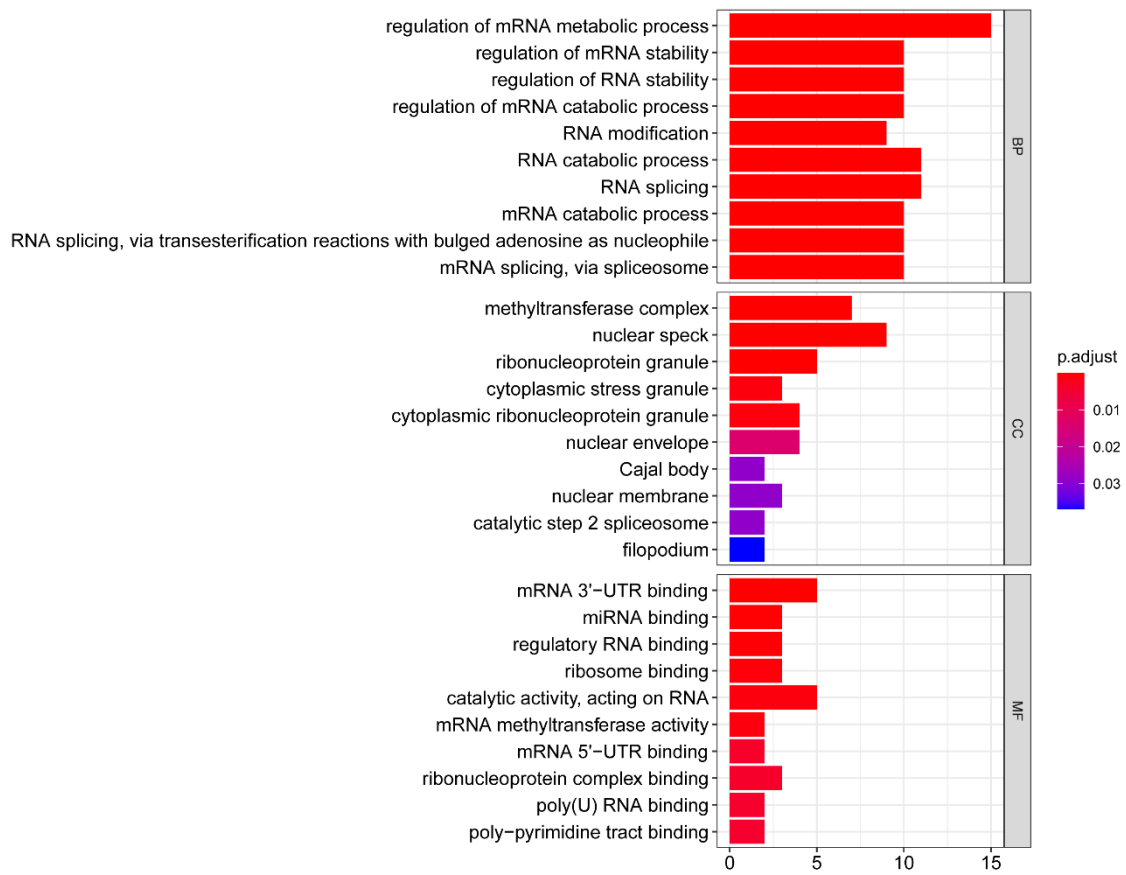


Supplemental information

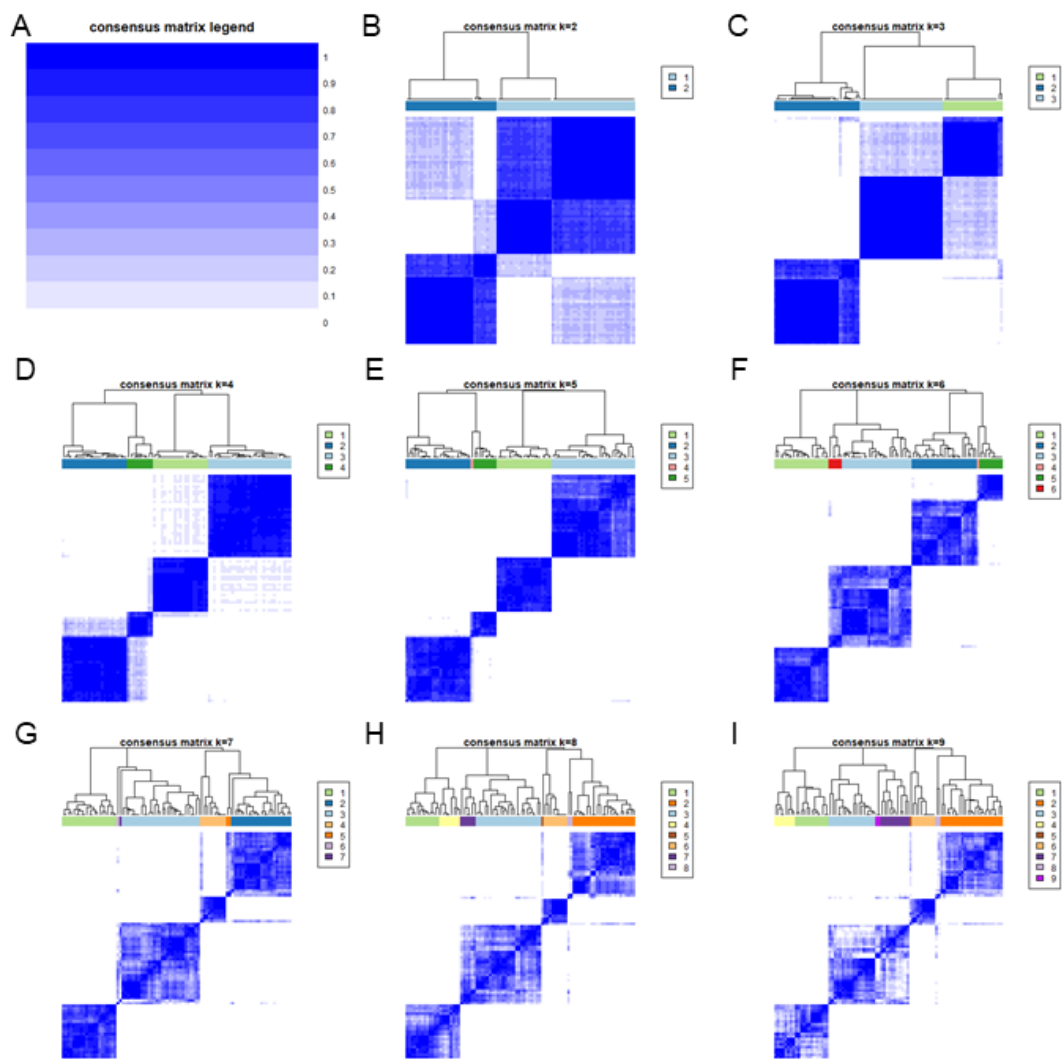
Analysis and identification of m⁶A RNA

methylation regulators in metastatic osteosarcoma

Hanji Huang, Xiaofei Cui, Xiong Qin, Kanglu Li, Guohua Yan, Dejie Lu, Mingjun Zheng, Ziwei Hu, Danqing Lei, Nihan Lan, Li Zheng, Zhenchao Yuan, Bo Zhu, and Jinmin Zhao

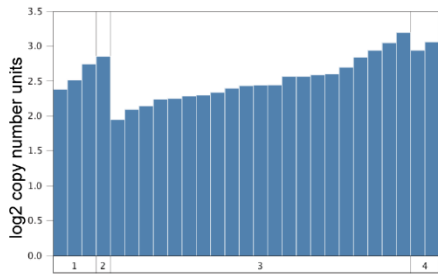


Supplementary Figure 1. Histogram for gene ontology analysis of the 21 N⁶-Methyladenosine modifiers.



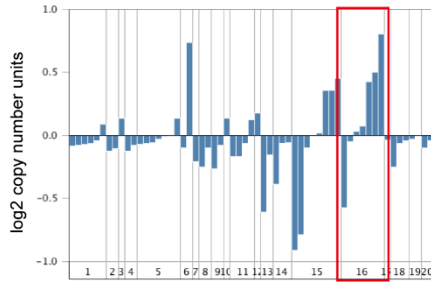
Supplementary Figure 2. Consensus clustering matrix when $k=1-9$.

A RBM15 Expression in Kobayashi Sarcoma



1. Chondroblastic Osteosarcoma (3)
2. Fibroblastic Osteosarcoma (1)
3. Osteoblastic Osteosarcoma (21)
4. Telangiectatic Osteosarcoma (2)

B RBM15 Copy Number in Osteosarcoma



1. Alveolar Rhabdomyosarcoma (6)
2. Anaplastic Ependymoma (2)
3. Anaplastic Renal Wilms Tumor (1)
4. Atypical Teratoid/Rhabdoid Tumor (2)
5. B-Cell Acute Lymphoblastic Leukemia (7)
6. Brain Glioblastoma (2)
7. Desmoplastic Medulloblastoma (1)
8. Embryonal Rhabdomyosarcoma (2)
9. Ependymoma (2)
10. Ewing's Sarcoma of Bone (1)
11. Extrasosseous Ewing's Sarcoma (4)
12. Giant Cell Glioblastoma (1)
13. Glioblastoma (2)
14. Medulloblastoma (3)
15. Neuroblastoma (8)
16. Osteosarcoma (7)
17. Pleomorphic Xanthoastrocytoma (1)
18. Renal Wilms Tumor (3)
19. Rhabdoid Tumor of the Kidney (2)
20. T-Cell Acute Lymphoblastic Leukemia (2)

Supplementary Figure 3. RBM15 copy number in different osteosarcoma samples and other cancer types. **(A)** The copy number of RBM15 in the recurrent osteosarcoma samples. **(B)** The copy number of RBM15 in different subtypes of OS samples.

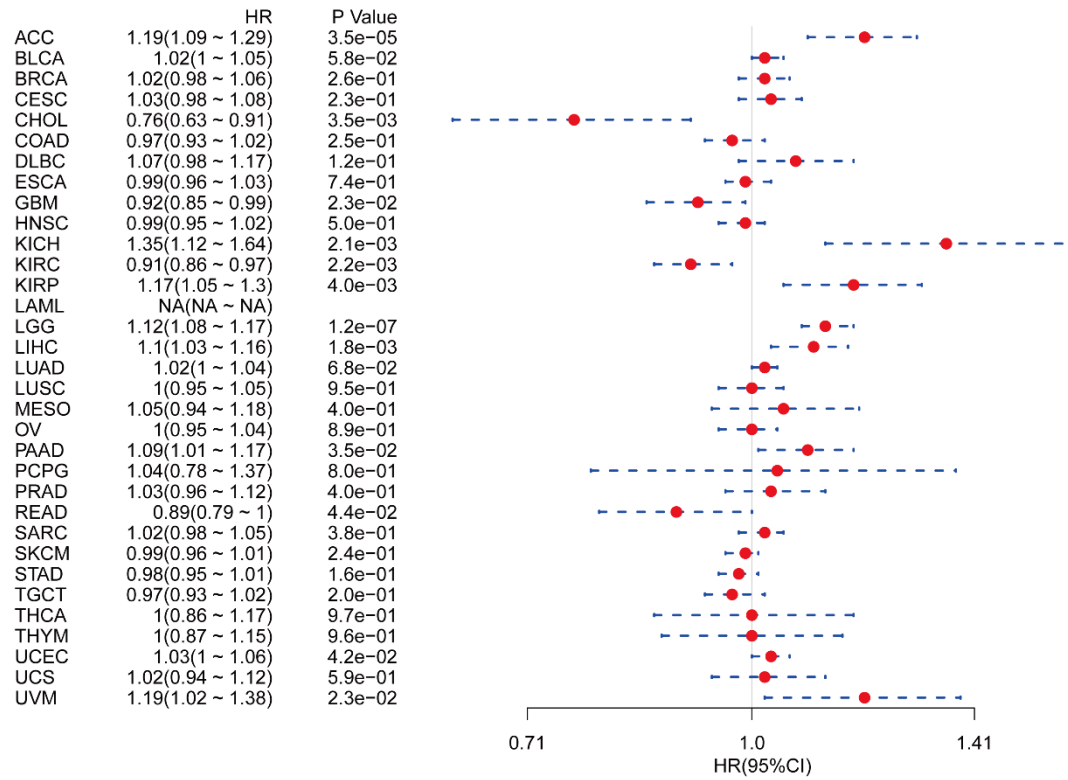
Disease Summary for RBM15

Analysis Type by Cancer	Cancer vs. Normal	Cancer vs. Cancer		Cancer Subtype Analysis											Cancer vs. Baseline (DNA only)	Pathway and Drug		Outlier	
		Cancer Histology	Multi-cancer	Clinical Outcome	Metastasis vs. Primary	Molecular Subtype Biomarker	Molecular Subtype Mutation	Pathology Subtype: Grade	Pathology Subtype: Stage	Patient Treatment Response	Recurrence Primary	Other	Drug Sensitivity	Perturbation					
Bladder Cancer																		1	1
Brain and CNS Cancer	1																	6	7
Breast Cancer																		5	11
Cervical Cancer	2																	2	2
Colorectal Cancer																		5	12
Esophageal Cancer																		1	3
Gastric Cancer																		3	3
Head and Neck Cancer																			8
Kidney Cancer																		1	2
Leukemia	1																	4	11
Liver Cancer																		1	2
Lung Cancer																		3	14
Lymphoma	3																	7	9
Melanoma																		3	3
Myeloma																		3	8
Other Cancer	1																	3	7
Ovarian Cancer																		2	3
Pancreatic Cancer																		2	5
Prostate Cancer																		7	5
Sarcoma		3	2															3	6
Significant Unique Analyses	7	1	3	2														59	116
Total Unique Analyses	380	627	216																824

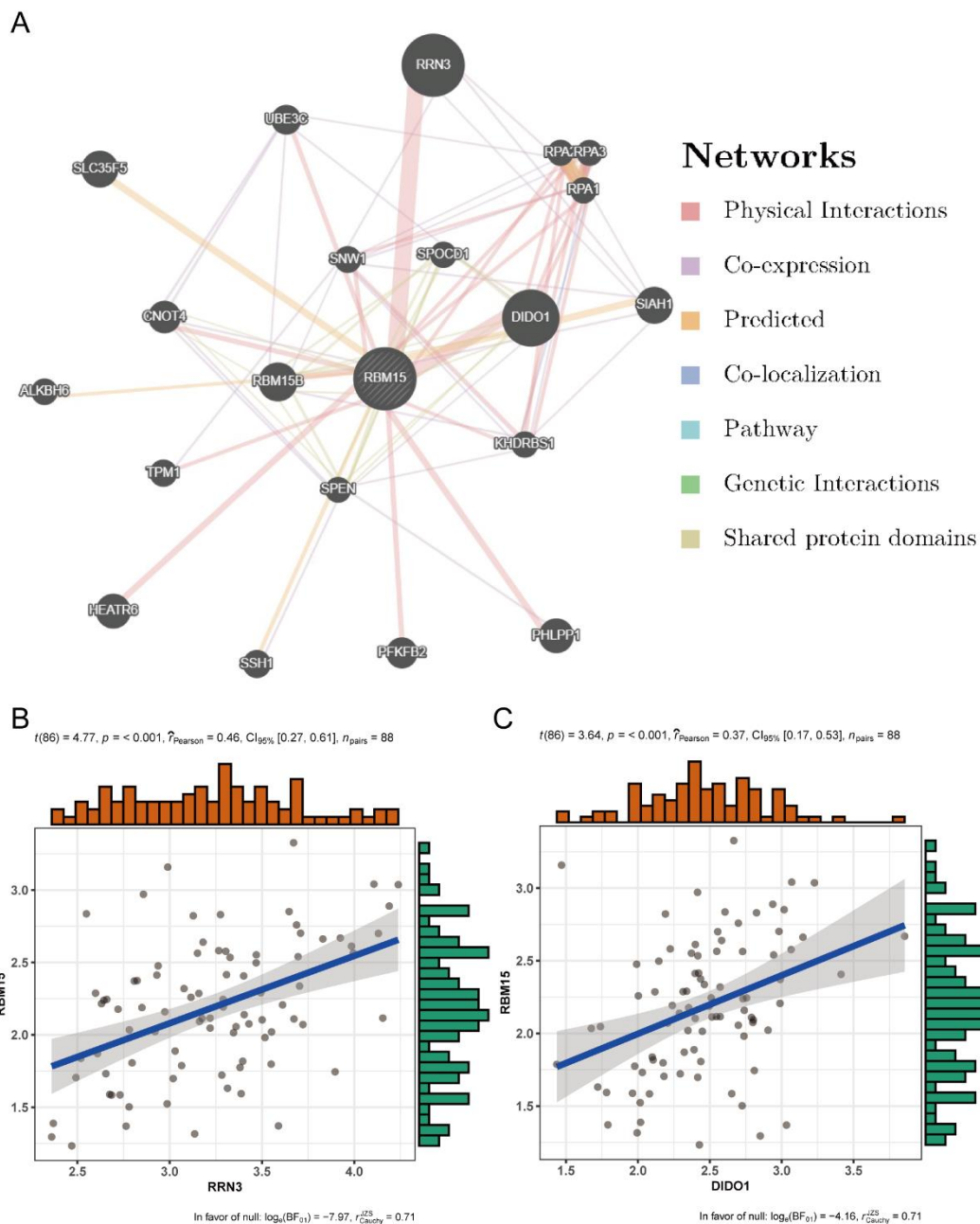


Cell color is determined by the best gene rank percentile for the analyses within the cell.
NOTE: An analysis may be counted in more than one cancer type.

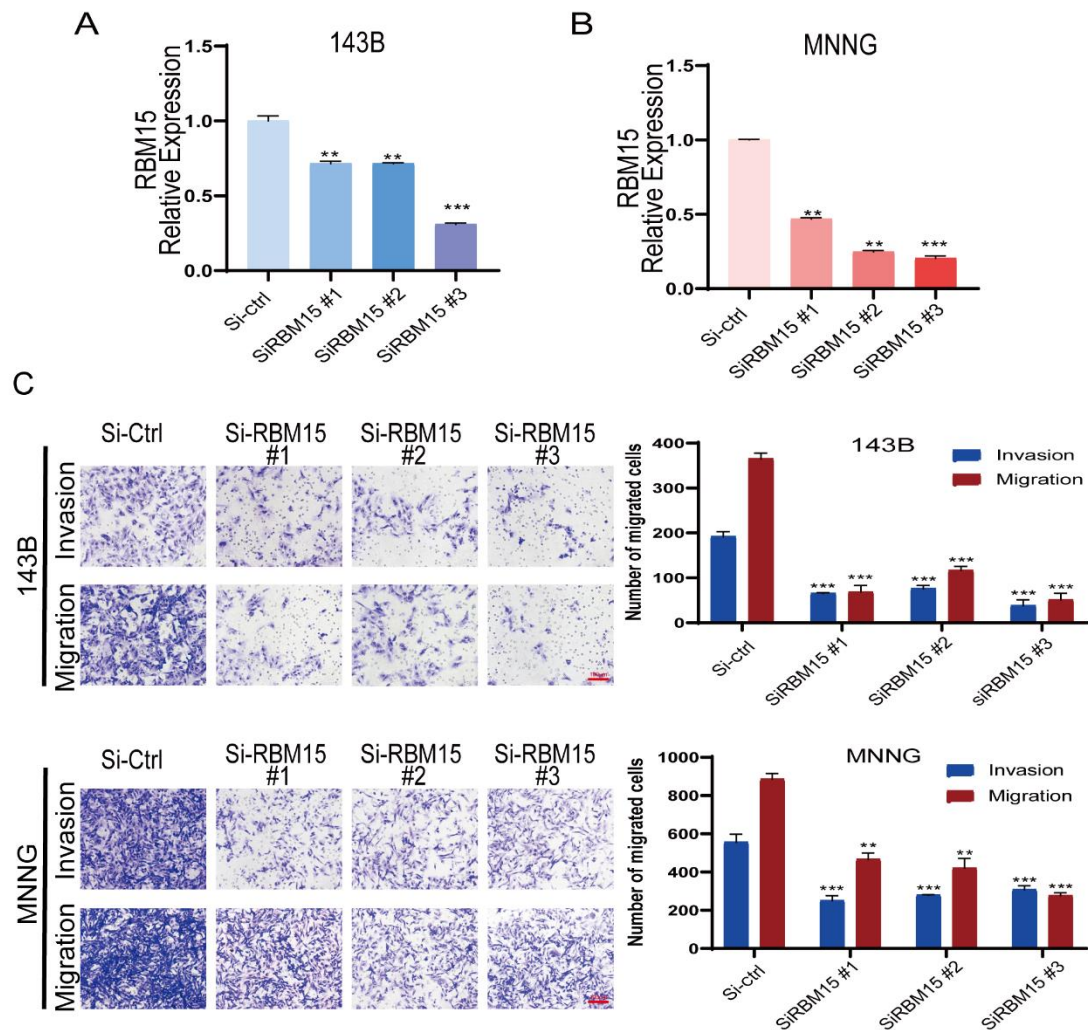
Supplementary Figure 4. Expression of RBM15 in different types of tumor.



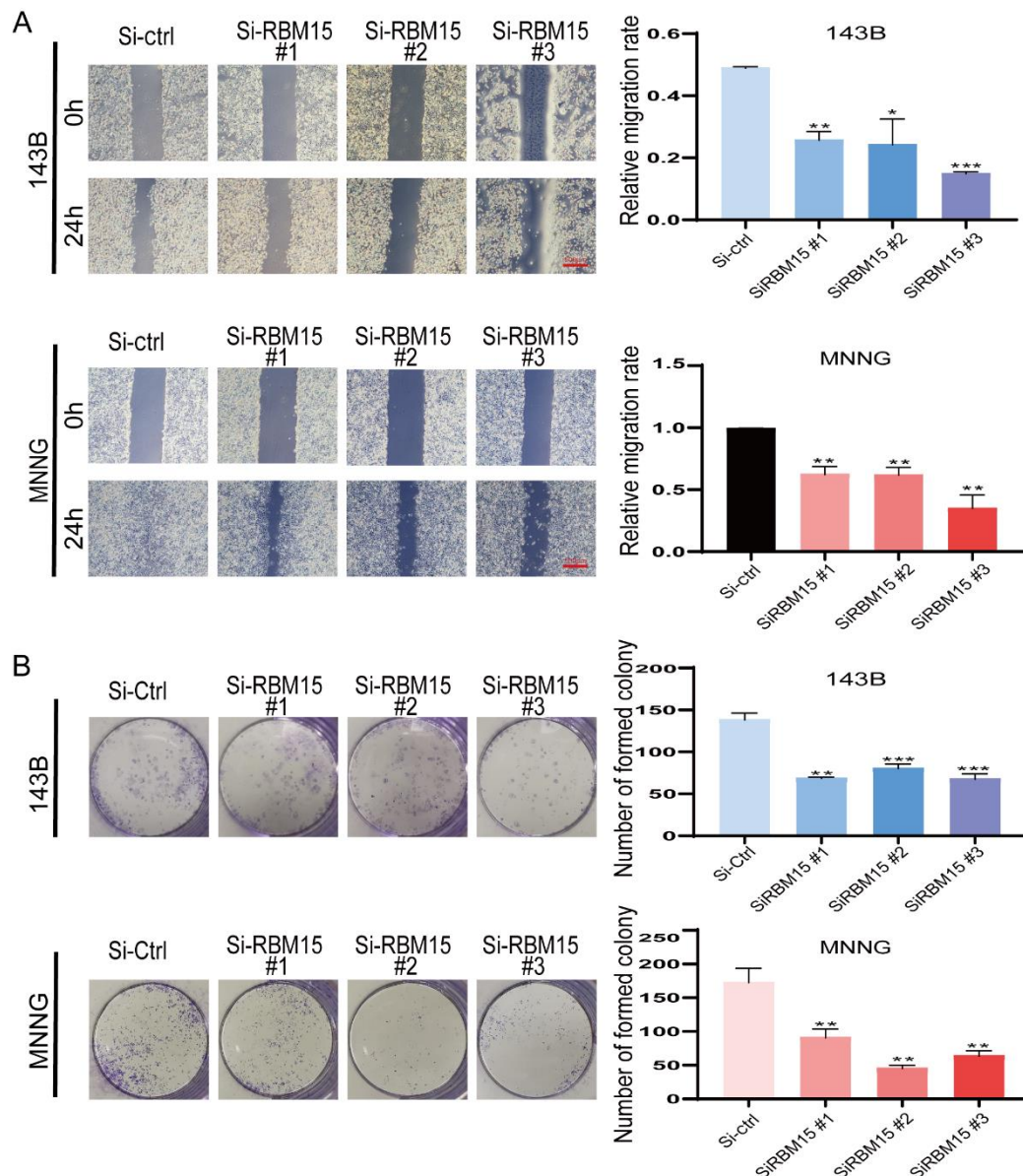
Supplementary Figure 5. Relationship between the expression of RBM15 and prognosis of disease-free interval analyzed in 33 tumors.



Supplementary Figure 6. Analysis of RBM15-interacting proteins. **(A)** The protein-protein interaction network analysis between the RBM15 and the other proteins. **(B)** The correlation analysis between RBM15 and RRN3. **(C)** The correlation analysis between RBM15 and DIDO1.



Supplementary Figure 7. Validation of RBM15 expression and its roles on the invasion and migration of metastatic osteosarcoma cell lines. **(A)** The effects of RBM15 knockdown were confirmed in 143B cells and **(B)** MNNG cells via qRT-PCR analysis. **(C)** The cell migration and invasion ability were investigated in metastatic OS cells after transfection with silencing si-Ctrl or si-RBM15 by Transwell assays. The left panel shows the representative images; scale bars: 100 μ m; the quantitative analyses of the cell migration and invasion level have been shown in the right panel. All bar plot data have been presented as mean \pm standard deviation of three independent experiments. ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure 8. Scratch and cell colony-forming experiments for the metastatic osteosarcoma cell lines. **(A)** The comparison of scratch experiments performed using the metastatic osteosarcoma cell lines with or without RBM15 knockdown. The left panel shows the representative images acquired under an inverted microscope, scale bars: 100 μ m. The right panel shows the percentage of areas exhibiting relevant healing. **(B)** The colony-forming assay performed using the 143B and MNNG cell lines with or without RBM15 knockdown. The left panel shows the overall view of the colony formation in the entire dish, scale bars: 100 μ m. The right panel shows the number of cell colonies in each dish. All bar plot data are presented as mean \pm standard deviation of three

independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.