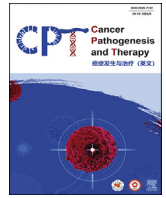




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## Research article

# Pan-cancer analysis of the prognostic and immunological role of nucleophosmin/nucleoplasmin 3 (*NPM3*) and its potential significance in lung adenocarcinoma

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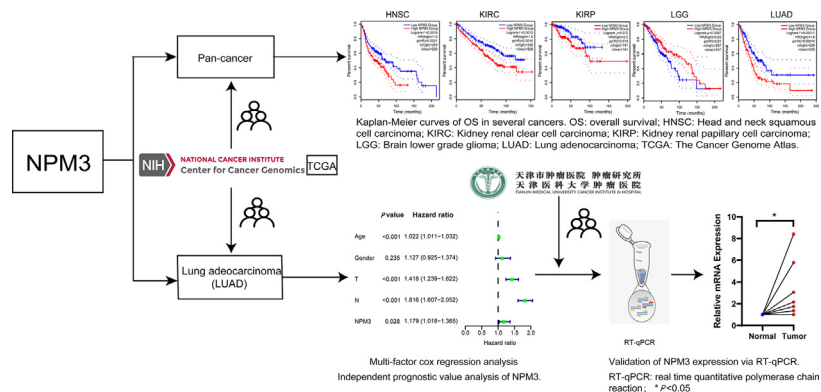
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## HIGHLIGHTS

- A systematic pan-cancer analysis revealed increased nucleophosmin/nucleoplasmin 3 (*NPM3*) expression in 20 types of cancer.
- Overall survival analysis indicates that *NPM3* expression was correlated with poor prognosis in five types of cancer.
- *NPM3* expression was significantly associated with the infiltration of various immune and stromal cells in various cancers.
- *NPM3* expression is an independent prognostic marker for lung adenocarcinoma.
- *NPM3* could serve as a potential biomarker for predicting prognosis and immunotherapy efficacy in various cancers.

## GRAPHICAL ABSTRACT



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## ABSTRACT

**Background:** Nucleophosmin/nucleoplasmin 3 (*NPM3*), a member of the NPM protein family, is widely expressed in various human tissues. Although previous studies identified elevated *NPM3* expression in several cancers, a systematic pan-cancer analysis remains lacking. In this study, we conducted a comprehensive analysis of *NPM3* to determine its role in tumorigenesis and tumor development.

**Methods:** Using data from The Cancer Genome Atlas (TCGA) and various bioinformatics analysis tools, we conducted a pan-cancer analysis of *NPM3*. Additionally, we collected gene expression and clinical data from 890 patients with lung adenocarcinoma (LUAD) from TCGA and the Gene Expression Omnibus database. We performed Cox regression analyses to explore the independent prognostic value of *NPM3* expression in LUAD and

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plotted a nomogram to predict patient survival. We also used real-time quantitative polymerase chain reaction (RT-qPCR) to examine the expression levels of *NPM3* in seven pairs of LUAD and paraneoplastic tissue samples. **Results:** *NPM3* expression was significantly increased in 20 types of cancer and was associated with poor prognosis in five types ( $P < 0.05$ ). *NPM3* expression was negatively correlated with DNA methylation and positively correlated with copy number variation. *NPM3* was also significantly associated with immune cell infiltration in various cancers. Cox regression analyses revealed that *NPM3* expression could serve as an independent prognostic marker of LUAD. Moreover, our nomogram demonstrated good predictive ability for the prognosis of patients with LUAD. Finally, the high expression of *NPM3* in LUAD was verified using RT-qPCR. **Conclusion:** *NPM3* is a promising biomarker for predicting pan-cancer prognosis and immunotherapeutic efficacy.

## Introduction

In the 21st century, cancer is a prominent cause of death in several countries.<sup>1</sup> The morbidity and mortality burdens of cancer are rapidly growing,<sup>2</sup> with lung cancer serving as the second most common cancer and leading cause of death worldwide.<sup>2</sup> However, cancer treatment has made great progress, and the 5-year survival rate has consistently improved over the last decade.<sup>3</sup> Particularly, immunotherapy has revolutionized cancer treatments<sup>4</sup> and achieved promising clinical results in several cancer types.<sup>5–8</sup> Unfortunately, its complex, heterogeneous, and uncertain nature poses significant challenges to effective cancer treatment.<sup>9</sup> Therefore, a thorough understanding of its underlying mechanisms is key to achieving breakthroughs in tumor immunotherapy.

The tumor immune microenvironment (TME) plays a critical role in anticancer immune mechanisms.<sup>10</sup> The TME consists of tumor cells, innate immune cells, adaptive immune cells, endothelial cells, stromal cells, and extracellular components that interact with and regulate responses to promote tumor growth.<sup>11</sup> The TME is divided into different subclasses that are crucial for tumor development, metastasis, and immune escape.<sup>12</sup> Consequently, a comprehensive analysis of the TME is necessary to elucidate its regulatory mechanisms and to identify new targets for immunotherapy to achieve accurate and effective cancer treatment.

Nucleophosmin/nucleoplasmin 3 (*NPM3*) is primarily a nuclear protein, widely expressed in various somatic tissues.<sup>13</sup> *NPM3* belongs to the NPM protein family, which comprises *NPM1* and *NPM3*, commonly expressed in somatic tissues, and *NPM2*, specifically expressed in oocytes.<sup>14</sup> The NPM family plays vital roles in DNA replication, histone chaperone activity, ribosome biogenesis, and chromatin remodeling,<sup>15</sup> which may influence tumorigenesis and progression.<sup>16</sup> Numerous studies have suggested that *NPM3* is strongly associated with cancer, as evidenced by its upregulation in various types of cancers, such as myxoinflammatory fibroblastic sarcoma (MIFS),<sup>17</sup> gastric cancer (GC),<sup>18</sup> and diffuse large B-cell lymphoma (DLBCL).<sup>19</sup> *NPM3* is a potential biomarker for various malignant tumors; however, its specific association and underlying mechanisms remain unclear. While previous studies each focus on only one specific form of cancer, a systematic analysis of the function of *NPM3* in pan-cancer is currently lacking.

Our study comprehensively analyzed for the first time the pan-cancer role of *NPM3* using multiple databases to analyze correlations between the differential expression of *NPM3* and survival, DNA methylation, copy number variation (CNV), immune infiltration, related cellular pathways, and drug sensitivity rarely. Our findings suggest that *NPM3* expression is an independent risk factor for lung adenocarcinoma (LUAD) prognosis and that *NPM3* could serve as a promising target for tumor treatment as it is connected with poor tumor prognosis and efficacy of tumor immunotherapy.

## Methods

### Data collection and processing

We obtained ribonucleic acid (RNA) sequencing data and clinical information, including survival status, age, sex, tumor stage, tumor size,

presence or absence of lymph node metastasis, and presence of distant metastasis from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>). The dataset GSE68465 was downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/>). The two datasets were merged, and the “sva” package in R software version 4.1.3 was employed for batch correction. Samples with incomplete clinical information were excluded from the analysis, resulting in the inclusion of 890 LUAD and 59 normal samples in this study.

### Gene and protein expression analysis

Tumor immune estimation resource version 2 (Timer 2.0; <http://timer.cistrome.org/>) is a detailed platform for systematically analyzing immune infiltrates in various cancers. It allows for the investigation of various genetic, immunological, and clinical aspects of tumors.<sup>20–22</sup> In our study, we used the “Gene Differential Expression (DE)” module of TIMER 2.0 to analyze *NPM3* differential expression in tumor and paraneoplastic tissues of 33 tumors from TCGA.

Gene Expression Profiling Interactive Analysis 2 (GEPIA 2.0; <http://gepia2.cancer-pku.cn/>) is a web-based tool that conducts extensive analysis using RNA sequencing data from TCGA and the Genotype-Tissue Expression (GTEx) database.<sup>23</sup> In this study, we used GEPIA 2.0, matching TCGA normal data and GTEx data, to perform an *NPM3* differential expression analysis for cancers lacking normal tissue samples in TCGA. In addition, we assessed the association between *NPM3* expression and the pathological stage of cancer using GEPIA 2.0. The results are presented as violin plots or box plots using  $\log_2[\text{transcripts per million (TPM)} + 1]$  transformed expression data. Statistical significance was set at  $P < 0.05$ .

The University of Alabama at Birmingham Cancer (UALCAN) data analysis portal (<http://ualcan.path.uab.edu/analysis-prot.html>) is a web-based tool that performs genetic and proteomic analyses using various databases, including the Clinical Proteomic Tumor Analysis Consortium (CPTAC).<sup>24,25</sup> We used this tool to examine the differential expression of *NPM3* in cancer and paraneoplastic tissues.

### Immunohistochemical staining analysis

The Human Protein Atlas (HPA; <http://www.proteinatlas.org/>) is an interactive platform that maps numerous proteins across cells, tissues, and organs by leveraging multiomics data and various omics technologies.<sup>26,27</sup> We obtained and analyzed immunohistochemistry (IHC) images of *NPM3* protein expression in normal tissues and five types of carcinoma tissues: breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), liver hepatocellular carcinoma (LIHC), LUAD, and prostate adenocarcinoma (PRAD).

### Survival prognosis analysis

We investigated the association between *NPM3* expression and cancer prognosis using the “Survival” module of GEPIA 2.0. We used Kaplan–Meier (KM) plots, made with the KM plotter tool ([kmplot.com](http://kmplot.com)), and survival maps to demonstrate this association. The statistical significance of our hypothesis was tested using the log-rank test, with statistical significance set at  $P < 0.05$ .

### Analysis of the relationship between nucleophosmin/nucleoplasmin 3 expression and methylation

Gene Set Cancer Analysis (GSCA; <http://bioinfo.life.hust.edu.cn/GSCA/>) is a public platform for genomic, pharmacogenomic, and immunogenomic cancer analysis.<sup>28</sup> Using this platform, we examined the relationship between *NPM3* methylation and messenger RNA (mRNA) expression levels in TCGA tumors.

### Analysis of the relationship between nucleophosmin/nucleoplasmin 3 expression and copy number variations

We assessed the correlation between the mRNA expression levels and CNVs of *NPM3* in genomic data using the GSCA platform.

### Immune infiltration analysis

We used the TIMER 2.0 tool to comprehensively evaluate the association between *NPM3* and immune cell infiltration. Specifically, we input “*NPM3*” into the “gene” module of the “immune” section, then applied different algorithms to derive the Spearman correlation. The algorithms used were CIBERSORT, CIBERSORT-ABS, EPIC, MCPOUNTER, QUANTISEQ, TIDE, TIMER, and XCELL. We analyzed B cells, cluster of differentiation (CD)<sup>4+</sup> and CD8<sup>+</sup> T cells, regulatory T cells (Treg) cells, mast cells, cancer-associated fibroblasts, myeloid-derived suppressor cells (MDSCs), monocytes, macrophages, and dendritic cells (DCs).

### Analysis of the relationship between nucleophosmin/nucleoplasmin 3 expression and both tumor mutation load and microsatellite instability

Tumor mutation load (TMB) refers to the overall number of somatic gene coding errors, base substitutions, insertions, and deletions discovered per million bases.<sup>29</sup> Microsatellite instability (MSI) is a heritable mutational state resulting from defects in DNA mismatch repair function.<sup>30</sup> We downloaded TMB and MSI data from TCGA. Next, we explored the relationship among *NPM3* mRNA expression, TMB expression, and MSI using Spearman's correlation analysis.

### Nucleophosmin/nucleoplasmin 3-related gene enrichment analysis

The STRING website (<https://string-db.org/>) was used to identify proteins that bind to *NPM3* and conduct a follow-up protein–protein interaction (PPI) network analysis. We inserted *NPM3* into the “protein by name” section under the “search” module and selected “homo intelligence.” Subsequently, we established several primary conditions: the meaning of network edges (“evidence”), active interaction sources (“experiments”), minimum required interaction score (“low confidence [0.150]”), and max number of interactors to show (“no more than 20 interactors” in the first shell).

Based on the datasets from TCGA and GTEx, we utilized GEPIA 2.0, to retrieve the top 100 *NPM3*-related genes. Subsequently, we plotted a heatmap of the Spearman correlations between *NPM3* and its top-5 correlated genes using TIMER 2.0.

We utilized “cluster Profiler” R package to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of the 100 *NPM3*-related genes.

### Drug sensitivity analysis

CellMiner (<http://discover.nci.nih.gov/cellminer/>) is an online cancer analysis tool that integrates and studies the molecular and pharmacological data of the United States National Cancer Institute (NCI)-60 cancer cell line. The NCI-60 cell line database contains comprehensive analytical data for 60 distinct human cancer cell lines.<sup>31</sup> Using this resource, we extracted data on the correlation between *NPM3* expression

and drug sensitivity. The statistical analysis was performed via R4.1.3, and “ggplot 2” R package was applied for visualization.

### Prognostic value and association with clinical characteristics of nucleophosmin/nucleoplasmin 3 in lung adenocarcinoma

Univariate and multivariate Cox regression analyses were conducted on *NPM3* expression and associated clinical characteristics to evaluate the independent prognostic significance of *NPM3* expression and associated clinical characteristics. To predict the survival outcomes at 1, 3, and 5 years for patients with LUAD, the independent prognostic factors were employed as variables to develop a nomogram utilizing the “rms” R package. Additionally, calibration curves of the column line graphs were generated using R software (version 4.1.3) to assess the reliability of the nomogram.

### Tissue sample collection and real-time quantitative polymerase chain reaction analysis

We collected seven pairs of LUAD tumor tissue and corresponding paraneoplastic tissue samples from patients who underwent lung cancer resection at the Tianjin Medical University Cancer Hospital.

Total RNA was extracted from fresh frozen tissues using TRIzol reagent (#9109, TaKaRa, Tokyo, Japan). Total RNA was reverse transcribed to complementary DNA by the StarScript III All-in-one RT Mix with genomic DNA remover kit (#A230-10, GenStar, Beijing, China) with a 20  $\mu$ L system (37 °C, 2 min; 50 °C, 15 min; 85 °C, 2 min). Real-time quantitative polymerase chain reaction (RT-qPCR) analysis was performed on an ABI QuantStudio 5 (Q5) system (Applied Biosystems, United States). The internal control was glyceraldehyde 3-phosphate dehydrogenase (GADPH). The relative expression was determined using the  $2^{-\Delta\Delta C_t}$  method. The primer sequences were 5'-GAGGGAGC-CAAAGACGAGTG-3' (forward) and 5'-CATCCAGACTGAGCATGGG-3' (reverse). A paired Student's *t*-test was used to compare the relative expression of *NPM3* in LUAD tumor and paraneoplastic samples.

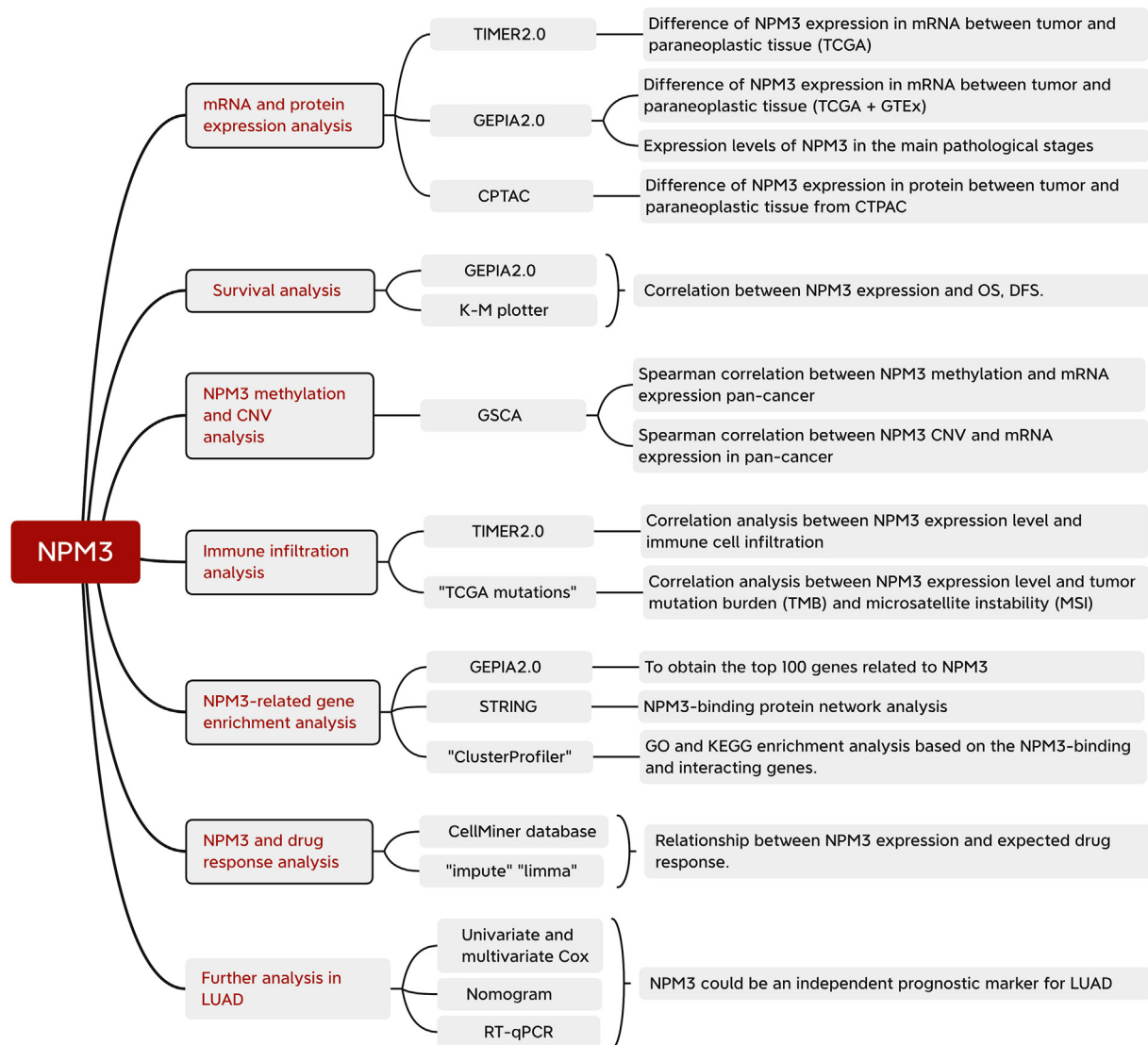
## Results

### Expression levels of nucleophosmin/nucleoplasmin 3 in pan-cancer

We utilized multiple databases, including TCGA, GEO, Clinical Proteomic Tumor Analysis Consortium (CTPAC), and CellMiner, to conduct a thorough gene- and protein-level pan-cancer analysis of *NPM3*. We performed analyses of the correlations between *NPM3* expression and survival, methylation, CNV, immune cell infiltration, gene enrichment, and drug sensitivity in pan-cancer. Our goal was to investigate the possible pathogenic mechanisms underlying the role of *NPM3* in tumorigenesis and to identify novel targets and potential drugs to improve tumor therapy. The methodology and process framework employed in this study are shown in Figure 1.

We conducted a pan-cancer analysis of *NPM3* expression using the TIMER 2.0 database. As shown in Figure 2A, *NPM3* has significantly expressed in tumor tissues of bladder urothelial carcinoma (BLCA), BRCA, cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), kidney renal papillary cell carcinoma (KIRP), LIHC, LUAD, lung squamous cell carcinoma (LUSC), PRAD, rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC) ( $P < 0.05$ ). Notably, *NPM3* was weakly expressed in the tumor tissues of kidney chromophobes (KICH) and kidney renal clear cell carcinoma (KIRC), whereas there was no differential expression in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), mesothelioma (MESO), pancreatic adenocarcinoma (PAAD), and pheochromocytoma and paraganglioma (PCPG).

To evaluate the differential expression of *NPM3* in cancers without paraneoplastic tissues in TCGA, we used normal tissues from the GTEx



**Figure 1.** Layout of this study, including the parameters investigated and databases or tools used for the analyses. CNV: Copy number variation; CPTAC: Clinical Proteomic Tumor Analysis Consortium; DFS: Disease-free survival; GEPIA 2.0: Gene Expression Profiling Interactive Analysis 2; GO: Gene Ontology; GSCA: Gene Set Cancer Analysis; GTEx: Genotype-tissue expression; KEGG: Kyoto Encyclopedia of Genes and Genomes; LUAD: Lung adenocarcinoma; *NPM3*: Nucleophosmin/nucleoplasmin 3; OS: Overall survival; RT-qPCR: Real-time quantitative polymerase chain reaction; TCGA: The Cancer Genome Atlas; TIMER 2.0: Tumor immune estimation resource, version 2.

database for comparison. As shown in Figure 2B, *NPM3* was highly expressed in lymphoid neoplasm DLBCL, brain lower grade glioma (LGG), testicular germ cell tumors (TGCT), thymoma (THYM), uterine carcinosarcoma (UCS), and skin cutaneous melanoma (SKCM) ( $P < 0.05$ ), whereas *NPM3* expression was reduced for acute myeloid leukemia (LAML;  $P < 0.05$ ). Additionally, no significant differences were observed for adrenocortical carcinoma (ACC), ovarian serous cystadenocarcinoma (OV), or sarcoma (SARC) [Supplementary Figure 1].

At the protein level, we used large-scale proteomic data made available by CPTAC to compare *NPM3* expression levels across several cancers. Our analysis revealed that *NPM3* was significantly overexpressed in BRCA, COAD, LUAD, and UCEC and significantly reduced in clear cell renal cell carcinoma (ccRCC;  $P < 0.05$ ; Figure 2C).

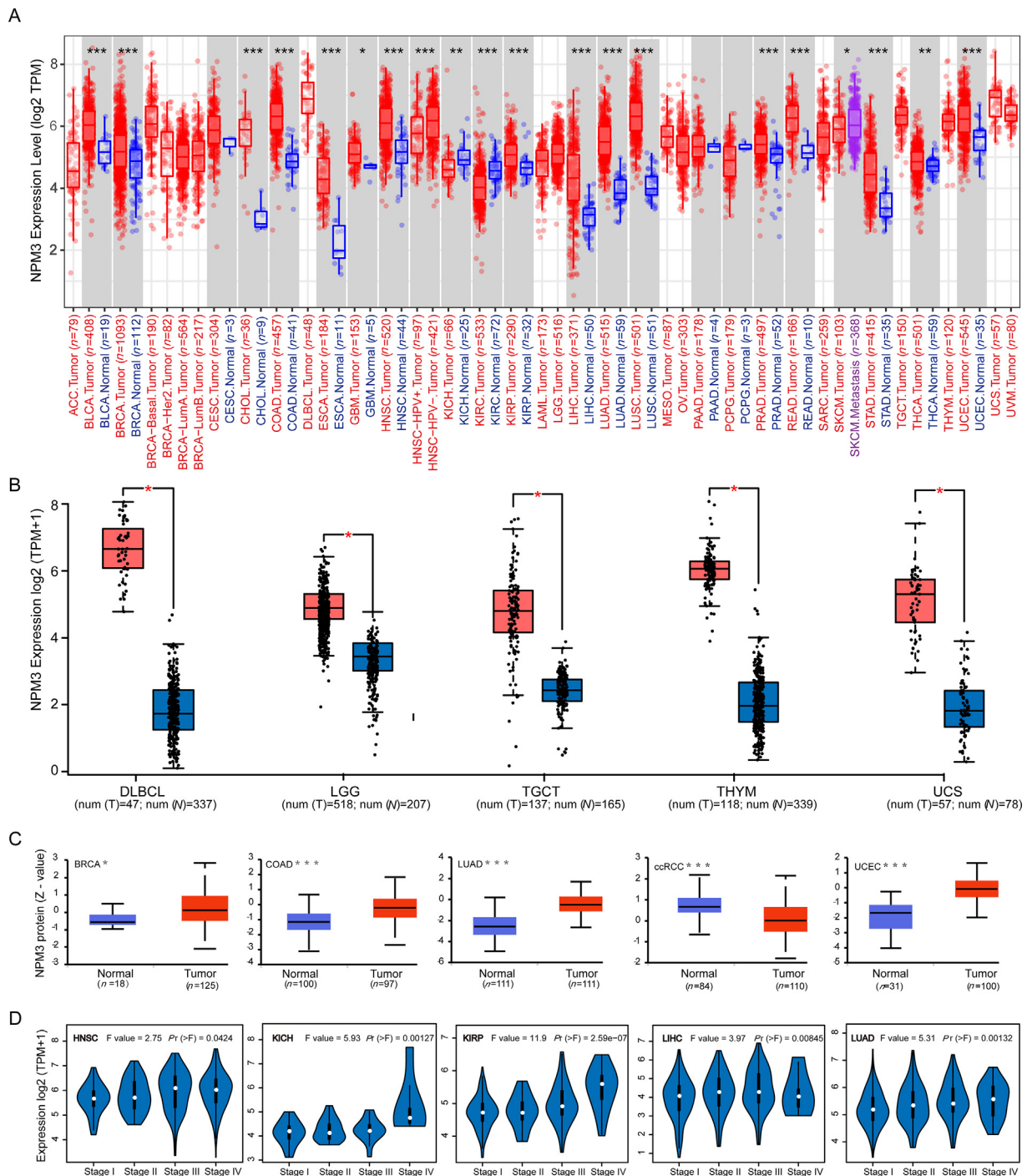
The association between *NPM3* expression and tumor pathological stage was then evaluated using GEPIA 2.0, which revealed a positive correlation between *NPM3* expression and pathological stage progression in head and neck squamous cell carcinoma (HNSC), KICH, KIRP, LIHC, and LUAD, with significantly higher *NPM3* expression levels in late-stage tumors than in early-stage tumors ( $P < 0.05$ ; Figure 2D). These findings

suggest a potential role for *NPM3* in cancer development. No significant correlation between *NPM3* expression and the pathological stage was observed in other cancer types [Supplementary Figure 2].

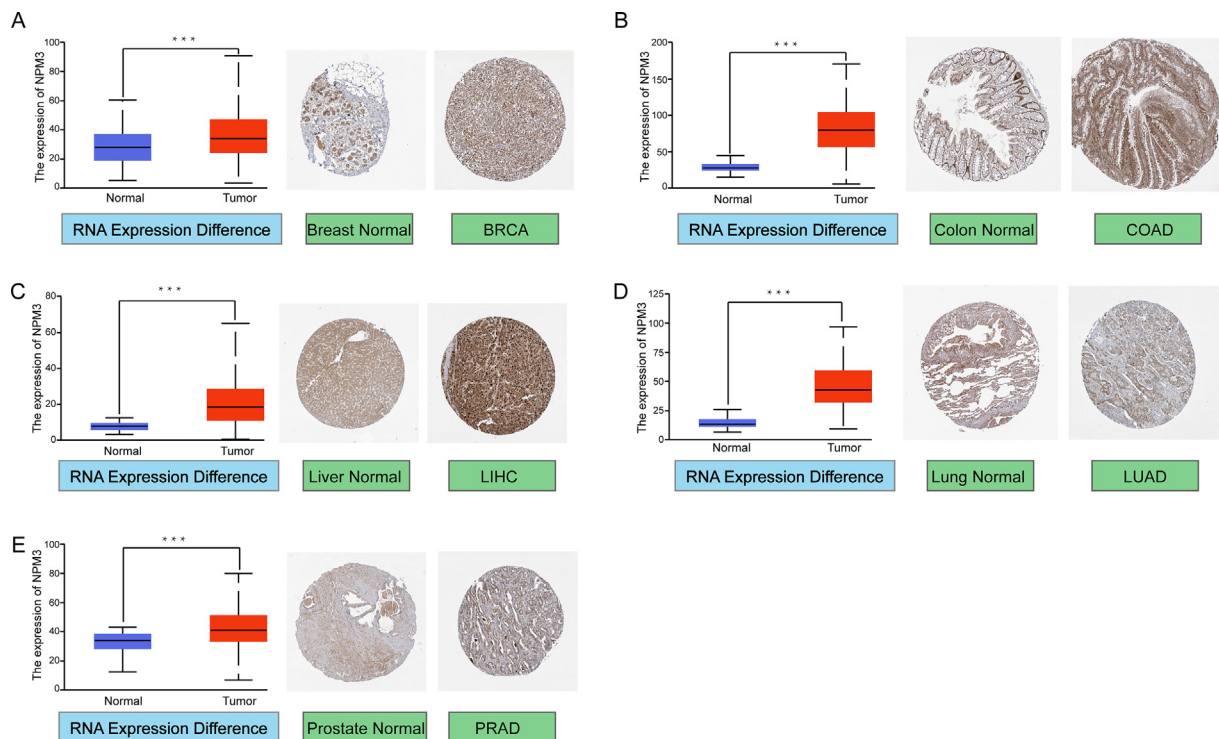
In addition, we analyzed the protein expression of *NPM3* using immunohistochemistry (IHC) data from the HPA database and compared it with *NPM3* RNA differential expression data from TCGA. Notably, for BRCA, COAD, LIHC, LUAD, and PRAD, IHC analysis revealed that *NPM3* was low or moderately positive in normal tissues but moderately or strongly positive in tumor tissues [Figures 3A–E].

#### Survival analysis of nucleophosmin/nucleoplasmin 3 in pan-cancer

We analyzed the relationship between *NPM3* expression and prognosis by conducting overall survival (OS) and disease-free survival (DFS) analyses using GEPIA 2.0. Patients were categorized into high and low *NPM3* mRNA expression groups. Furthermore, we investigated the relationship between *NPM3* expression and patient prognosis by integrating data from the GEO and TCGA databases. The results of the OS analysis [Figure 4A] revealed that high *NPM3* expression levels were associated



**Figure 2.** Messenger ribonucleic acid expression and protein levels of nucleophosmin/nucleoplasmin 3 in pan-cancer. (A) Comparison of *NPM3* expression levels in tumor and paraneoplastic tissues (where available) from TCGA in TIMER 2.0. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . (B) Box plot presentation of *NPM3* mRNA expression levels in DLBCL, LGG, TGCT, THYM, and UCS from TCGA and the GTEx database in GEPIA 2.0. \* $P < 0.05$ . (C) Differences in protein expression levels between normal tissue and tumor samples of BRCA, COAD, LUAD, ccRCC, and UCEC in CPTAC. The results are presented in box plots; the Z-value represents the standard deviation of the sample from the median for the type of cancer, and “n” represents the number of samples. \* $P < 0.05$ ; \*\* $P < 0.001$ . (D) Expression levels of *NPM3* in the main pathological stages (stage I, II, III, and IV) of HNSC, KICH, KIRP, LIHC, and LUAD. Log<sub>2</sub>(TPM+1) was used for logarithmic scaling. ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; ccRCC: Clear cell renal cell carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; CPTAC: Clinical Proteomic Tumor Analysis Consortium; DLBCL: Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; GEPIA 2.0: Gene Expression Profiling Interactive Analysis 2; GTEx: Genotype-tissue expression; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRP: Kidney renal papillary cell carcinoma; KIRC: Kidney renal clear cell carcinoma; LAML: Acute myeloid leukemia; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma; NPM3: Nucleophosmin/nucleoplasmin 3; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; TCGA: The Cancer Genome Atlas; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; TIMER 2.0: Tumor immune estimation resource, version 2; TPM: Transcripts per million; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma; UVM: Uveal melanoma.



**Figure 3.** Comparison of Nucleophosmin/nucleoplasmin 3 messenger ribonucleic acid expression between normal and tumor tissues in TCGA (left), and immunohistochemical images of *NPM3* expression in normal (middle) and tumor (right) tissues from the HPA database. Representative immunohistochemical images of *NPM3* were taken at 100X magnification. (A) Breast (B) Colon (C) Liver (D) Lung (E) Prostate. \*\*\* $P < 0.001$ . BRCA: Breast invasive carcinoma; COAD: Colon adenocarcinoma; HPA: Human Protein Atlas; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; *NPM3*: Nucleophosmin/nucleoplasmin 3; PRAD: Prostate adenocarcinoma; TCGA: The Cancer Genome Atlas.

with poor prognosis in HNSC ( $P = 0.0019$ ), KIRC ( $P = 0.0013$ ), KIRP ( $P = 0.012$ ), and LUAD ( $P = 0.00011$ ), and low *NPM3* expression was observed in LGG ( $P = 0.0097$ ). Additionally, our DFS analysis [Figure 4B] revealed that high *NPM3* expression was associated with worse prognosis in HNSC ( $P = 0.029$ ), KICH ( $P = 0.022$ ), KIRP ( $P = 0.028$ ), LUAD ( $P = 0.007$ ), and TGCT ( $P = 0.011$ ). Conversely, low *NPM3* expression was associated with a poor prognosis in LGG ( $P = 0.014$ ), STAD ( $P = 0.044$ ), and UCEC ( $P = 0.034$ ).

Subsequently, we performed a survival analysis of the selected cancer types using the KM plotter tool. According to these findings, higher *NPM3* expression was associated with worse OS ( $P = 0.045$ ), disease-specific survival (DSS;  $P = 0.034$ ), progression-free survival (PFS;  $P = 0.0014$ ), and recurrence-free survival (RFS;  $P = 0.01$ ) in LIHC, as well as worse OS ( $P = 1.6e-08$ ) and first progression (FP;  $P = 0.00028$ ) in LUAD. In contrast, lower *NPM3* expression was associated with poor post-progression survival (PPS;  $P = 0.033$ ) in OV and poor OS ( $P = 0.031$ ), FP ( $P = 0.019$ ), and PPS ( $P = 1.6e-08$ ) in STAD [Supplementary Figure 3].

#### Correlation between nucleophosmin/nucleoplasmin 3 expression and methylation

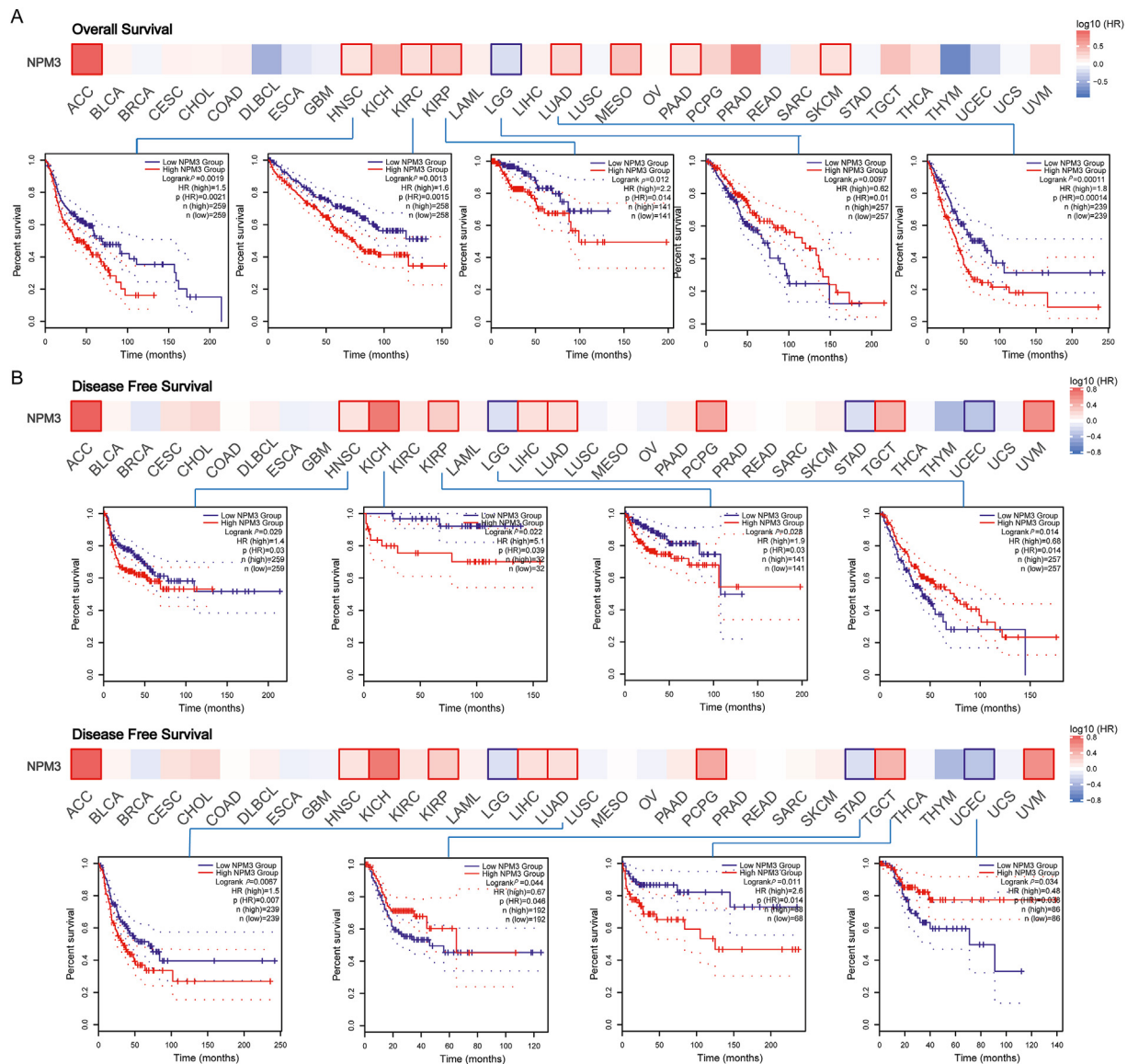
DNA methylation is common in numerous cancer types and is closely associated with tumorigenesis.<sup>32</sup> Therefore, we used GSCA to investigate the association between *NPM3* methylation and mRNA expression. As shown in Figure 5A, *NPM3* methylation was negatively correlated with mRNA expression in most cancer types, and its relevance in LIHC, SARC, BRCA, LGG, LUSC, and BLCA was particularly significant ( $P < 0.01$ ; Figure 5B). However, in only a few cancers, including PAAD, KIRC, LAML, READ, CHOL, KIRP, THYM, and GBM, no significant relationship was observed [Figure 5A].

#### Correlation between nucleophosmin/nucleoplasmin 3 expression and copy number variation

We also used GSCA to examine the Spearman's correlation between *NPM3* CNV and mRNA expression. *NPM3* CNV showed a positive association with mRNA expression, except in LAML, KIRC, GBM, THYM, PCPG, ACC, uveal melanoma (UVM), DLBCL, and CHOL ( $P < 0.05$ , Figure 5C). This correlation was significantly higher in SARC, UCEC, SKCM, COAD, OV, and CESC ( $P < 0.05$ ; Figure 5D).

#### Analysis of nucleophosmin/nucleoplasmin 3 expression and immune cell infiltration

The level of immune cell infiltration plays a significant role in predicting the prognosis of patients with tumors and determining the efficacy of immunotherapy.<sup>33</sup> We used several different algorithms to investigate the connection between *NPM3* expression and the infiltration of various immune and stromal cells in pan-cancer. As shown in Figure 6A, *NPM3* expression was negatively associated with B-cell infiltration in HNSC, LUAD, and LUSC, whereas it was positively correlated with LIHC. For CD4<sup>+</sup>T cells [Figure 6B], the correlation between *NPM3* expression and cell infiltration was mostly positive in various types of cancer. However, for CD8<sup>+</sup>T cells, the connection was negative in LGG and SKCM [Figure 6C], and for Treg cells, it was negative in HNSC [Figure 6D]. For mast cells, *NPM3* expression was negatively correlated with activated mast cells but positively correlated with resting mast cells in HNSC and LUAD [Figure 6E]. For cancer-associated fibroblasts, a negative relationship between *NPM3* expression and cell infiltration in LUSC and PRAD was observed, but a positive relationship was observed in KIRC [Figure 6F]. The association between *NPM3* expression and MDSC cell infiltration was generally positive in various cancer types



**Figure 4.** The prognostic significance of nucleophosmin/nucleoplasmin 3 in multiple human cancers. GEPIA 2.0 was utilized to evaluate the connection between *NPM3* and the overall survival (A) and disease-free survival (B) of various tumors in TCGA. The results were presented with Survival Map and Kaplan–Meier plots. ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; ccRCC: Clear cell renal cell carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; DLBCL: Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: Esophageal carcinoma; GEPIA 2.0: Gene Expression Profiling Interactive Analysis; GBM: Glioblastoma multiforme; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MECO: Mesothelioma; *NPM3*: Nucleophosmin/nucleoplasmin 3; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PRAD: Prostate adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; TCGA: The Cancer Genome Atlas; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma; UVM: Uveal melanoma.

[Figure 6G]. Furthermore, *NPM3* expression negatively correlated with monocyte infiltration in both KIRC and SARC [Figure 6H]. In macrophages, the relationship was negative for LUSC and READ, whereas PCPG showed a positive relationship [Figure 6I]. The expression of *NPM3* in READ was negatively correlated with DCs [Figure 6J].

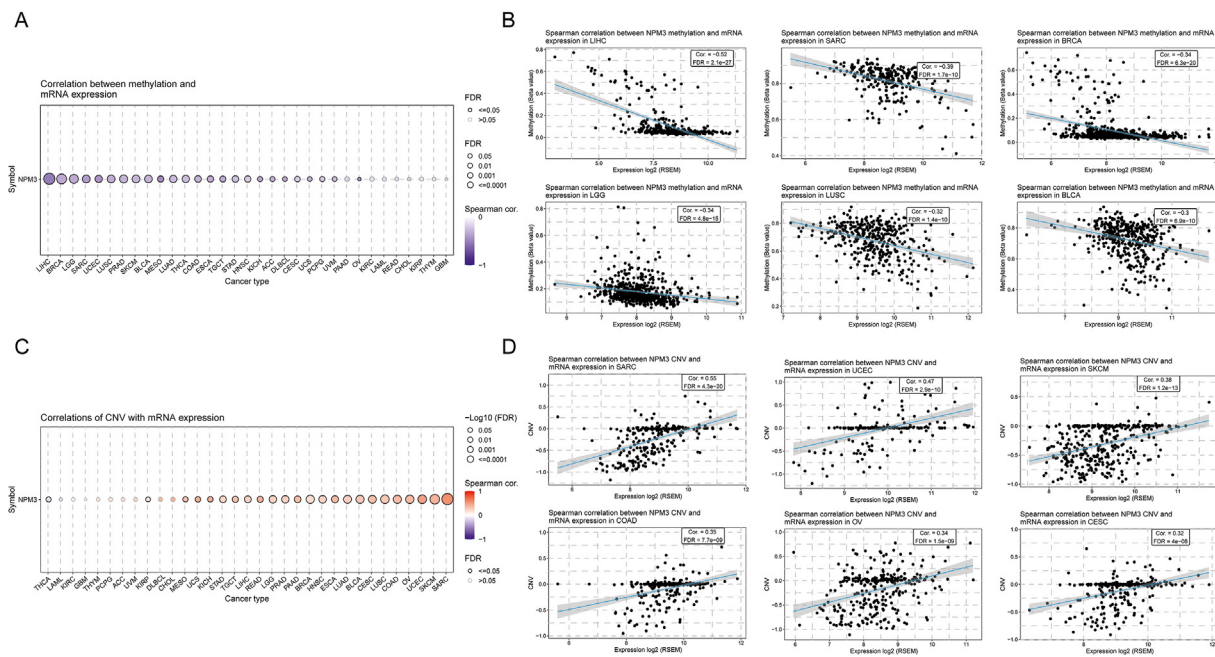
*Association of nucleophosmin/nucleoplasmin 3 expression and tumor mutation load and microsatellite instability in various cancers*

TMB and MSI have recently identified biomarkers for tumor diagnosis that have shown a strong correlation with the effectiveness of immunotherapy in patients with cancer.<sup>34</sup> Therefore, we explored the correlation

between *NPM3* expression, TMB, and MSI in pancreatic cancers. Figure 7A illustrates the positive correlation between *NPM3* expression and TMB in 10 cancer types: ACC, BRCA, COAD, HNSC, KICH, LUAD, LUSC, SKCM, PAAD, and STAD. Figure 7B shows a positive connection between *NPM3* expression and MSI in BRCA, DLBCL, KICH, KIRC, KIRP, and STAD and a negative connection with LUAD.

*Protein–protein interaction network of nucleophosmin/nucleoplasmin 3-binding proteins and enrichment analysis*

To investigate the possible involvement of *NPM3* expression in carcinogenesis and development, we utilized STRING to obtain 20 *NPM3*-



**Figure 5.** Association between Nucleophosmin/nucleoplasmin 3 expression and Nucleophosmin/nucleoplasmin 3 methylation and copy number variation. (A) Spearman correlation between *NPM3* methylation and mRNA expression in pan-cancer. (B) The top six cancers with the highest correlation score between *NPM3* methylation and mRNA expression. (C) Spearman correlation between *NPM3* CNV and mRNA expression in pan-cancer. (D) The top six cancers with the highest correlation score between *NPM3* CNV and mRNA expression. ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; ccRCC: Clear cell renal cell carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; CNV: Copy number variation; DLBCL: Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MECO: Mesothelioma; *NPM3*: Nucleophosmin/nucleoplasmin 3; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma; UVM: Uveal melanoma.

binding proteins supported by experimental data and established a PPI network [Figure 8A]. Subsequently, we identified the top five genes with the highest relevance to *NPM3* and generated a heatmap of the association between *NPM3* and these five genes across all cancers. The results revealed that *NPM3* expression positively correlated with chaperonin containing TCP1 subunit 7 (CCT7), glutaredoxin 3 (GLRX3), MRT4 homolog ribosome maturation factor (MRTO4), programmed cell death 11 (PDCD11), and Ras-related nuclear (RAN) expression in various cancers [Figure 8B].

We utilized the “cluster Profiler” R package to perform GO and KEGG enrichment analyses to explore the possible molecular functions, cellular environment, biological processes, and potential carcinogenic pathways associated with *NPM3*. GO analysis indicated that *NPM3* was mainly related to biological processes such as “structural constituent of ribosome,” “ribonucleoprotein complex binding,” “ribosomal RNA binding,” “small nucleolar RNA binding,” “small nuclear RNA binding,” “U6 small nuclear RNA binding,” and “histone kinase activity” [Figure 8C]. KEGG analysis revealed that *NPM3* may be involved in “ribosome,” “Coronavirus disease-COVID-19,” “spliceosome,” “ribosome biogenesis in eukaryotes,” “nucleocytoplasmic transport,” and “non-homologous end-joining” pathways [Figure 8D].

#### Nucleophosmin/nucleoplasmin 3 expression and drug response

We utilized the CellMiner database and R4.1.3 to investigate the connection between *NPM3* expression and sensitivity to antineoplastic agents. This analysis revealed a significant positive correlation between *NPM3* expression and sensitivity to 8-Chloro-adenosine, cytarabine,

methotrexate, AT-13387, cladribine, dexrazoxane, parthenolide, RH1, obatoclax, idarubicin, nitrogen mustard, oxaliplatin, amonafide, and ribavirin. Conversely, a negative correlation was observed between *NPM3* expression and the response to AZD-8055 [Figure 9].

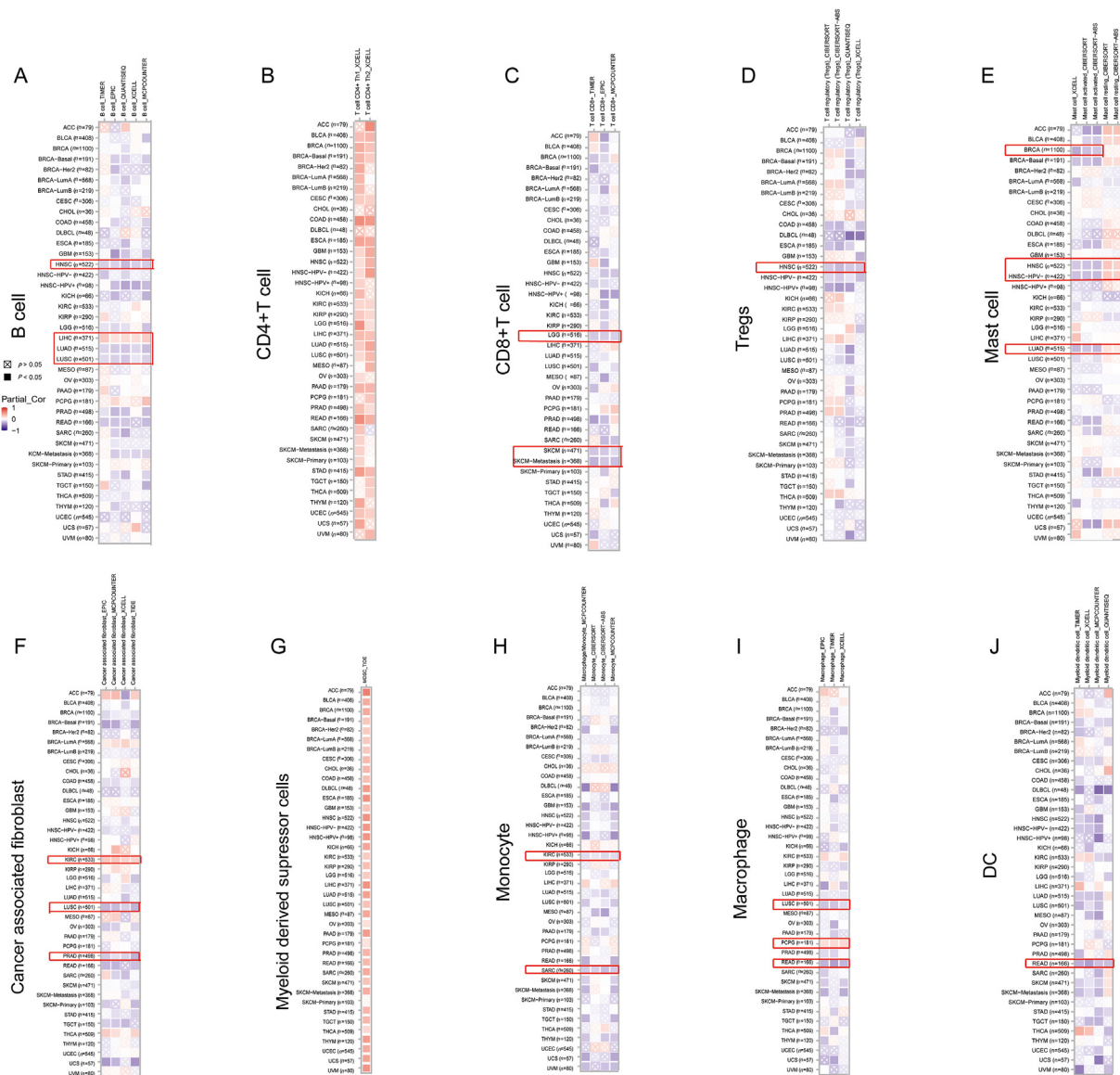
#### Prognostic value and association with clinical characteristics of nucleophosmin/nucleoplasmin 3 expression in lung adenocarcinoma

*NPM3* expression, age, sex, tumor size, and the presence of lymph node metastasis were included as variables in the univariate and multivariate Cox regression analyses. The results showed that age, tumor size, presence of lymph node metastasis, and *NPM3* expression were independent risk factors affecting the prognosis of patients with LUAD ( $P < 0.05$ ; Figures 10A and B). These risk factors were included in the construction of a nomogram to predict the survival of patients with LUAD at 1, 3, and 5 years [Figure 11A]. The calibration curves of the nomogram demonstrated reliable predictive performance for predicting the actual likelihood of 1-year, 3-year, and 5-year OS in patients with LUAD [Figures 11B–D].

#### Validation of the relative expression of nucleophosmin/nucleoplasmin 3 in lung adenocarcinoma by real-time quantitative polymerase chain reaction

To confirm the differential expression of *NPM3* in LUAD, we quantified the mRNA levels of *NPM3* in seven tumor and matched normal tissue samples using RT-qPCR. The results demonstrated a significant upregulation of *NPM3* expression in tumor tissues compared to the corresponding normal tissues ( $P < 0.05$ ; Figures 12A and B).



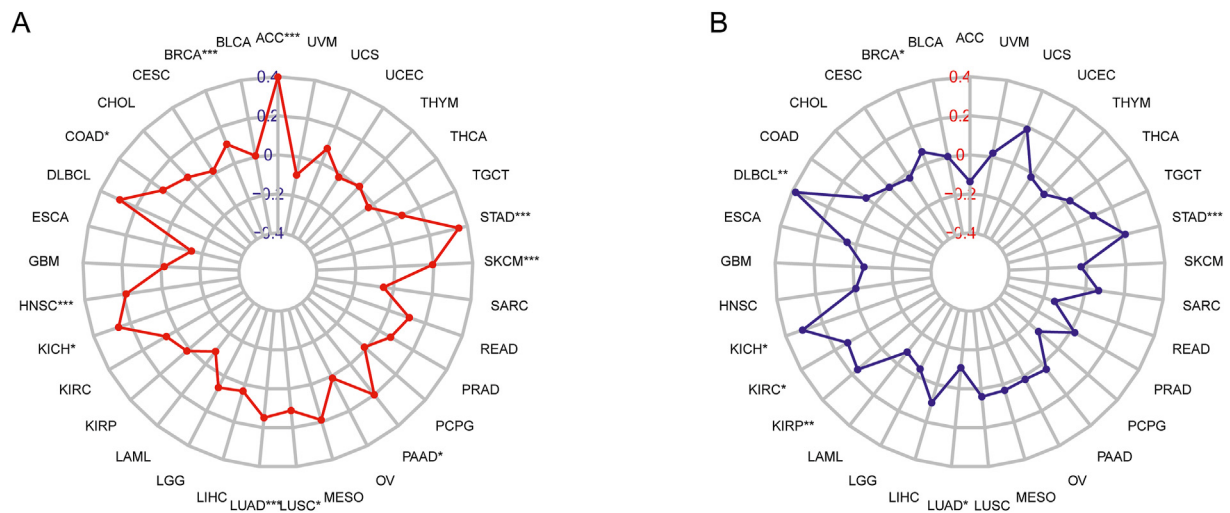


**Figure 6.** Correlation analysis between nucleophosmin/nucleoplasmin 3 expression level and immune cell infiltration. (A–J) Correlative analysis between *NPM3* expression level and the infiltration of B cells, CD4<sup>+</sup>T cells, CD8<sup>+</sup> T cells, Tregs, mast cells, cancer-associated fibroblasts, MDSCs, monocytes, macrophages, and DCs using TIMER, CIBERSORT, CIBERSORT-ABS, EPIC, MCPCCOUNTER, QUANTISEQ, and XCELL algorithms. Correlations with *P* values < 0.05 are considered statistically significant and are indicated by black rectangles, and statistically insignificant correlation values are marked by crosses. Red indicates a positive correlation (0–1), while blue indicates a negative correlation (-1-0). ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; ccRCC: Clear cell renal cell carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; DC: Dendritic cell; DLBCL: Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MECO: Mesothelioma; MDSC: Myeloid-derived suppressor cell; *NPM3*: Nucleophosmin/nucleoplasmin 3; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; TIMER: Tumor immune estimation resource; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine Carcinosarcoma; UVM: Uveal melanoma.

**Discussion**

*NPM3* was initially identified among the genes activated by pro-viral insertions of the mouse tumor virus and is closely linked to the proto-oncogene fibroblast growth factor 8 (FGF8), which is often co-activated.<sup>35</sup> *NPM3* belongs to the NPM family and is involved in many important biological processes such as DNA replication and transcriptional regulation, histone storage, histone chaperone activity, ribosome biogenesis, and chromatin remodeling.<sup>15</sup> Several studies have investigated the association between *NPM3* expression and cancer. For example,

deep sequencing of MIFS has shown significant upregulation of *NPM3*.<sup>17</sup> Additionally, a study conducted on a transgenic mouse model suggested that c-Myc could enhance gene expression through chromatin remodeling and post-transcriptional RNA editing and identified *NPM3* as a new c-Myc-responsive gene. Presently, changes in *NPM3* levels have been linked to human LUAD.<sup>36</sup> Similarly, a high-resolution gene microarray analysis of 27 patients with GC revealed deletions in *NPM3*.<sup>18</sup> Moreover, a study analyzing gene expression in follicular and diffuse large B-cell lymphomas using RT-qPCR showed a significant upregulation of *NPM3* in patients with DLBCL.<sup>19</sup>



**Figure 7.** Correlation of nucleophosmin/nucleoplasmin 3 expression with tumor mutation burden and microsatellite instability in pan-cancer. The results are presented with radar diagrams. (A) Correlation between TMB and *NPM3* expression. (B) Association between MSI and *NPM3* expression. Spearman's correlation coefficients are shown above ( $P < 0.05$  was considered statistically significant,  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ). ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; ccRCC: Clear cell renal cell carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; DLBCL: Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MECC: Mesotheioma; MSI: Microsatellite instability; *NPM3*: Nucleophosmin/nucleoplasmin 3; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; TMB: Tumor mutation burden; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma; UVM: Uveal melanoma.

In line with previous studies, our findings indicate that *NPM3* expression is upregulated in many malignancies, including DLBCL and LUAD.<sup>18,19</sup> *NPM3* may be a valuable prognostic marker due to the significant correlation between high *NPM3* expression and poor prognosis in several malignancies, as shown by KM analysis. Conversely, we observed the downregulation of *NPM3* expression in KICH, KIRC, and LAML cells, suggesting that *NPM3* may have different functions in different malignancies. Furthermore, we found significant changes in *NPM3* expression levels during HNSC, KIRP, and LUAD progression, implying a role for *NPM3* in malignancy progression. Notably, *NPM3* expression levels were higher in the late stages of these tumors than in the early stages.

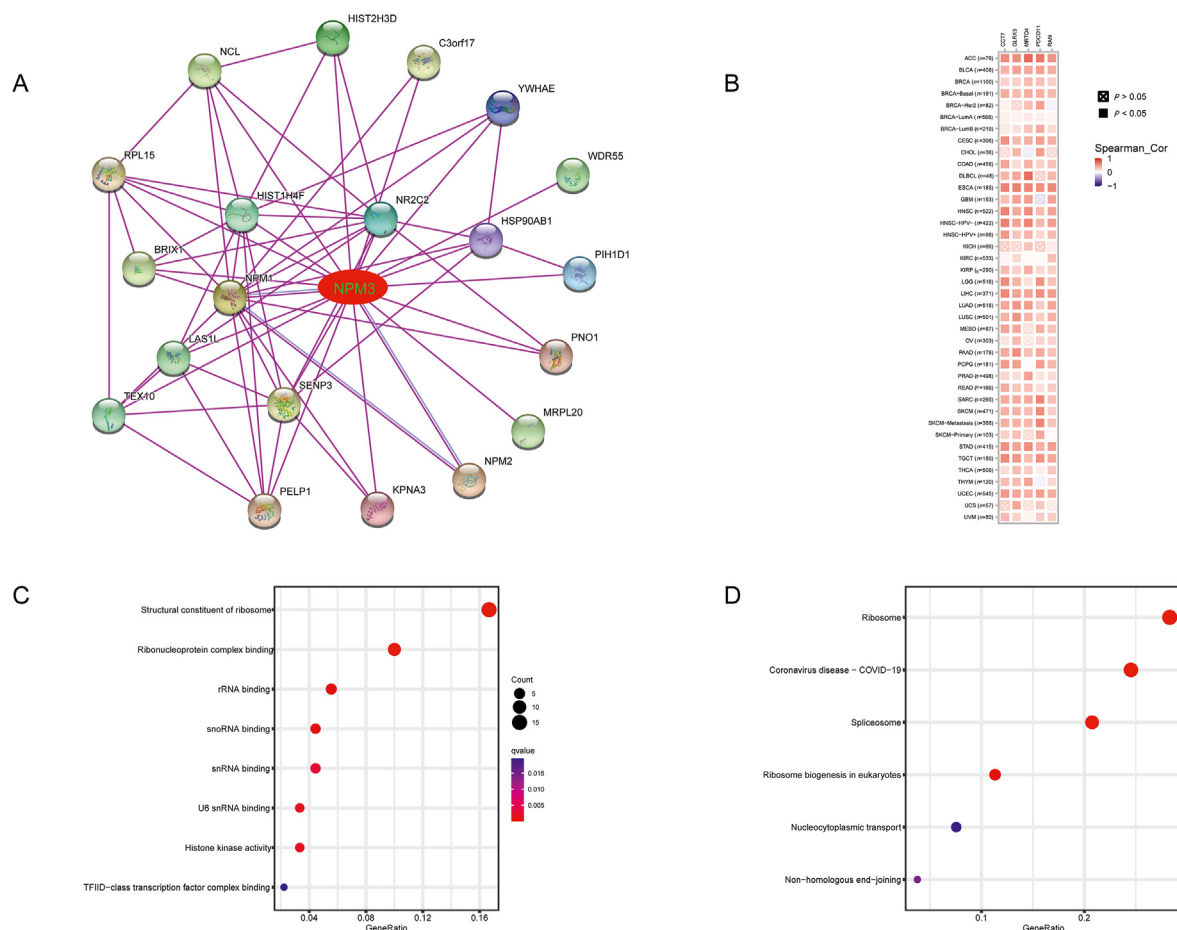
Tumor development is largely affected by genetic variations.<sup>37</sup> Therefore, we investigated the relationship between *NPM3* methylation, CNV, and *NPM3* expression. In most cancer types, our findings depicted a positive association between *NPM3* expression and methylation but showed a negative relationship with CNV.

A deeper understanding of immune cell infiltration is crucial for improving the outcomes of patients with tumors and increasing their response rates to immunotherapy.<sup>4</sup> Immunological cell-infiltrating tumors are generally comprised of T and B lymphocytes, natural killer cells, DCs, macrophages, neutrophils, eosinophils, MDSCs, and mast cells.<sup>33</sup> T cells have a strong tumor-killing ability and serve as the cellular basis of cancer immunotherapy.<sup>38</sup> According to our research, *NPM3* expression was positively correlated with the proportion of infiltrating Th1 and Th2 cells in most cancers but negatively correlated with the infiltration of CD8+T cells in LGG and SKCM. Th cells can remove tumor cells by direct cytotoxicity or indirectly regulating the tumor microenvironment.<sup>39</sup> CD8+T cells can be primed and activated into effector cytotoxic T cells, generating a potent and durable antitumor immune response.<sup>40</sup> Additionally, our investigation revealed a significant correlation between *NPM3* expression and MDSC infiltration in most malignancies. MDSCs are strongly linked to negative clinical outcomes of cancer and have been biologically established as potent immunosuppressive cells.<sup>41</sup> Furthermore, we observed a correlation between *NPM3* expression and levels of

immune and stromal cell infiltration in certain types of cancer. Our findings suggest that *NPM3* may play a role in modulating the TME, but the specific regulation mechanism seems to be intricate and unexplainable with the present data. Recently, single-cell technologies, including single-cell RNA sequencing and mass spectrometry, have advanced dramatically<sup>4</sup> and would enable obtaining a more systematic and thorough understanding of the interactions between different TME components in future research.

TMB and MSI are biomarkers that provide insights into potential responses to immunotherapy.<sup>42,43</sup> In various types of cancers, a combination of TMB and programmed cell death ligand 1 (PD-L1) is frequently employed to determine the need for immune checkpoint blockade selection, whereas MSI can serve as a biosignature for programmed cell death protein 1 (PD-1) blockade.<sup>44</sup> Our study revealed a significant correlation between *NPM3* and TMB/MSI in various cancers, including LUAD, suggesting a potential link between *NPM3* and tumor immunity, which motivated our in-depth study of *NPM3*.

To understand the potential role of *NPM3* in cancer development, we established a PPI network for *NPM3* and conducted GO and KEGG enrichment analyses. PPI network analysis revealed a strong association between *NPM3* and NPM1. During the cell cycle, nucleolar phosphoprotein NPM1 shuttles continuously between the cytoplasm and nucleus.<sup>45</sup> NPM1 has dual functions as a proto-oncogene and a tumor suppressor.<sup>46</sup> NPM1 regulates the ARF-p53 pathway.<sup>45</sup> Numerous studies have shown a strong correlation between NPM1 expression and several types of cancer. NPM1 has been identified as a prognostic biomarker for breast cancer,<sup>47</sup> colon cancer,<sup>48–50</sup> GC,<sup>51,52</sup> hepatocellular carcinoma,<sup>53</sup> ovarian cancer<sup>54</sup> and prostate cancer<sup>55,56</sup> and is one of the most commonly affected genes in hematological cancers.<sup>57</sup> Notably, some studies have shown that *NPM3* promotes the nucleocytoplasmic shuttling of NPM1 in the somatic nucleus by inhibiting the RNA-binding activity of NPM1. Furthermore, the NPM1-*NPM3* complex may regulate the chromatin structure at specific sites that are RNA polymerase II promoter-driven.<sup>58</sup> Other studies have shown that *NPM3* inhibits ribosome biogenesis and prevents NPM1 from acting as a histone chaperone.<sup>59</sup> In



**Figure 8.** Nucleophosmin/nucleoplasm 3-related gene enrichment analysis. (A) A STRING PPI network for *NPM3*. Colored nodes represent experimentally confirmed proteins that interact with *NPM3*. (B) Correlations of *NPM3* with the top-5 related genes in a heatmap. (C) KEGG enrichment analysis based on *NPM3*-binding and interacting genes. (D) GO analysis based on *NPM3*-interacting and correlated genes. ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; ccRCC: Clear cell renal cell carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; DLBCL: Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; GO: Gene Ontology; HNSC: Head and neck squamous cell carcinoma; KEGG: Kyoto Encyclopedia of Genes and Genomes; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MECO: Mesothelioma; NPM3: Nucleophosmin/nucleoplasm 3; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; PPI: Protein–protein interaction; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine Carcinosarcoma; UVM: Uveal melanoma.

summary, the optimal balance between *NPM1* and *NPM3* expression levels plays a critical role in maintaining the dynamic equilibrium of cells and preventing malignant transformation, which is closely linked to oncogenesis.<sup>59</sup> The results of our GO and KEGG analyses confirmed previous findings by demonstrating that high levels of *NPM3* expression in eukaryotes are primarily linked to rRNA binding and ribosome biogenesis.<sup>59</sup> Tumorigenesis is characterized by the uncontrolled proliferation of cancer cells<sup>60</sup> and is intimately linked to ribosome biogenesis,<sup>61</sup> and ribosome biogenesis is thought to play an important role in tumor metastasis and drug resistance.<sup>62</sup> Several traditional, highly efficient chemotherapeutic drugs have been shown to target ribosome biogenesis.<sup>63</sup> However, the underlying mechanism remains unclear.<sup>64</sup> Therefore, our analysis of *NPM3* is highly significant because it provides opportunities to understand the complex mechanisms of ribosome biogenesis and opens the door for new targeted cancer therapies.

Lung cancer is the leading cause of cancer-related deaths worldwide,<sup>2</sup> with LUAD as the most prevalent pathological type.<sup>65</sup> Therefore, it is imperative to investigate the fundamental mechanisms that drive the progression of LUAD and identify novel biomarkers. Our Cox regression analyses identified *NPM3* expression as an independent risk

factor for poor LUAD prognosis. Therefore, we constructed a nomogram based on independent prognostic factors for LUAD, such as *NPM3* expression, which can effectively predict the survival of patients with LUAD. Furthermore, the high expression of *NPM3* was validated using RT-qPCR in seven pairs of LUAD and paraneoplastic tissues. Our study indicates that *NPM3* expression is a potential biomarker for the prognosis of LUAD.

Despite conducting an initial exploration of the carcinogenic role of *NPM3* via several bioinformatics approaches, our research has certain limitations. Our study was based on several public databases with varying data collection and processing methods, which may have influenced our findings. Although we used biomolecular methods to validate the high expression of *NPM3* in LUAD, further *in vivo* and *in vitro* experiments are necessary to confirm and investigate our findings.

In conclusion, we performed a novel comprehensive pan-cancer analysis of *NPM3* that revealed significant associations between *NPM3* expression and multiple cancer prognoses, DNA methylation, CNV, immune cell infiltration, TMB, and MSI. Our investigation also included a preliminary exploration of the underlying mechanisms involved. Notably, we identified *NPM3* expression as an independent prognostic

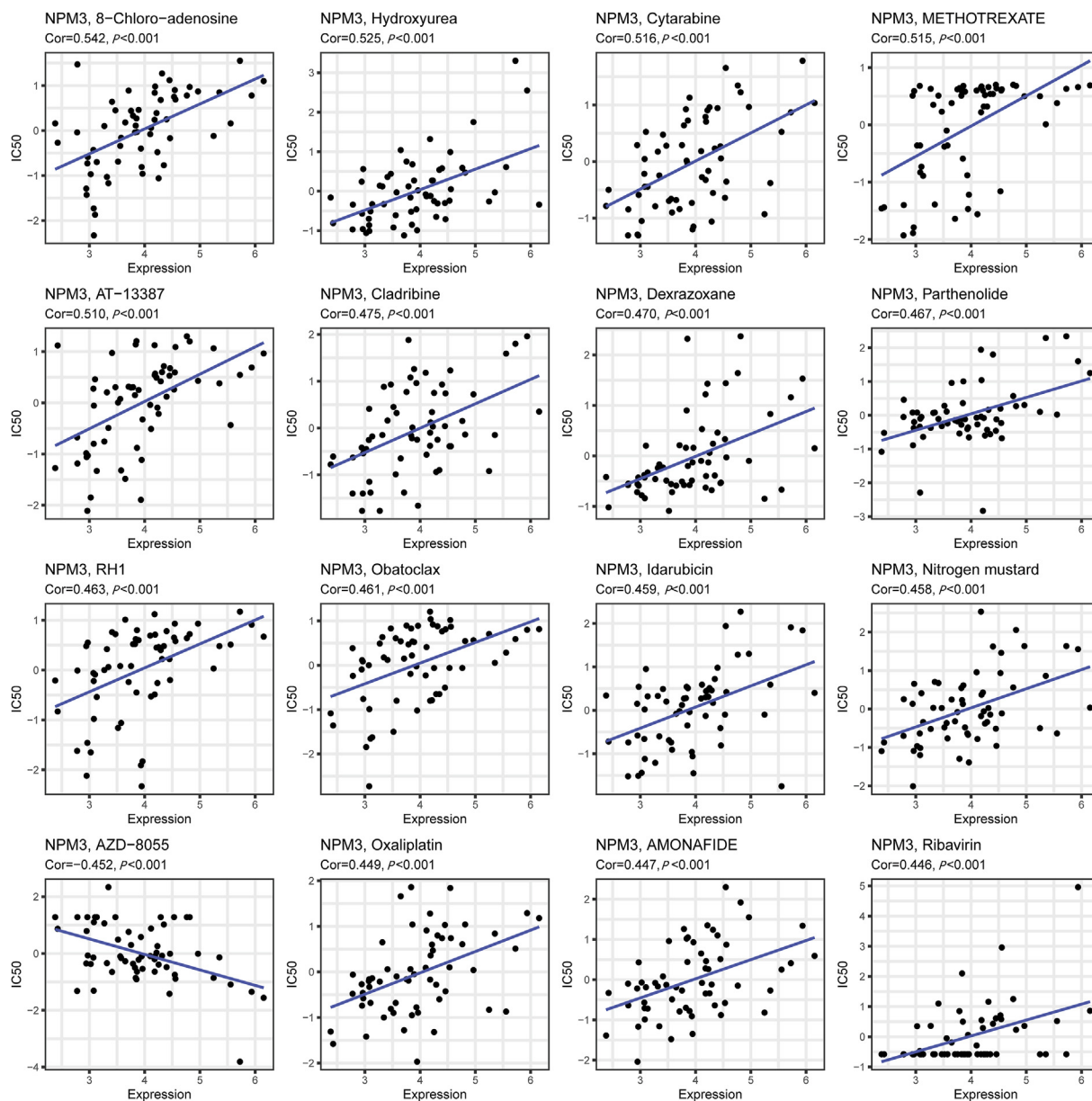


Figure 9. Scatter plots of the connection between nucleophosmin/nucleoplasmin 3 expression and expected drug response.

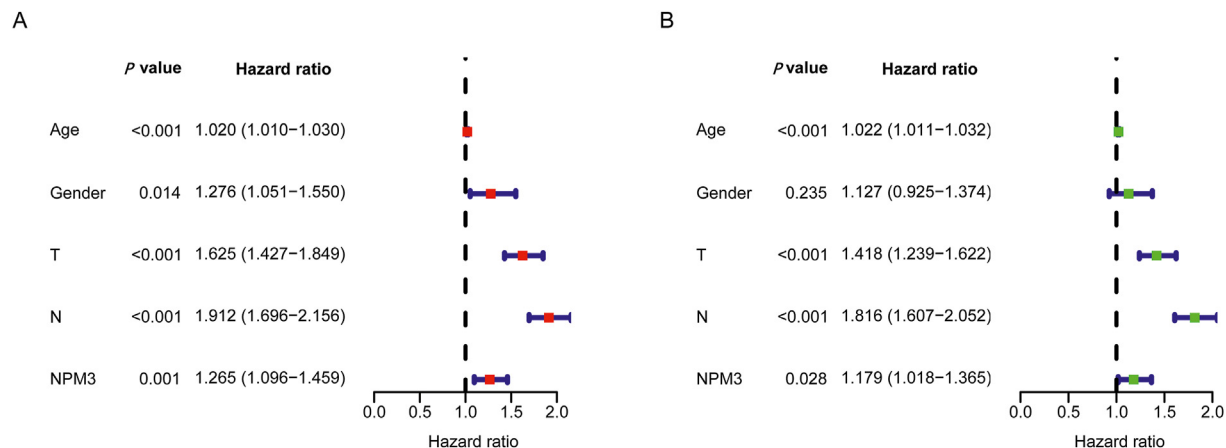
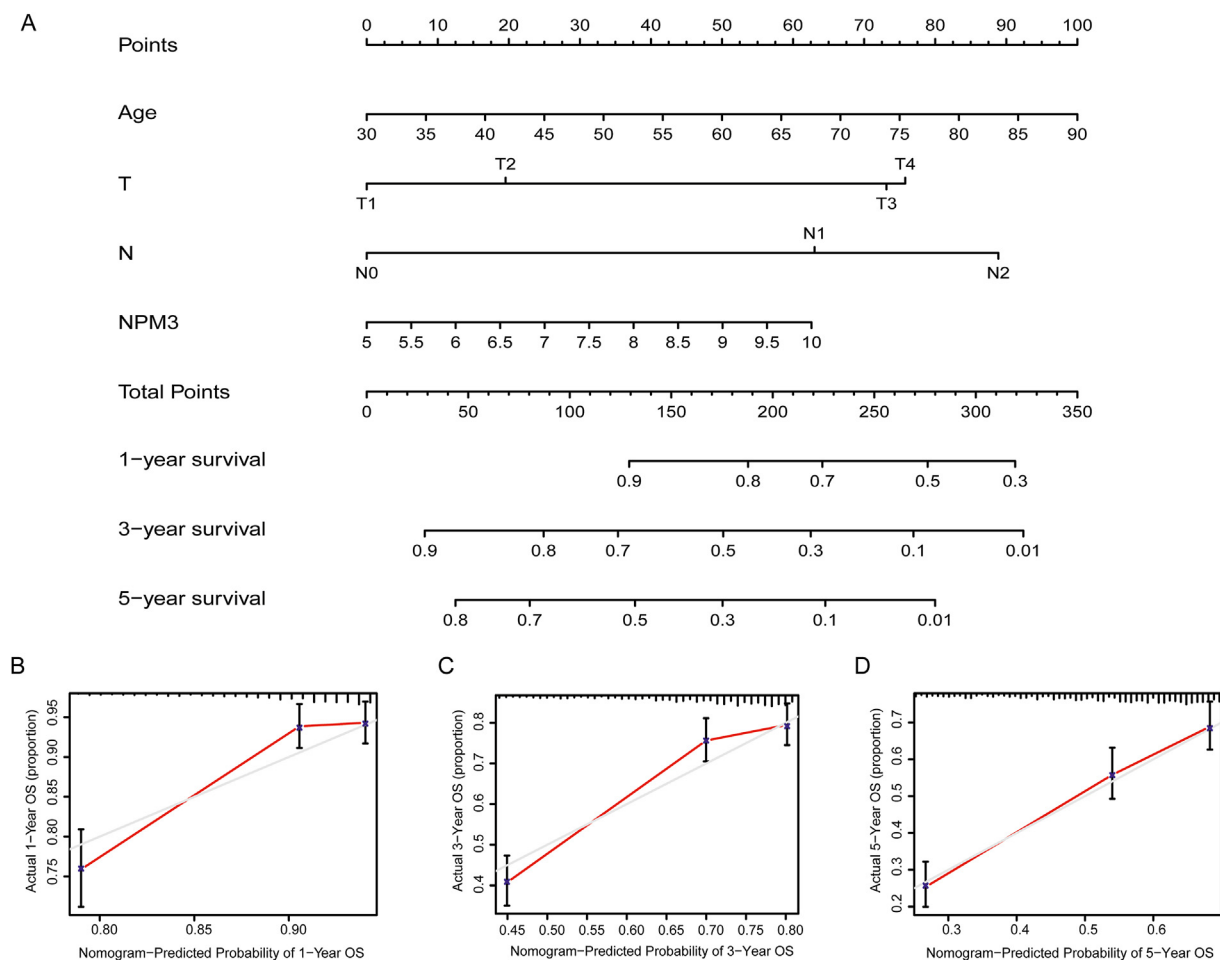
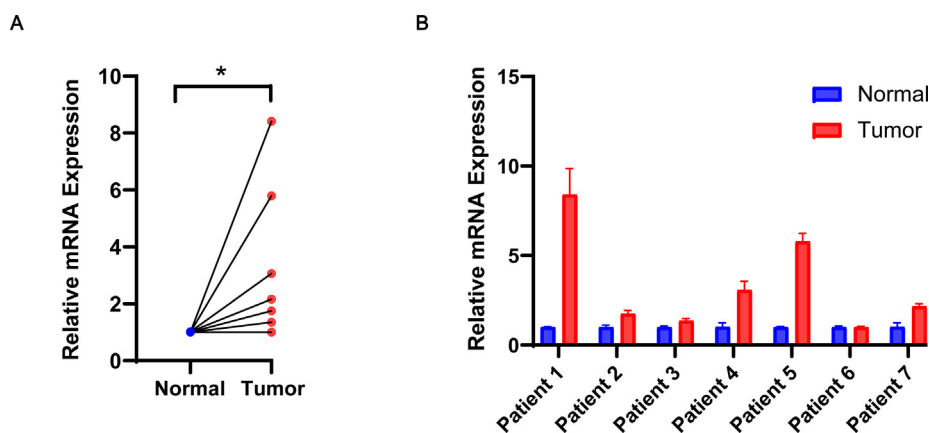


Figure 10. Independent prognostic value analysis of nucleophosmin/nucleoplasmin 3. (A) Single-factor Cox regression analysis. (B) Multi-factor Cox regression analysis.



**Figure 11.** Nomogram and calibration curves for patients with lung adenocarcinoma. (A) Nomogram. (B) Calibration curve for 1-year OS predicted by nomogram. (C) calibration curve for 3-year OS predicted by nomogram. (D) calibration curve for 5-year OS predicted by nomogram (the gray line represents the ideal prediction, and the red line denotes the actual fit). LUAD: Lung adenocarcinoma; N: Lymph node; OS: Overall survival; T: Tumor.



**Figure 12.** Validation of nucleophosmin/nucleoplasmin 3 expression: via real-time quantitative polymerase chain reaction. (A) The relative mRNA expression of *NPM3* in tumor and paraneoplastic tissues is presented in the form of a paired scatter graph. (B) Comparison of the relative mRNA expression of *NPM3* in tumor and paraneoplastic tissues of seven patients, respectively.  $P < 0.05$  was considered statistically significant;  $*P < 0.05$ . *NPM3*: Nucleophosmin/nucleoplasmin 3; RT-qPCR: Real-time quantitative polymerase chain reaction.

marker for LUAD and developed a nomogram that could effectively predict the prognosis and clinical outcomes of patients with LUAD. Our study indicates that *NPM3* could be a promising biomarker for predicting prognosis and immunotherapy efficacy in pan-cancer, thus potentially providing an antitumor strategy that targets *NPM3*.

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## Authors contribution

Qianhui Wei: Conceptualization, Writing- Original draft preparation. Jing Zhou: Data curation. Xinyue Wang: Methodology, Software. Zhaona Li: Visualization. Xiuqiong Chen: Software, Validation. Kaidi Chen: Data curation. Richeng Jiang: Supervision.

## Ethics statement

This project was reviewed and approved by the Institutional Ethics Committee of Tianjin Medical University Cancer Institute and Hospital (No. bc2022263). All participants provided written informed consent to participate in this study. All the procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the *Helsinki Declaration* of 1975, as revised in 2000.

## Data availability statement

The proposed dataset can be found in an online repository. Please refer to the article/supplementary material for the name and accession number of the repository. For further inquiries, the appropriate author can be contacted directly.

## Conflicts of interest

None.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cpt.2023.06.004>.

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