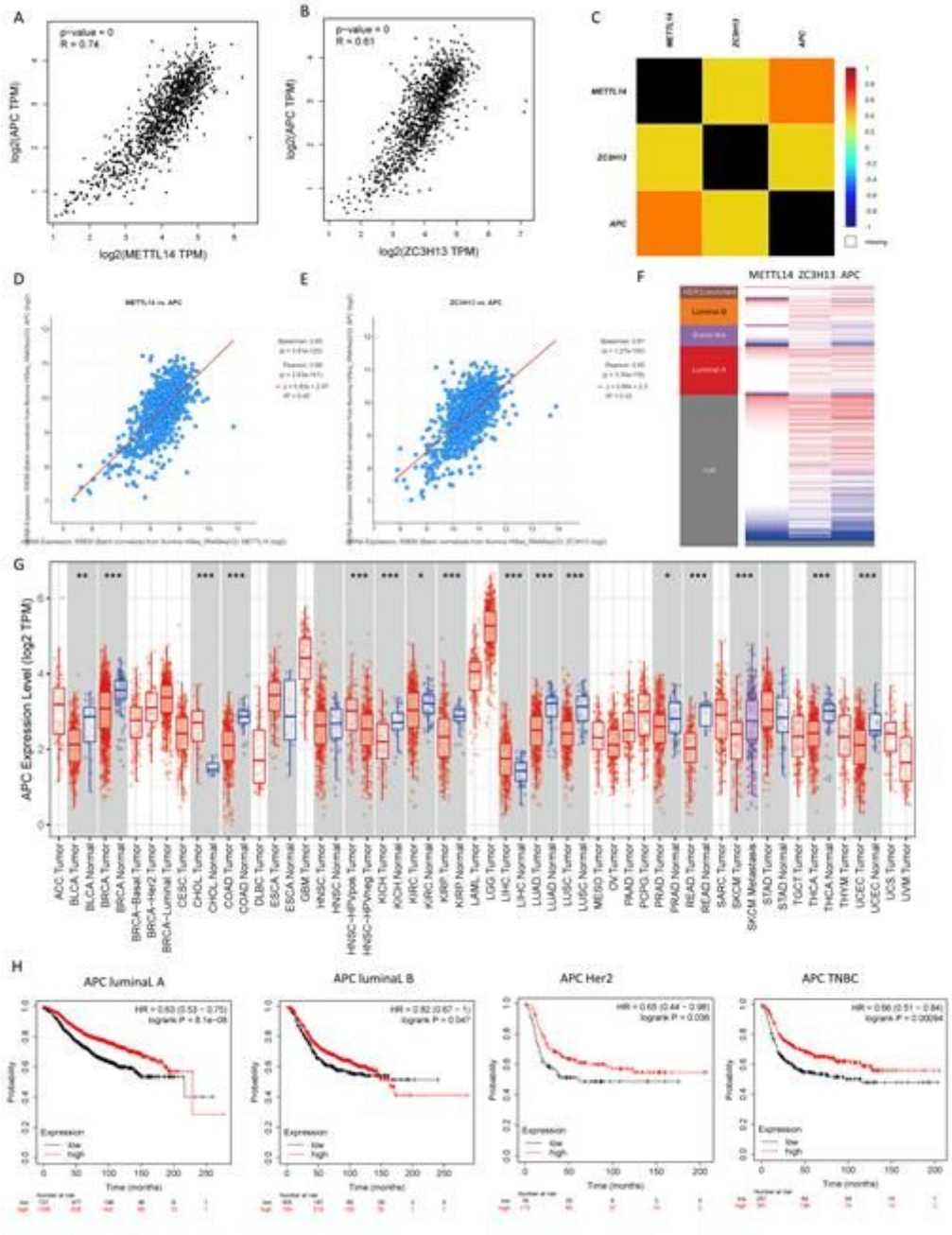
KEGG and GO enrichment of co-expressed genes. (A) The top 200 positively co-expressed genes of METTL14 and ZC3H13 in breast cancer (TCGA, PanCancer Atlas) were used intersection to build a set of 76 common co-expressed genes. (B, C, D) GO enrichment analysis of the 76 common co-expressed genes, including biological process, cellular component and molecular function. (E) KEGG pathway enrichment analysis of the the 76 common co-expressed genes.




## Figure 6

METTL14 and ZC3H13 associated PPI network construction. (A) The PPI network of was construction based on the 76 co-expressed genes using the STRING database. (B) The function analysis of this PPI network. (C) Three crucial modules selected by Cytoscape from the PPI network. (D) The clustered heatmap of the 10 genes in crucial modules and METTL14, ZC3H13 in TCGA BRCA based UCSC Xena.

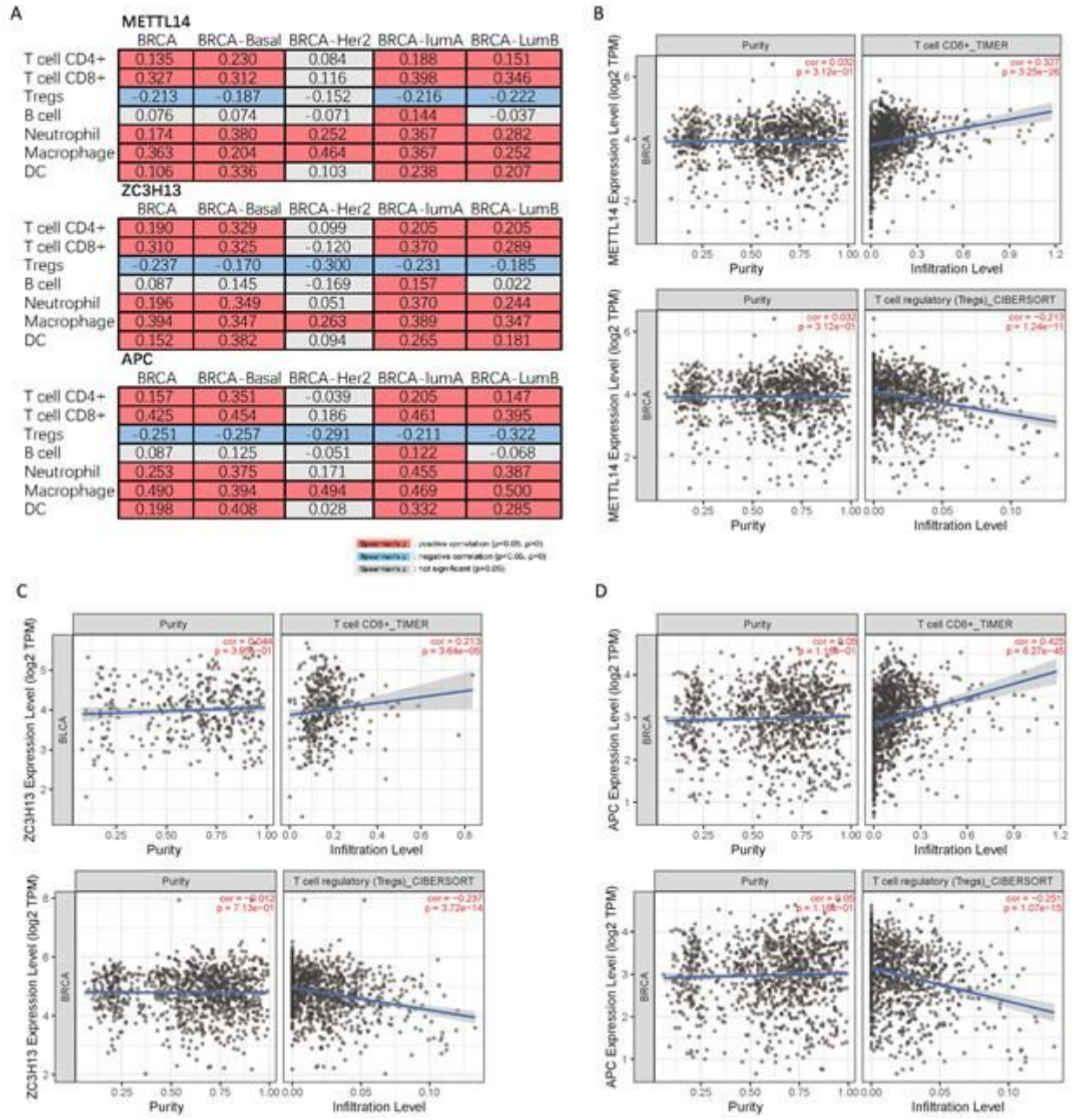
(E) Prognostic significances of hub genes expression in breast cancer patients based on the KM plotter database.




## Figure 7

Co-expression of APC and METTL14, ZC3H13. (A, B) The correlation between APC and METTL14, ZC3H13 mRNA expression determined using GEPIA. (C) The relationship between APC and METTL14, ZC3H13 in breast cancer analyzed using bc-GenExMiner v4.0. (D, E) The correlation between APC and METTL14, ZC3H13 mRNA expression via the cBioPortal online database (TCGA, PanCancer Atlas). (F) Heat map of APC expression and METTL14, ZC3H13 mRNA expression across PAM50 breast cancer subtypes in the TCGA using UCSC Xena. (G) The expression of APC in cancer tissues and normal control in TCGA database generated by TIMER database. (F) The RFS curves of APC gene expression in four

types breast cancer patients, including luminal type A, luminal type B, HER2 enriched type and triple negative type (TNBC). * * *P < 0.001, * * P < 0.01, * P < 0.05.




## Figure 8

Correlation of METTL14 and ZC3H13 expression with 7 types of immune cells. (A) Spearman' s correlation of METTL14 and ZC3H13 expression with 7 types of immune cells in breast cancer patients. (B) The correlation between METTL14 expression level and infiltrating CD8+T cells, Treg cells in breast cancer. (C). The correlation between ZC3H13 expression level and infiltrating CD8+T cells, Treg cells in breast cancer. (D). The correlation between APC expression level and infiltrating CD8+T cells, Treg cells in breast cancer.

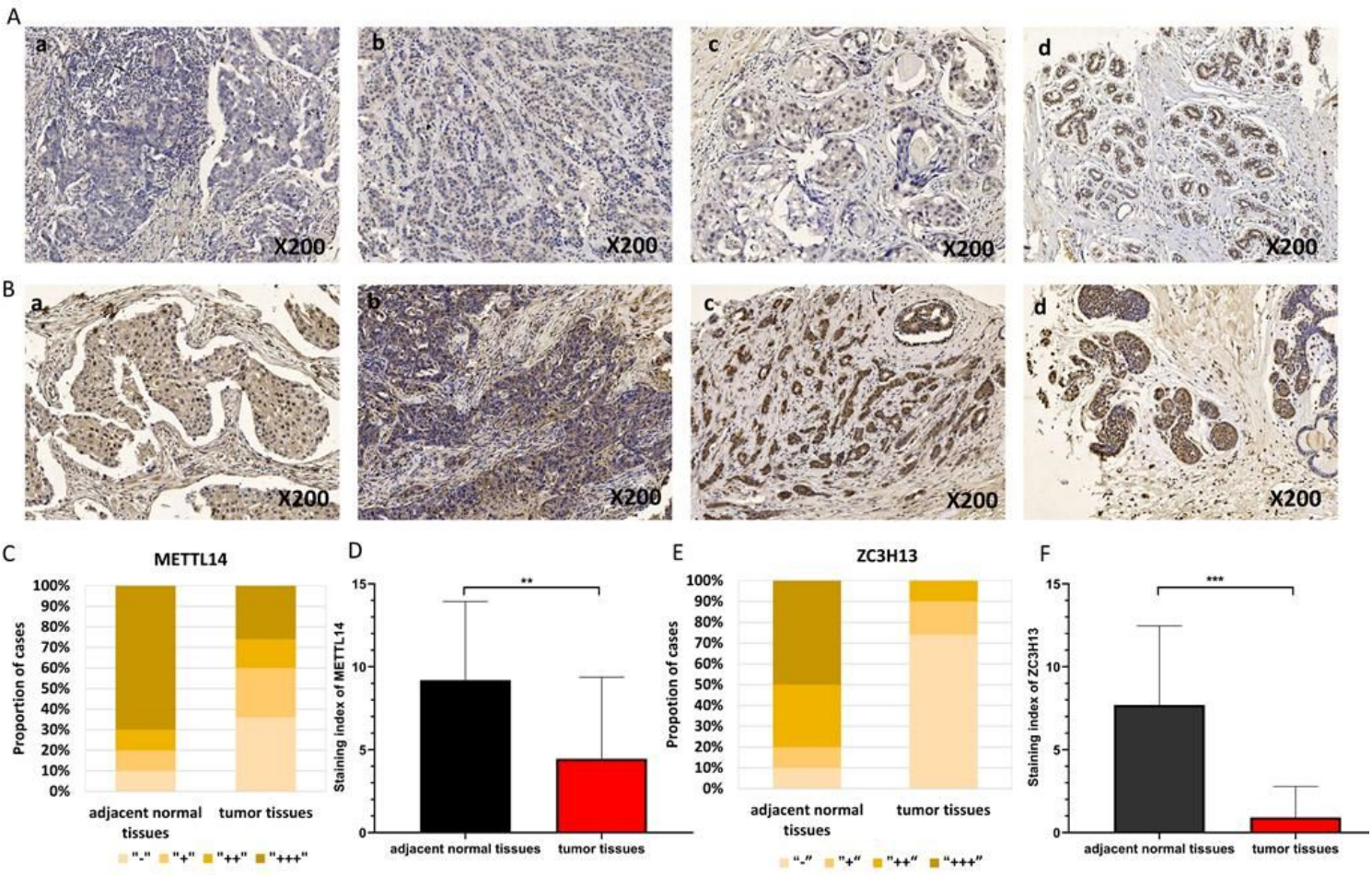



**Figure 9**

Validation of METTL14 and ZC3H13 expression in Breast cancer tissues. (A) Representative immunohistochemical (IHC) staining showing METTL14 expression in breast tumor and adjacent normal tissues (a: tumor tissue, nucleus-; b: tumor tissue, nucleus+; c: tumor tissue, nucleus++; d: adjacent normal tissue, nucleus+++). (B) Representative immunohistochemical (IHC) staining showing ZCH313 expression in breast tumor and adjacent normal tissues (a: tumor tissue, nucleus-, cytoplasm-; b: tumor tissue, nucleus+, cytoplasm+; c: tumor tissue, nucleus++, cytoplasm+; d: adjacent normal tissue, nucleus+++, cytoplasm+). (C, E) Proportion of cases for staining intensity of METTL14 and ZC3H13 in nucleus. (D, F) Staining index of METTL14 and ZC3H13 in nucleus in breast tumor and adjacent normal tissues. * * *P < 0.001, * * P < 0.01, * P < 0.05.

# Supplementary Files

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

- SupplementaryMaterial.pdf