A Prognostic Molecular Signature of N⁶-Methyladenosine Methylation Regulators for Soft-Tissue Sarcoma from The Cancer Genome Atlas Database

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Soft-tissue sarcomas are a group of heterogeneous and rare mesenchymal tumors with aggressive behavior. We aimed to identify the molecular signatures of N⁶-methyladenosine (m⁶A) methylation regulators associated with patient prognosis using The Cancer Genome Atlas (TCGA) database.

Material/Methods: To evaluate the role of m⁶A in soft-tissue sarcomas, genomic and clinical data were downloaded from TCGA. The copy number variations (CNVs) and mutations of m⁶A regulators were analyzed.

Results: Alterations of m⁶A regulators were common, and ALKBH5 showed the highest frequency of copy number gain, while ZC3H13 had the highest frequency of loss. CNVs and mutations were closely correlated with histology ($P<0.001$) and tumor size ($P=0.040$), and CNVs were correlated with mRNA expression. Furthermore, patients with gains of METTL16, RMB15, RMB15B, YTHDC, and YTHDF3 displayed poorer overall survival (OS), and patients with gains of RBM15 and YTHDC2 and loss of IGF2BP1 had poorer disease-free survival (DFS). Further analysis indicated that CNVs and mutations of KIAA1429, YTHDF3, and IGF2BP1 were independent risk factors predicting OS and DFS. Gain of "writers" with loss of "erasers" led to worse OS than gain of "writers". Genes involved in JAK2 oncogenic signature were enriched in cases of higher expressions of METTL16, YTHDC2, and YTHDF3. Similarly, the core serum response signature was enriched in patients with higher expressions of IGF2BP1, METTL16, RBM15, and YTHDC2.

Conclusions: Our study provides a useful molecular tool to predict the outcome of soft-tissue sarcomas and deepens our understanding of the molecular mechanisms of the development of the disease.

MeSH Keywords: DNA Copy Number Variations • Methylation • Mutation • Prognosis • Sarcoma

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DATABASE ANALYSIS

Background

Soft-tissue sarcomas are a group of heterogeneous and rare mesenchymal tumors with aggressive behavior. Tumor resection and radiotherapy are still the main treatments for non-metastatic sarcomas [1]. Although the 5-year overall survival (OS) rate is about 70% in sarcomas [2], poor prognosis is common with local recurrence [3]. Currently, the most useful features to predict prognosis and recurrence are tumor grade, tumor size, histology, margin status, and tumor aggressiveness [4,5]. However, there is much heterogeneity even among localized high-risk tumors. It has been shown that approximately 50% of patients with these tumors achieve long-term remission, while the other 50% develop recurrence within 5 years [6]. Therefore, it is imperative to identify molecular tools to treat sarcomas and predict patient prognosis.

Methylation of N^6-adenosine (m⁶A) is the most common type of RNA modification and is involved in a variety of cancer behaviors [7]. m⁶A is controlled by various types of regulators, including methyltransferases ("writers"), RNA-binding proteins ("readers"), and demethylases ("erasers"). The m⁶A mediated by these regulators plays crucial roles in cancer cell malignancy and leads to many disorders [8]. Identification of these different m⁶A regulators has helped elucidate the effect of RNA methylation on gene regulation [9].

However, there are few studies on m⁶A in sarcomas. In this study, we evaluated the copy number variations (CNVs) and mutations of these m⁶A regulators and aimed to provide a useful molecular tool to predict the outcome of sarcomas and deepen our understanding of the molecular mechanisms of the development of sarcomas.

Material and Methods

Ethics statement

Soft-tissue sarcomas (leiomyosarcoma, liposarcoma, pleomorphic sarcoma, myxofibrosarcoma, synovial sarcoma, and malignant peripheral nerve sheath tumor) clinical data and genomic data were downloaded from the adult soft-tissue sarcomas program of The Cancer Genome Atlas (TCGA, Cell 2017) from the cBio-portal platform (*https://www.cbioportal.org/*), which is publicly available. Therefore, all written informed consent was obtained. According to the Cancer Genome Atlas Research Network (*https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/history/timeline*), participants were recruited into the study in November 2017 and participants' data and samples were collected. The present study was conducted from July 1 to 22, 2020. Authors had no access to information that could identify individual participants during or after data collection.

Data processing

A total of 18 m⁶A regulators, which had been identified in previous papers [10,11], were analyzed in this study: writers, METTL3, METTL14, METTL16, WTAP, RBM15, RBM15B, ZC3H13, and KIAA1429; erasers, FTO and ALKBH5; and readers, YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, HNRNPC, HNRNPA2B1, and IGF2BP1. We collected and identified 206 soft-tissue sarcoma patient cases with genomic and clinical data from the TCGA project. The GISTIC segmentation algorithm was used to identify the CNV status of the m⁶A regulators. The 206 patients were divided into 2 groups according to the presence of CNVs and mutations in the m⁶A regulators. Clinicopathological features were compared between the 2 groups. The relationship between CNVs and mRNA was evaluated after calculating and log scaling of mRNA expression data from the V2 RNA-Seq by expectation-maximization (RSEM).

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) application was available with Java software with MSigDB v6.1 and was downloaded from the Broad Institute website. Subjects were divided into 2 groups based on the median mRNA expression value. Hallmark gene set "c6.all.v7.1.symbols.gmt" was applied in our research. Gene sets with a normalized *P* value <0.05, and a false discovery rate <0.25 were considered to be significantly enriched.

Statistical analysis

Statistical analysis was performed with R version 4.0.2 or SPSS 20.0 (IBM, Chicago, IL, USA). Figures were produced by R (version 4.0.2) or GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA). The chi-square test and Fisher exact test were used to analyze categorical variables. The log-rank and Kaplan-Meier method were used to analyze survival data. All statistical results with a *P* value <0.05 were considered to be significant.

Results

CNVs and mutations of m⁶A regulators in soft-tissue sarcoma

Mutations of m⁶A regulators were detected in only 11 of the 206 samples of patients with genomic data (Table 1). However, CNVs of the 18 m⁶A regulators were commonly found in all 206 samples of patients with CNV data (Figure 1A), with the number of events of loss (941/3708) and gain (1023/3708) being similar (Figure 1B) (Table 2). The loss and gain percentages of each m⁶A regulator were also analyzed (Figure 1C, 1D). Among all regulators, ALKBH5 had the highest frequency of copy number gain (Figure 1C), while ZC3H13 had the highest frequency of copy number loss (Figure 1D).

Table 1. Mutations of m⁶A regulatory genes in 206 patients with soft-tissue sarcoma.

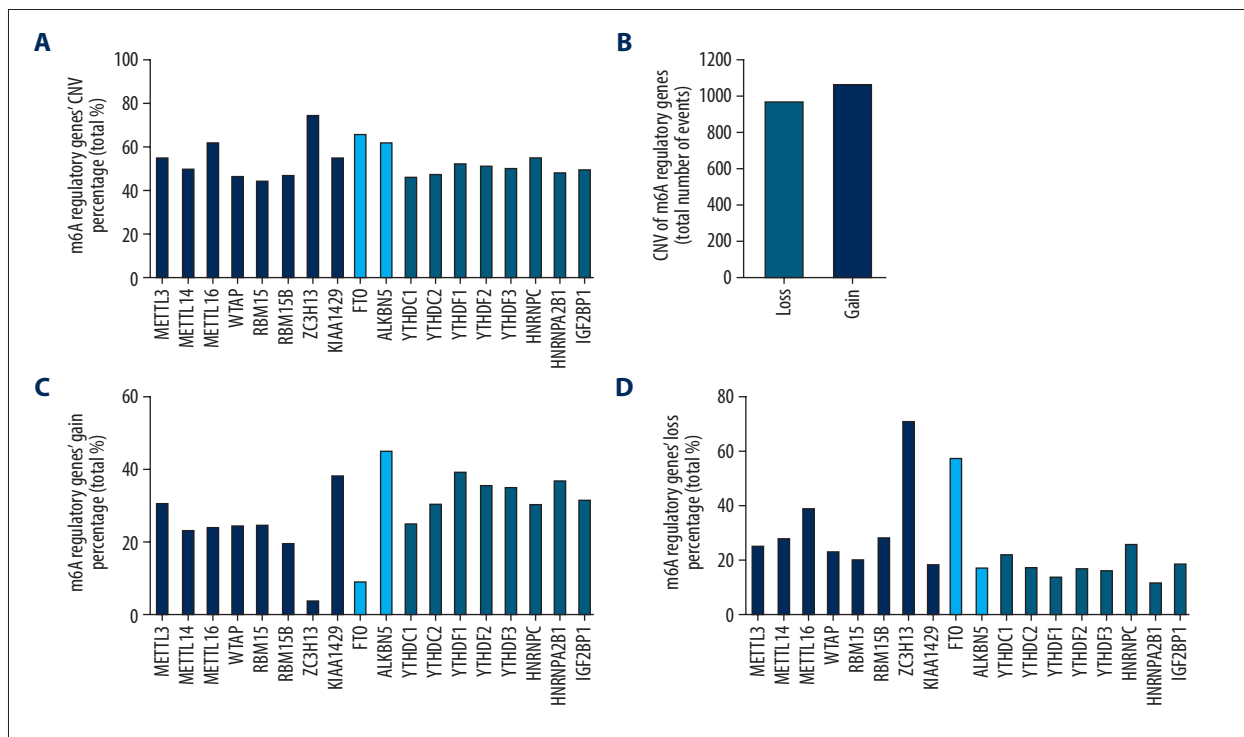| SST sample ID	KIAA1429	RBM15	ZC3H13	YTHDC1	YTHDC2	YTHDF3	IGF2BP1
TCGA-FX-A48G-01	p.V128E						
TCGA-DX-A8BK-01	p.V28=						
TCGA-DX-AB32-01	p.R1640H						
TCGA-FX-A76Y-01		p.E387D					
TCGA-3B-A9HT-01			p.Q935H				
TCGA-DX-A6BA-01							p.I420T
TCGA-IW-A3M6-01							p.T249S
TCGA-DX-A8BM-01							p.R174Q
TCGA-X6-A7W8-01				p.E240=			
TCGA-X6-A8C2-01					p.S347Y		
TCGA-IF-A4AJ-01						p.E572=	

Figure 1. Mutations and copy number variations (CNVs) of m⁶A regulatory genes in patients with soft-tissue sarcoma. (**A**) CNV percentages of the 18 m⁶A regulators. (**B**) Loss and gain events of the 18 m⁶A regulators. (**C, D**) Gain and loss percentages of the 18 m⁶A regulators.

Relationship between CNVs and mutations of m⁶A regulators and clinicopathological features

To determine the role of m⁶A regulators in soft-tissue sarcoma, we analyzed the correlation of CNVs and mutations of m⁶A regulators with patient clinicopathological characteristics including age, sex, histologic diagnosis, presence of metastasis, tumor size, and FNCLCC grade. The results showed that m⁶A regulators were closely correlated with histologic diagnosis (*P*<0.001) and tumor size (*P*=0.040), but not with age, sex, metastatic status, and FNCLCC grade (Table 3).

Table 2. Different copy number variation (CNV) patterns occurring in soft-tissue sarcoma samples (n=206).

		Diploid	Deep deletion	Shallow deletion	Copy number gain	Amplification	CNV* sum	Percentage
Writer	METTL3	94	0	50	57	5	112	54.37%
	METTL14	104	1	55	44	2	102	49.51%
	METTL16	79	0	79	45	3	127	61.65%
	WTAP	111	0	46	45	4	95	46.12%
	RBM15	116	4	36	50	0	90	43.69%
	RBM15B	110	2	55	39	0	96	46.60%
	ZC3H13	54	2	143	7	0	152	73.79%
	KIAA1429	93	2	34	73	4	113	54.85%
Eraser	FTO	71	4	113	17	1	135	65.53%
	ALKBH5	80	0	35	73	18	126	61.17%
Reader	YTHDC1	111	0	44	50	1	95	46.12%
	YTHDC2	110	1	33	57	5	96	46.60%
	YTHDF1	99	0	27	76	4	107	51.94%
	YTHDF2	101	1	32	70	2	105	50.97%
	YTHDF3	104	1	30	66	5	102	49.51%
	HNRNPC	94	0	51	57	4	112	54.37%
	HNRNPA2B1	108	0	23	68	7	98	47.57%
	IGF2BP1	105	0	37	61	3	101	49.03%

* CNV – copy number variation.

Table 3. Clinical pathological parameters of patients with soft-tissue sarcoma with or without mutations and CNVs of m⁶A regulatory genes.

		With mutation and/or CNV*	Without mutation and CNV*	P
Sex	Female	105	7	0.313
	Male	84	10	
Age	≤60	93	11	0.312
	>60	96	6	
Histologic diagnosis	DDLPS	40	10	**<0.001**
	UPS	43	1	
	MFS	17	0	
	LMS	79	1	
	MPNST	4	1	
	SS	6	4	
Metastatic	YES	45	1	0.264
	NO	82	7	
	N/A**	62	9	
Tumor size (cm)	≥12.7***	69	11	**0.040**
	<12.7	112	6	
	N/A**	8	0	
FNCLCC grade	1	13	1	0.322
	2	100	12	
	3	76	4	

N/A – not applicable. * With mutation and/or copy number variation (CNV): The Cancer Genome Atlas (TCGA) soft tissue sarcoma patients with mutant or CNV or mutant + CNV; Without mutation and CNV: TCGA soft tissue sarcoma patients with neither mutant nor CNV; ** ambiguous variables (N/A) were excluded from chi-square test or Fisher exact test; *** the average of tumor size is 12.7 cm.
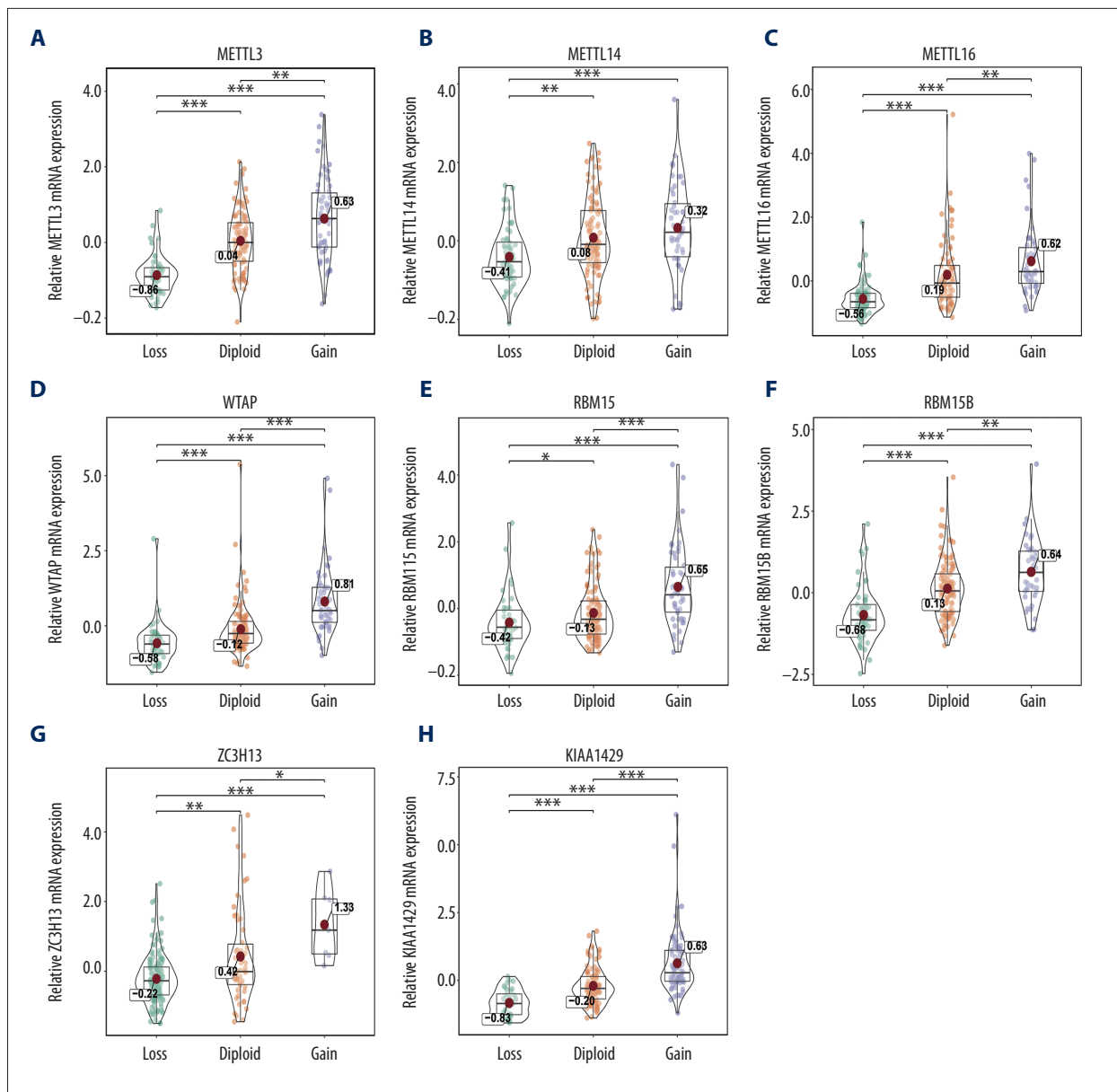
Figure 2. (**A–H**) Correlation of writer copy number variations (CNVs) with mRNA expression. The writer mRNA expression differences among cases with different CNVs. * *P*<0.05; ** *P*<0.01; *** *P*<0.001.

Correlation of CNVs with mRNA expression

The relationship between alterations in m6A regulators and corresponding mRNA expression levels was then analyzed. The data showed that various CNV levels were correlated with mRNA expression in the 206 patients with soft-tissue sarcoma. Copy number gains were correlated with higher mRNA expression, while copy number status loss was correlated with a decrease in mRNA expression (Figures 2-4). We also validated the findings in bladder cancer and found that copy number gains were correlated with higher mRNA expression in bladder cancer (Supplementary Figure 1).

Role of CNVs of m6A regulators in survival of patients with soft-tissue sarcoma

The roles of CNVs in the disease-free survival (DFS) and OS of patients with soft-tissue sarcoma were then explored to analyze the prognostic value of the m6A regulators. The results showed no difference between patients with and without CNVs of m6A regulators in terms of OS (*P*=0.64) and DFS (*P*=0.75) (Figure 5A, 5B). Interestingly, patients affected by gains of the m6A writer genes METTL16, RBM15, and RMB15B and reader genes YTHDC and YTHDF3 separately displayed poorer OS (Figure 5C–5G), and patients with gains of writers
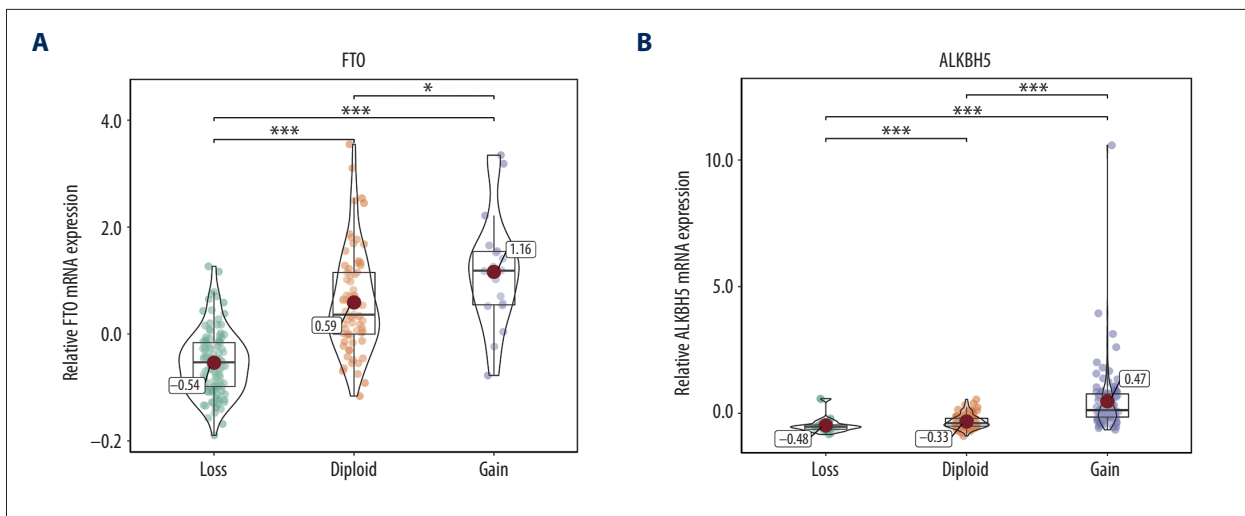
Figure 3. (**A, B**) Correlation of eraser copy number variations (CNVs) to mRNA expression. The eraser mRNA expression differences among cases with different CNVs. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

RBM15 and YTHDC2 and loss of reader IGF2BP1 had poorer DFS (Figure 5H–5J). Multivariate Cox regression analyses indicated that CNVs and mutations of writer KIAA1429 and readers YTHDF3 and IGF2BP1 were independent risk factors predicting OS and DFS (Table 4). To further validate the results in other cancers, a similar analysis was conducted on bladder cancer data, which showed that gains of some of the regulators were also related with poor survival of bladder cancer cases (Supplementary Figure 2).

Writers are a cluster of methyltransferases that play crucial roles in the m6A modification process. The above findings suggest that a higher expression of writers could result in poor prognosis. To validate the above conclusion, we then tested it in patients who were affected by 2 types of CNVs, copy number gain of writers and copy number loss of erasers.

Patients were further divided into 4 groups based on writer gain status and eraser loss status. As shown in Figure 6A, patients with a copy number gain of writers in combination with a loss of erasers had worse OS than those with only a copy number gain of writers (Figure 6A, 6B, Table 4). This provided more evidence for the link between an upregulated m6A level of writer genes and poor survival.

Pathways enriched in m6A regulation

To further understand the role of m6A regulators in the regulation of oncogenic pathways, we performed GSEA analysis. Patients were divided into 2 groups based on the median expression of mRNA. As shown in Figure 7A, the genes involved in the JAK2 oncogenic signature were enriched in patients with higher expressions of METTL16, YTHDC2, and YTHDF3 and were not enriched in patients with lower expressions.

Similarly, the core serum response (CSR) signature was also enriched only in patients with higher expressions of IGF2BP1, METTL16, RBM15, and YTHDC2 (Figure 7B).

Discussion

Previous studies have described the role of m6A regulators in sarcoma. For example, METTL3 can promote osteosarcoma progression by regulating the m6A level of LEF1 [12]. Despite these data, there is still a lack of studies on m6A regulators in sarcoma. In the present study, we systematically evaluated the roles of the CNVs of m6A regulators in sarcoma, especially in the prognosis of sarcoma. To the best of our knowledge, this is the first study to analyze the effects of CNVs of m6A regulators in sarcoma, and we hope to provide useful information to future researchers.

As indicated by our analysis, all genes of the m6A regulators developed CNVs in sarcoma; in particular, more than 60% of patients acquired METTL16, ZC3H13, FTO, and ALKBH5 CNVs, showing that CNVs of m6A regulators are common in sarcoma. In fact, genetic alteration is considered one of many features of sarcomas, and it has been demonstrated that CNVs and other alterations could result in dysregulated gene expression, subsequently leading to the development of sarcoma [13]. Our results consistently showed that CNVs closely correlate with mRNA expression and an outcome of sarcoma.

When we focus on high-risk soft-tissue sarcoma (high-grade, deep, large tumors), the mortality rate exceeds 50% [14]. Local recurrences of soft-tissue sarcoma of the trunk and extremities ranges from 7% to 15% and are associated with a poor prognosis, with a 2-year survival rate ranging from 50%
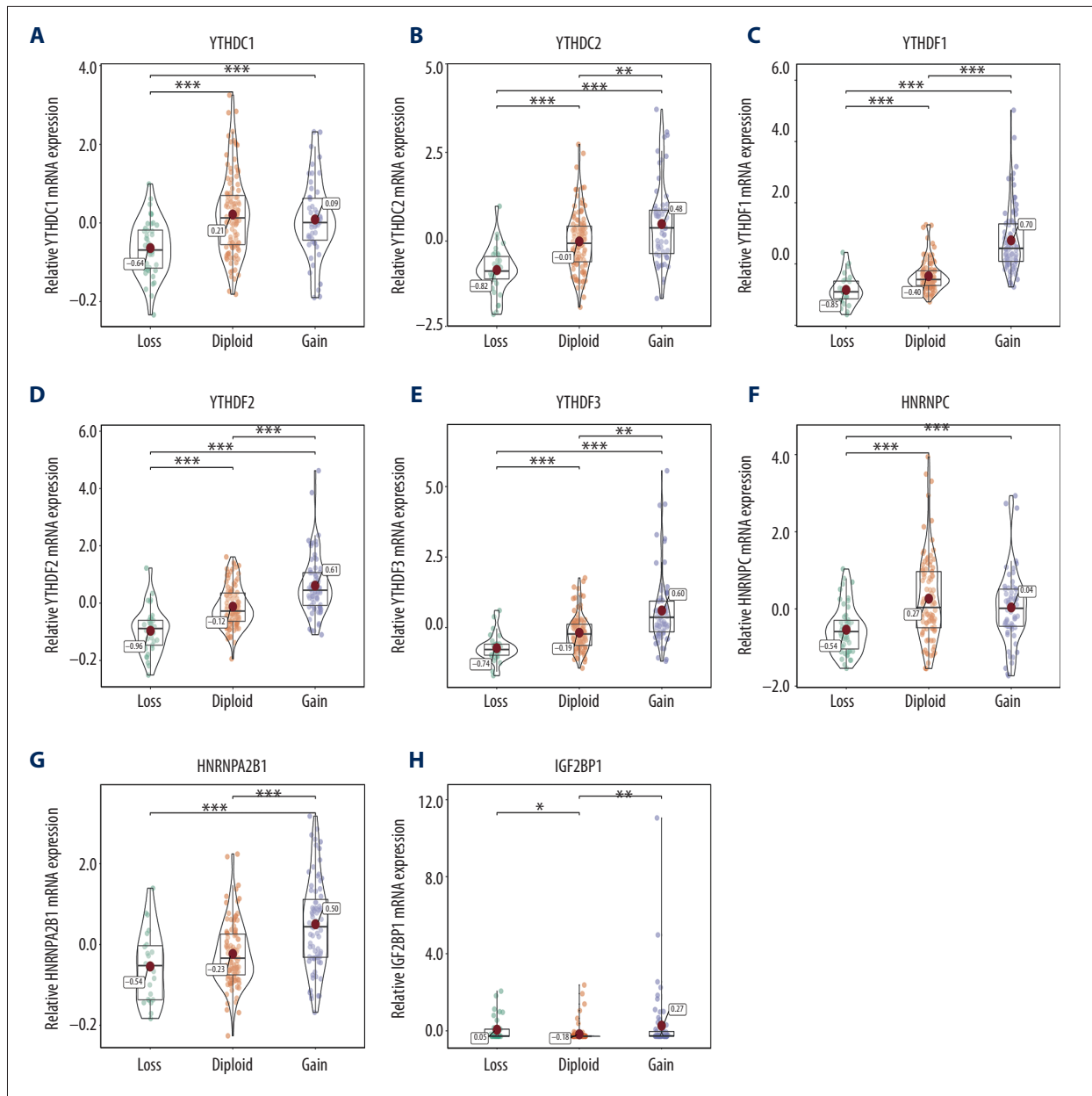
Figure 4. (**A–H**) Correlation of reader copy number variations (CNVs) to mRNA expression. The reader mRNA expression differences among cases with different CNVs. * *P*<0.05; ** *P*<0.01; *** *P*<0.001.

to 70% [15]. In the soft-tissue sarcoma cohort of the present study, we found the m⁶A regulator genes correlated with tumor size and histology. According to previous studies, the most important clinical risk factors for recurrence are high-grade, larger tumor size, and aggressive histology, as found in undifferentiated pleomorphic sarcoma, leiomyosarcoma, and dedifferentiated liposarcoma [16]. Further, the present analysis revealed the alterations of RBM15 (writer), YTHDC2 (reader), and IGF2BP1 (reader) were associated with an adverse prognosis.

These data can potentially help stratify patients at the highest risk of relapse and patients who would benefit from adjuvant chemotherapy. Especially in soft-tissue sarcoma, a high degree of heterogeneity contributes to considerable uncertainty about the clinical value of adjuvant chemotherapy on unselected patients.

Previous researchers have identified many other potentially prognostic molecules in sarcoma. The prognosis of patients with dedifferentiated liposarcoma and soft-tissue leiomyosarcoma from TCGA was evaluated and prognostic markers were
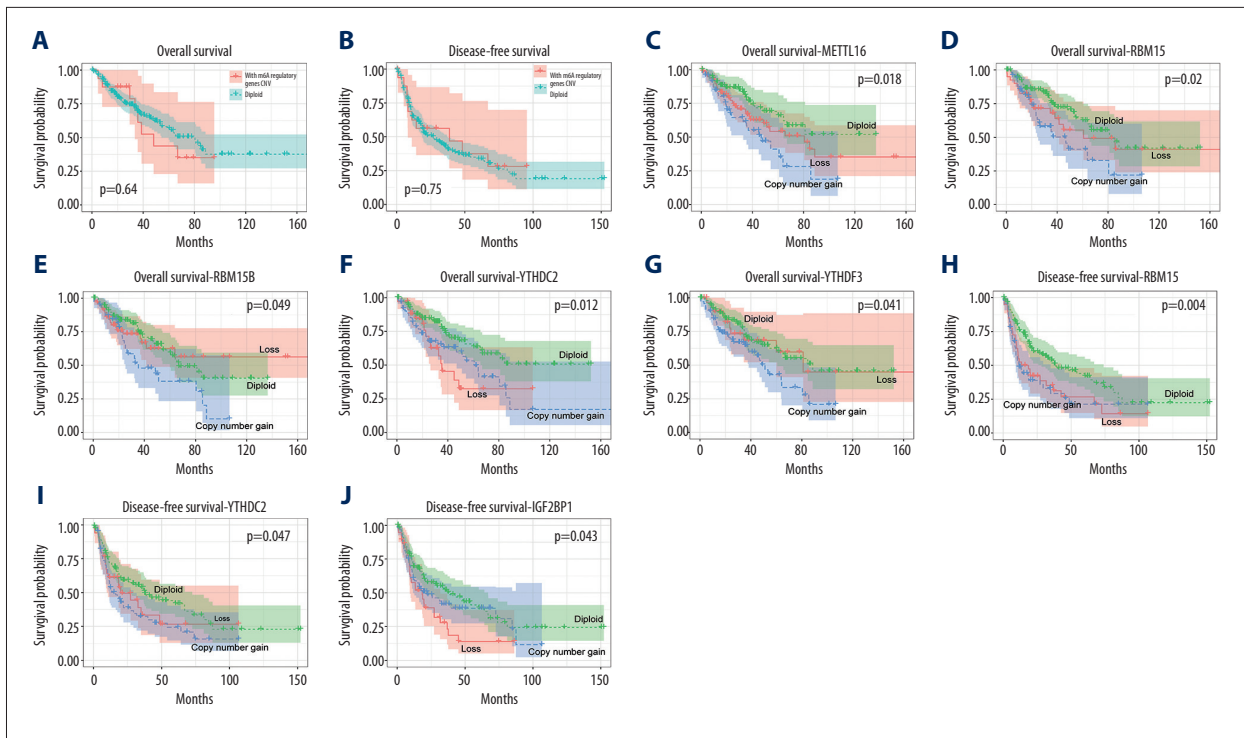
Figure 5. Association between copy number variation (CNVs) of m6A regulatory genes and survival of patients with soft-tissue sarcoma. Effects of CNVs (diploid *vs.* nondiploid) of m6A regulators on (**A**) overall survival (OS) and (**B**) disease-free survival (DFS). Effect of CNVs of (**C**) METTL16, (**D**) RBM15, (**E**) RBM15B, (**F**) YTHDC2, and (**G**) YTHDF3 on OS. Effect of CNVs of (**H**) RBM15, (**I**) YTHDC2, and (**J**) IGF2BP1 on DFS.

identified, showing that hypermethylation and certain chromosomal amplifications were associated with poor outcomes of dedifferentiated liposarcoma, while miRNA-181b was related to the shorter survival time of soft-tissue leiomyosarcoma [17]. Also, researchers developed a set of gene-expression signatures using the high-throughput method, which can identify patients at high-risk [18,19]. In the present study, we found that the CNV level of several m6A regulators, namely METTL16, RBM15, RBM15B, YTHDC2, YTHDF3, and IGF2BP1, are significantly associated with DFS and OS. The CNVs of YTHDC2 and RBM15, in particular, are correlated with both DFS and OS. As these molecules are mainly writers and erasers, we further stratified patients according to the CNVs of the writers and erasers. Interestingly, patients with writer gain and eraser deletion had worse OS than the other groups. Because an eraser functions as an adverse regulator of a writer, the above result makes sense and indicates that writer gain is a powerful indicator of poor prognosis. As shown in Figure 2, copy number gain was significantly correlated with mRNA expression. Higher writer mRNA is expected to predict the poor outcome of sarcoma patients. Consistently, the expression levels of mRNA writers, in contrast to those of readers and erasers, were indicated as risk factors of patients' survival [10].

Based on the influence of the CNVs of these m6A regulators on prognosis, we further evaluated their underling mechanisms. Patients were divided into 2 groups according to the median value of the m6A regulators following GSEA analysis of the 2 groups. We mainly focused on the GSEA results of IGF2BP1, METTL16, RBM15, RBM15B, YTHDC2, and YTHDF3 owing to their performance in predicting survival. Among the dysregulated pathways and hallmarks, JAK2 (genes downregulated in HEL cells [erythroleukemia] after the knockdown of JAK2) and CSR were the most commonly upregulated oncogenic signatures. JAK2 is a key component of the JAK family of protein tyrosine kinases and is an important intracellular mediator of cytokine and hormone signaling. Also, JAK2 is ubiquitously expressed in almost every cancer cell type [20]. JAK2 signaling plays crucial roles in both pathology and physiology processes and is involved in inflammation and hemopoiesis, especially in cancer [21,22]. The CSR signature includes genes that are induced in the fibroblast serum-response program, and these genes are expressed in tumor cells and tumor-associated fibroblasts [23]. The genes from the CSR signature are related to metastasis and death in various types of cancer [24]; thus, the CSR signature is considered to be a useful predictor of the clinical course in several cancers. Therefore, more detailed studies about the mechanisms of CNVs of m6A regulators in

Table 4. Univariate and multivariate Cox regression analysis of m⁶A regulatory genes for the overall survival (OS) and disease-free survival (DFS) of patients with soft-tissue sarcoma.

Variable	OS			
	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
Age (continuous)	1.02 (1.00–1.03)	**0.049**	1.05 (1.02–1.08)	**0.002**
Sex (Male *vs.* Female)	0.95 (0.60–1.49)	0.817		
Pathological tumour size (mm) (continuous)	1.04 (1.02–1.07)	**0.001**		
Residual tumour (R1/R2/RX *vs.* R0)	2.72 (1.73–4.26)	**<0.001**	2.76 (1.17–6.51)	**0.018**
Pharmaceutical drug adjuvant (no *vs.* yes)	0.85 (0.51–1.40)	0.521		
Radiation treatment adjuvant (no *vs.* yes)	1.31 (0.79–2.18)	0.300		
FNCLCC grade (3 *vs.* 1/2)	1.64 (1.05–2.57)	**0.029**	2.42 (1.03–5.71)	**0.040**
Metastatic disease (no *vs.* yes)	3.02 (1.68–5.43)	**<0.001**	4.63 (1.95–10.99)	**0.001**
m⁶A regulator alteration (writer loss+eraser deletion *vs.* others)	1.62 (1.03–2.54)	**0.036**	0.79 (0.31–2.05)	0.631
METTL16 (*vs.* diploid)				
Gain	2.27 (1.27–4.06)	**0.006**		
Loss	1.52 (0.88–2.61)	0.133		
RBM15 (*vs.* diploid)				
Gain	2.07 (1.23–3.47)	**0.006**		
Loss	1.39 (0.78–2.48)	0.259		
RBM15B (*vs.* diploid)				
Gain	1.86 (1.10–3.15)	**0.021**		
Loss	1.04 (0.59–1.82)	0.890		
KIAA1429 (*vs.* diploid)				
Gain	1.59 (0.97–2.61)	**0.066**	0.17 (0.04–0.62)	**0.007**
Loss	1.48 (0.78–2.79)	0.227	0.85 (0.16–4.35)	0.842
YTHDC2 (*vs.* diploid)				
Gain	1.82 (1.11–2.99)	**0.018**		
Loss	2.19 (1.18–4.07)	**0.013**		
YTHDF1 (*vs.* diploid)				
Gain	1.42 (0.87–2.31)	0.160	1.10 (0.37–3.23)	0.869
Loss	2.06 (1.08–3.93)	**0.029**	3.12 (0.85–11.45)	0.086
YTHDF3 (*vs.* diploid)				
Gain	1.79 (1.11–2.89)	**0.016**	4.15 (1.06–16.27)	**0.041**
Loss	1.06 (0.52–2.15)	0.866	0.60 (0.10–3.58)	0.577
IGF2BP1 (*vs.* diploid)				
Gain	1.20 (0.72–2.02)	0.484	1.00 (0.38–2.59)	0.996
Loss	1.76 (1.00–3.11)	**0.051**	3.02 (0.93–9.80)	**0.066**

Table 4 continued. Univariate and multivariate Cox regression analysis of m⁶A regulatory genes for the overall survival (OS) and disease-free survival (DFS) of patients with soft-tissue sarcoma.

Variable	DFS				
	Univariate		Multivariate		
	HR (95% CI)		P	HR (95% CI)		P
Age (continuous)	1.00	(0.99–1.01)	0.795			
Sex (Male vs. Female)	1.10	(0.77–1.57)	0.592			
Pathological tumour size (mm) (continuous)	1.03	(1.01–1.05)	**0.002**	1.07	(1.03–1.11)	**<0.001**
Residual tumour (R1/R2/RX vs. R0)	2.24	(1.57–3.21)	**<0.001**	3.00	(1.21–7.44)	**0.010**
Pharmaceutical drug adjuvant (no vs. yes)	0.59	(0.40–0.88)	**0.009**			
Radiation treatment adjuvant (no vs. yes)	0.94	(0.63–1.39)	0.753			
FNCLCC grade (3 vs. 1/2)	1.53	(1.07–2.19)	**0.020**	2.76	(1.41–5.38)	**0.002**
Metastatic disease (no vs. yes)	5.05	(3.17–8.05)	**<0.001**	5.59	(2.81–11.13)	**<0.001**
m⁶A regulator alteration (writer loss+eraser deletion vs. others)	1.39	(0.97–1.99)	0.070			
METTL16 (vs. diploid)						
Gain	1.53	(0.95–2.44)	0.077			
Loss	1.33	(0.88–2.00)	0.174			
RBM15 (vs. diploid)						
Gain	1.88	(1.23–2.88)	**0.003**			
Loss	1.72	(1.10–2.68)	**0.017**			
RBM15B (vs. diploid)						
Gain	1.24	(0.79–1.96)	0.348			
Loss	0.96	(0.62–1.47)	0.840			
KIAA1429 (vs. diploid)						
Gain	1.42	(0.96–2.10)	**0.076**	0.11	(0.04–0.33)	**<0.001**
Loss	1.27	(0.76–2.11)	0.365	0.51	(0.16–1.60)	0.292
YTHDC2 (vs. diploid)						
Gain	1.62	(1.10–2.40)	**0.016**			
Loss	1.37	(0.83–2.29)	0.221			
YTHDF1 (vs. diploid)						
Gain	1.14	(0.78–1.68)	0.502			
Loss	1.40	(0.82–2.38)	0.216			
YTHDF3 (vs. diploid)						
Gain	1.43	(0.97–2.09)	**0.068**	7.33	(2.48–21.69)	**<0.001**
Loss	0.95	(0.55–1.66)	0.863	1.15	(0.32–4.11)	0.832
IGF2BP1 (vs. diploid)						
Gain	1.26	(0.84–1.90)	0.264	1.72	(0.83–3.53)	0.142
Loss	1.79	(1.13–2.84)	**0.013**	3.14	(1.27–7.81)	**0.014**

* Ambiguous variables (N/A, discrepancy) were excluded. OS – overall survival; DFS – disease free survival.
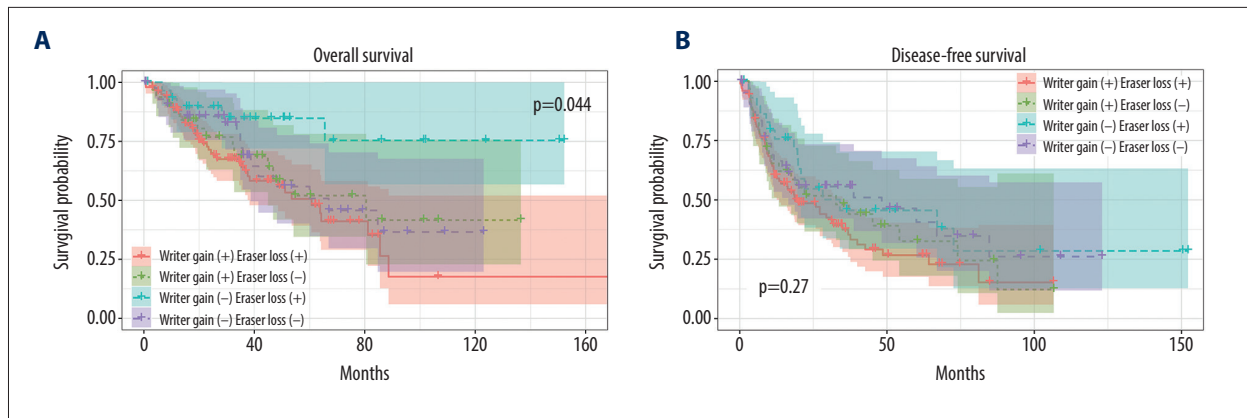
Figure 6. Association between copy number variations (CNVs) of m6A regulatory genes and survival of patients with soft-tissue sarcoma. Effects of writer gain status and eraser loss status on (**A**) overall survival (OS) and (**B**) disease-free survival (DFS).
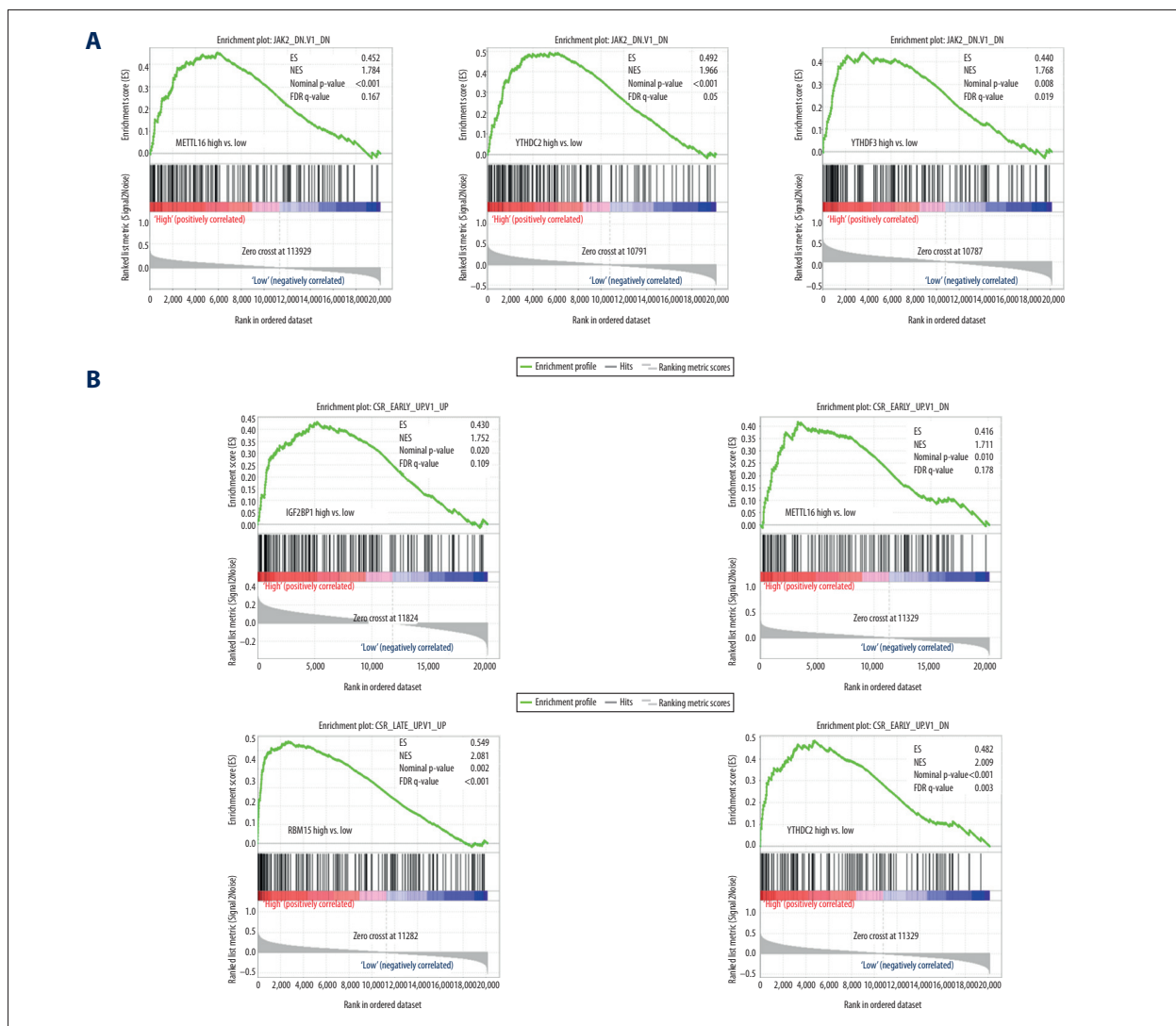

Figure 7. Pathways enriched in m6A regulation. (**A**) Gene set enrichment analysis (GSEA) results of group with higher expression *vs.* group with lower expression of METTL16, YTHDC2, or YTHDF3. (**B**) GSEA results of group with higher expression *vs.* group with lower expression of IGF2BP1, METTL16, RBM15, or YTHDC2.

sarcoma are warranted, and the CNVs of these m⁶A regulators might be used as targets to develop drugs to treat cancers.

Our study has some limitations. First, we studied the role of m⁶A regulators in the whole of soft-tissue sarcomas, and there are many types of sarcomas with varied clinical behaviors. Therefore, future studies should evaluate m⁶A methylation regulators in different types of sarcomas, such as angiosarcoma and liposarcoma. Second, the results from our study are based on data analysis and not experimental findings. In future studies, genomic methods could be used to specifically modify the m⁶A regulators and determine the effect of m⁶A regulator alterations on cancer cells *in vitro*. Third, to validate the definite target mRNAs of the m⁶A modification during the initiation and progression of soft-tissue sarcoma, future studies

should include a different study cohort with m⁶A-Seq and m⁶A MeRIP. Lastly, we used TCGA retrospective data and should validate our findings using prospective studies in the future.

Conclusions

In conclusion, we analyzed the mutation and CNV status of m⁶A regulators in sarcoma and found that CNVs were closely correlated with mRNA expression. These m⁶A regulators could also predict survival of patients with sarcoma and are involved in various oncogenic signatures. Our study provides a useful survival prediction model and contributes to the area of cancer drug development.

Supplementary Data
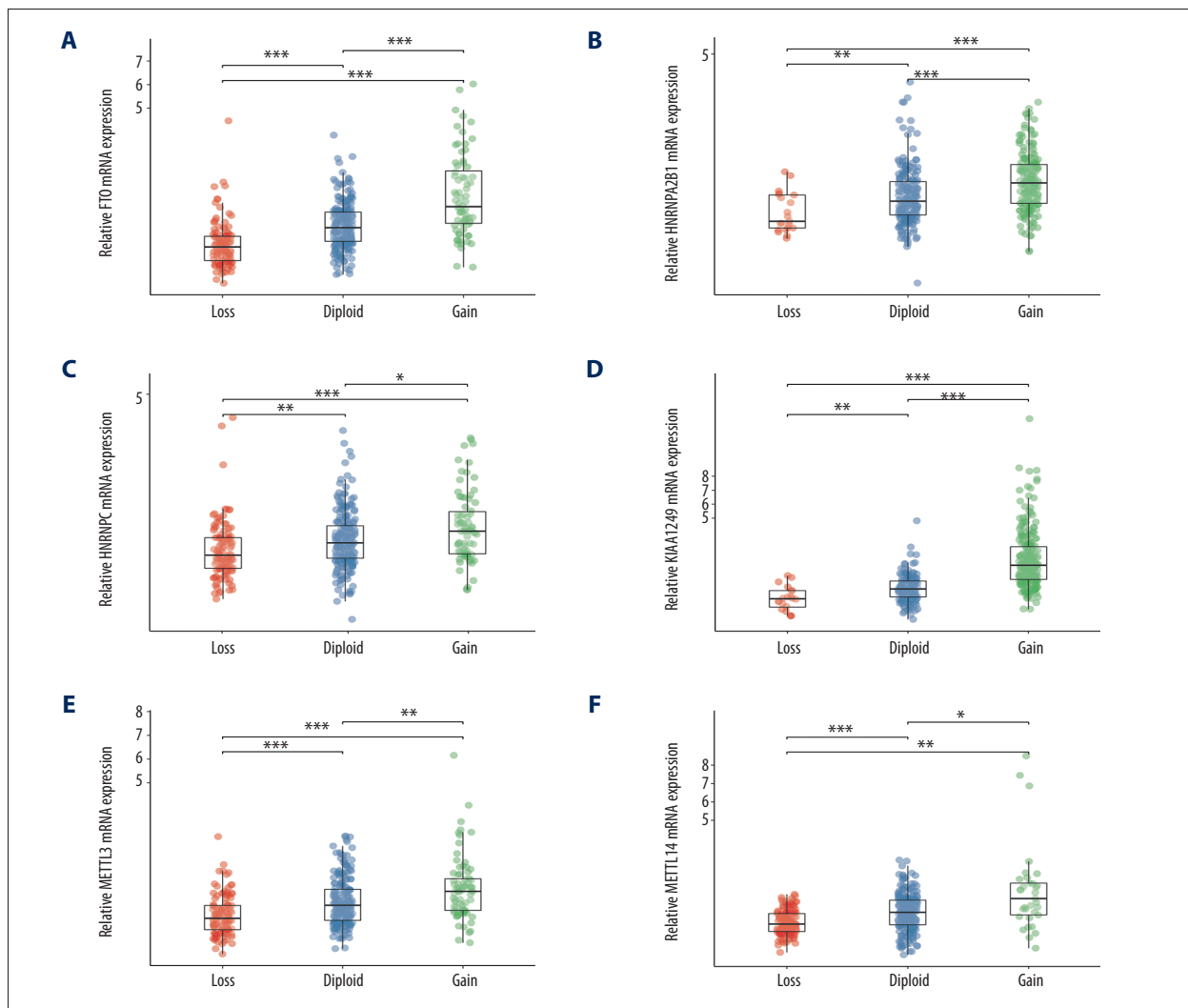
Supplementary Figure 1. (**A–F**) Correlation of copy number variations (CNVs) with mRNA expression in bladder cancer. The relative mRNA expression differences among bladder cancer cases with different CNVs. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.
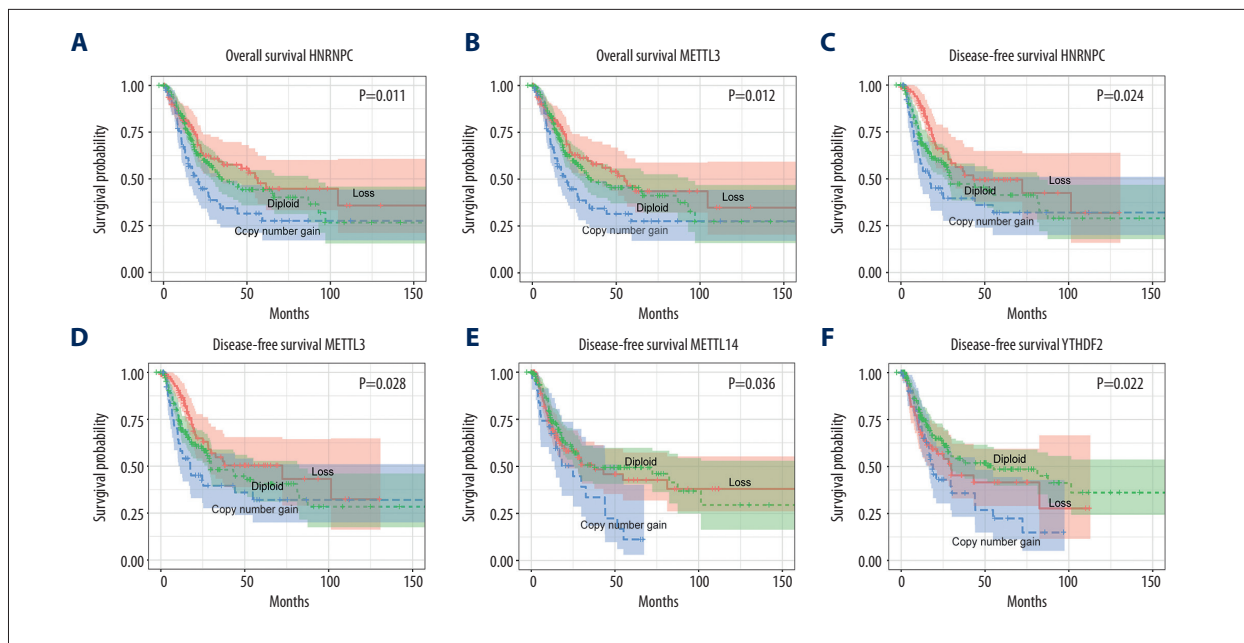
Supplementary Figure 2. Association between copy number variations (CNVs) of m⁶A regulatory genes and survival of patients with bladder cancer. Effect of CNVs of (**A**) HNRNPC and (**B**) METTL3 on overall survival (OS). Effects of CNVs of (**C**) HNRNPC, (**D**) METTL3, (**E**) METTL14, and (**F**) YTHDF2 on disease-free survival (DFS).

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