Supplementary

Table S1 The PCR and specific siRNA primers

Gene	Sequence (5'-3')	
	Forward	Reverse
GAPDH	CTGGGCTACACTGAGCAAC	AAGTGGTCGTTGAGGGCAATG
METTL3	AAGGAACACTGCTTGGTTGGT	CGAACCTCAGCTACGATCACAT
KIAA1429	TGACCTTGCCTCACCAACTGCA	TGACCTTGCCTCACCAACTGCA
PRRC2A	GTGACAAGGACTCAGACTTACG	CAGTCTCGTGATACAGGAATGG
RBMX	CTTCCCACCTTGTGAGTTCTCC	CTTCTTGTTCTCATACCTAACTCC
ZC3H3	ACAGTGGCAGAGCCTTTAG	CCGATTCACGAGGGAGTATTT
siRNA-KIAA1429	AAACAACUCAGGUUAUUGATT	UCAAUAACCUGAGUUGUUUGC
siRNA-NC	UUCUUCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Table S2 Biomarkers m6A expression between normal and tumor tissue in TCGA data

Gene	All samples (n=424)	Normal (n=50)	Tumor (n=374)
ALKBH5	11.70 (11.30–12.08)	11.72 (11.47–11.83)	11.70 (11.28–12.14)
CBLL1	9.66 (9.15–10.14)	9.056 (8.77–9.38)	9.74 (9.23–10.24)
FTO	10.08 (9.53–10.59)	9.81 (9.58–10.15)	10.14 (9.49–10.62)
HNRNPA2B1	14.38 (13.94–14.83)	13.84 (13.57–14.18)	14.47 (14.04–14.89)
HNRNPC	13.47 (13.07–13.88)	12.97 (12.57–13.15)	13.53 (13.17–13.91)
IGF2BP1	6.62 (2.0–9.42)	1.59 (0.0–2.519)	7.57 (3.0–9.862)
IGF2BP2	8.55 (6.22–10.0)	6.72 (6.17–7.21)	8.86 (6.54–10.16)
IGF2BP3	4.17 (2.32–7.26)	2.32 (1.59–3.0)	4.61 (2.59–7.67)
KIAA1429	11.02 (10.57–11.61)	10.19 (9.84–10.64)	11.14 (10.70–11.65)
METTL14	9.72 (9.21–10.09)	9.70 (9.35–9.97)	9.72 (9.20–10.11)
METTL16	9.23 (8.77–9.74)	8.88 (8.52–9.16)	9.31 (8.81–9.83)
METTL3	9.78 (9.19–10.33)	8.73 (8.32–9.22)	9.91 (9.37–10.36)
PRRC2A	12.67 (11.96–13.31)	11.77 (11.46–12.13)	12.77 (12.20–13.40)
RBM15	8.72 (8.29–9.07)	8.48 (8.14–8.91)	8.74 (8.31–9.09)
RBM15B	10.92 (10.46–11.39)	10.18 (9.96–10.54)	11.03 (10.58–11.44)
RBMX	11.70 (11.13–12.17)	10.91 (10.57–11.23)	11.77 (11.35–12.29)
WTAP	10.85 (10.37–11.29)	10.61 (10.31–10.97)	10.90 (10.37–11.33)
YTHDC1	10.82 (10.33–11.22)	10.66 (10.38–11.0)	10.86 (10.30–11.24)
YTHDC2	10.05 (9.48–10.53)	9.82 (9.45–10.09)	10.11 (9.49–10.56)
YTHDF1	11.11 (10.69–11.53)	10.60 (10.46–10.95)	11.18 (10.79–11.58)
YTHDF2	11.01 (10.60–11.39)	10.78 (10.48–11.13)	11.04 (10.65–11.41)
YTHDF3	11.48 (11.01–11.98)	11.21 (10.86–11.50)	11.54 (11.03–12.07)
ZC3H13	10.77 (10.11–11.36)	10.85 (10.56–11.28)	10.74 (10.02–11.36)
ZC3H3	10.46 (9.78–11.14)	9.134 (8.879–9.466)	10.61 (10.04–11.23)
ZCCHC4	8.0 (7.44–8.44)	7.508 (6.966–7.839)	8.06 (7.52–8.50)

Data are presented as median (interquartile range).

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Table S3 Biomarkers m6A expression between normal and tumor tissue in GSE45267 data

Gene	All samples (n=87)	Normal (n=41)	Tumor (n=46)
KIAA1429	6.76 (6.44–7.18)	6.54 (6.26–6.76)	7.16 (6.74–7.69)
METTL3	7.37 (7.03–7.69)	7.06 (6.86–7.33)	7.67 (7.38–7.93)
PRRC2A	6.95 (6.63–7.27)	6.82 (6.49–7.0)	7.20 (6.78–7.61)
RBMX	8.57 (8.34–8.95)	8.37 (8.21–8.46)	8.88 (8.61–9.23)
ZC3H3	6.31 (5.87–6.75)	6.01 (5.74–6.33)	6.52 (6.14–7.0)

Data are presented as median (interquartile range).

Table S4 Biomarkers m6A expression between normal and tumor tissue in GSE84402 data

Gene	All samples (n=28)	Normal (n=14)	Tumor (n=14)
KIAA1429	6.74 (6.39–7.02)	6.41 (6.08–6.68)	7.04 (6.83–7.44)
METTL3	7.16 (6.96–7.63)	6.95 (6.87–7.10)	7.54 (7.30–7.87)
PRRC2A	6.19 (5.70–6.63)	5.69 (5.45–5.88)	6.49 (6.26–6.95)
RBMX	8.09 (7.89–8.35)	7.89 (7.57–8.03)	8.38 (8.15–8.58)
ZC3H3	4.80 (4.33–5.29)	4.36 (3.58–4.68)	5.27 (5.05–5.50)

Data are presented as median (interquartile range).

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Table S5 Baseline according to TCGA patients

Parameter	All patients (n=368)
Age (years)	59.7±13.3
Gender	
Male	249 (67.7)
Female	119 (32.3)
Race	
White	183 (49.7)
Asian	157 (42.7)
African American	17 (4.6)
Not reported/others	11 (3)
Stage	
Stage I	172 (46.7)
Stage II	85 (23.1)
Stage III	83 (22.6)
Stage IV	4 (1.1)
Not reported	24 (6.5)
Grade	
G1	55 (14.9)
G2	176 (47.8)
G3	120 (32.6)
G4	12 (3.3)
Not reported	5 (1.4)
T	
T1	182 (49.5)
T2	92 (25.0)
T3	78 (21.2)
T4	13 (3.5)
Not reported/TX	3 (0.8)
M	
M0	265 (72.0)
M1	3 (0.8)
MX	100 (27.1)
N	
N0	250 (67.9)
N1	4 (1.1)
Not reported/NX	114 (31.0)

Data are presented as number (%) or mean ± standard deviation.

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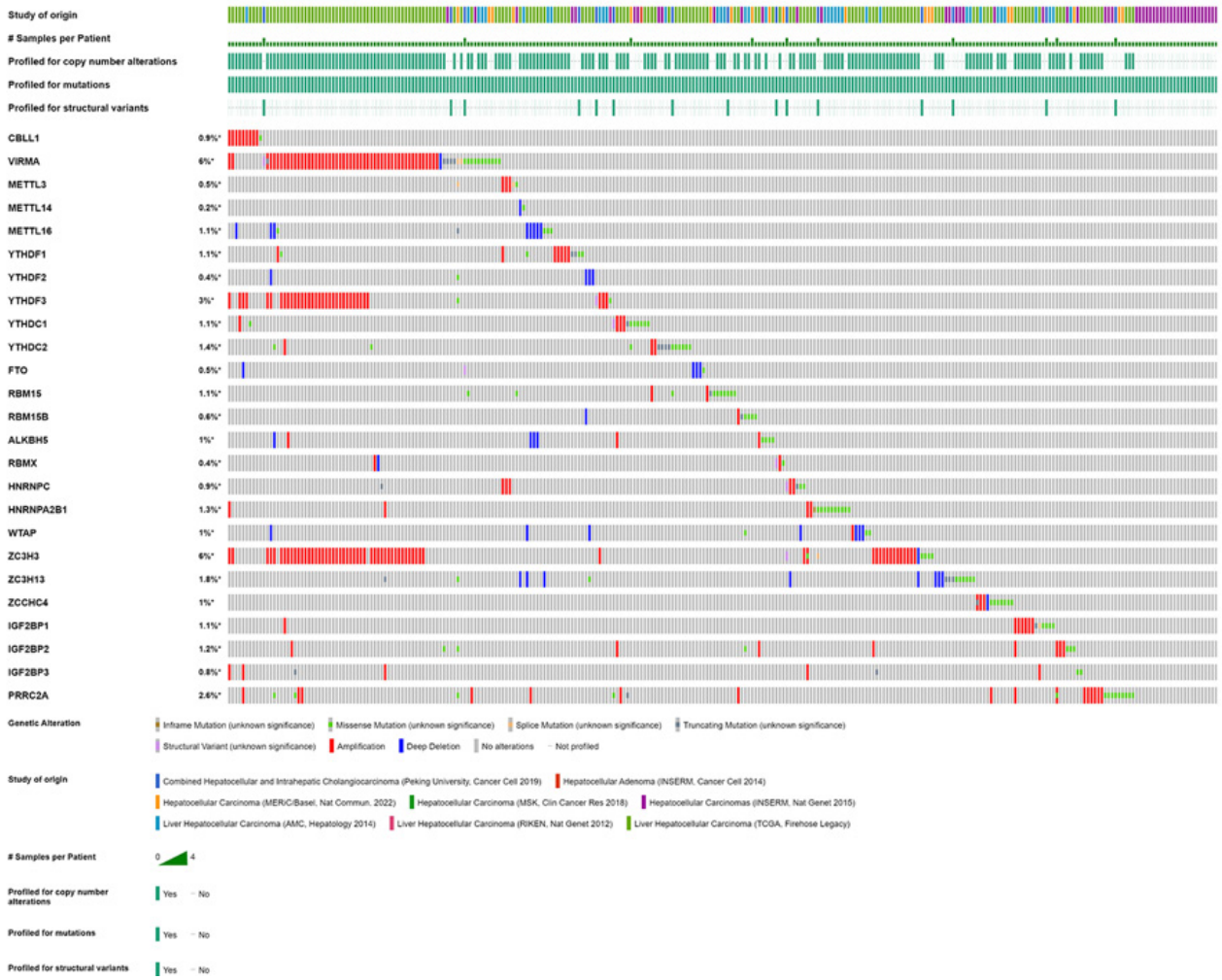

Figure S1 Mutation, amplification, deep deletion in HCC.

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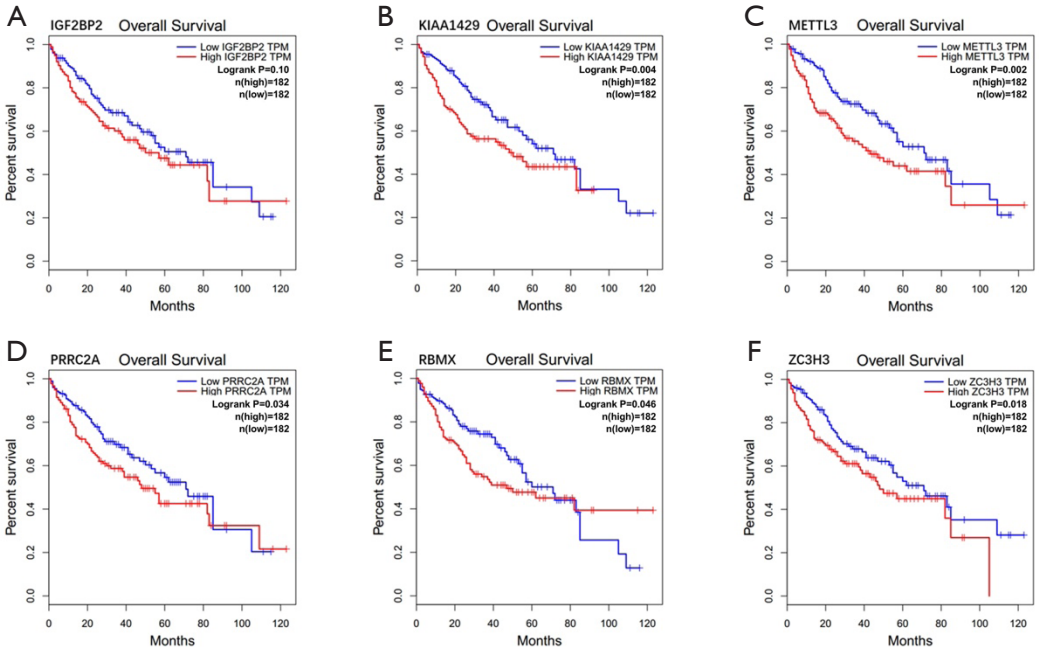

Figure S2 OS analysis in the GEPIA dataset. (A) IGF2BP2 expression was not reach statistical significance (P=0.10) in OS. (B-F) Upregulated m6A regulators expression like KIAA1429, METTL3, PRRC2A, RBMX and ZC3H3 were related to poor OS and DSS time in HCC.

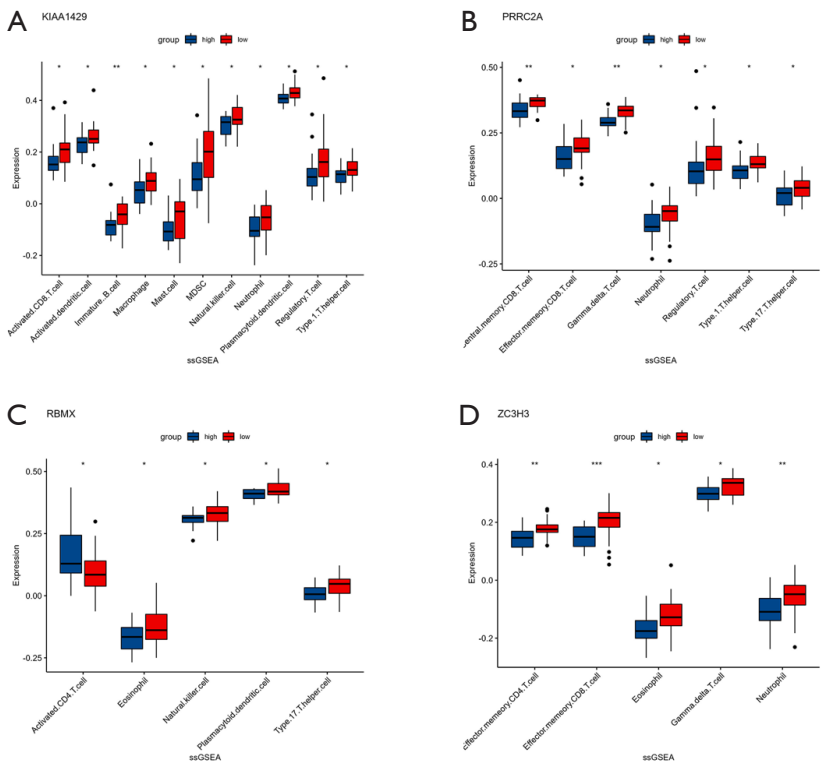

Figure S3 ssGSEA in dataset GSE45267 (*, P<0.05; **, P<0.01; ***, P<0.001). (A-D) Immune analysis by ssGSEA based on the high and low expression of m6A regulators in GSE45267.

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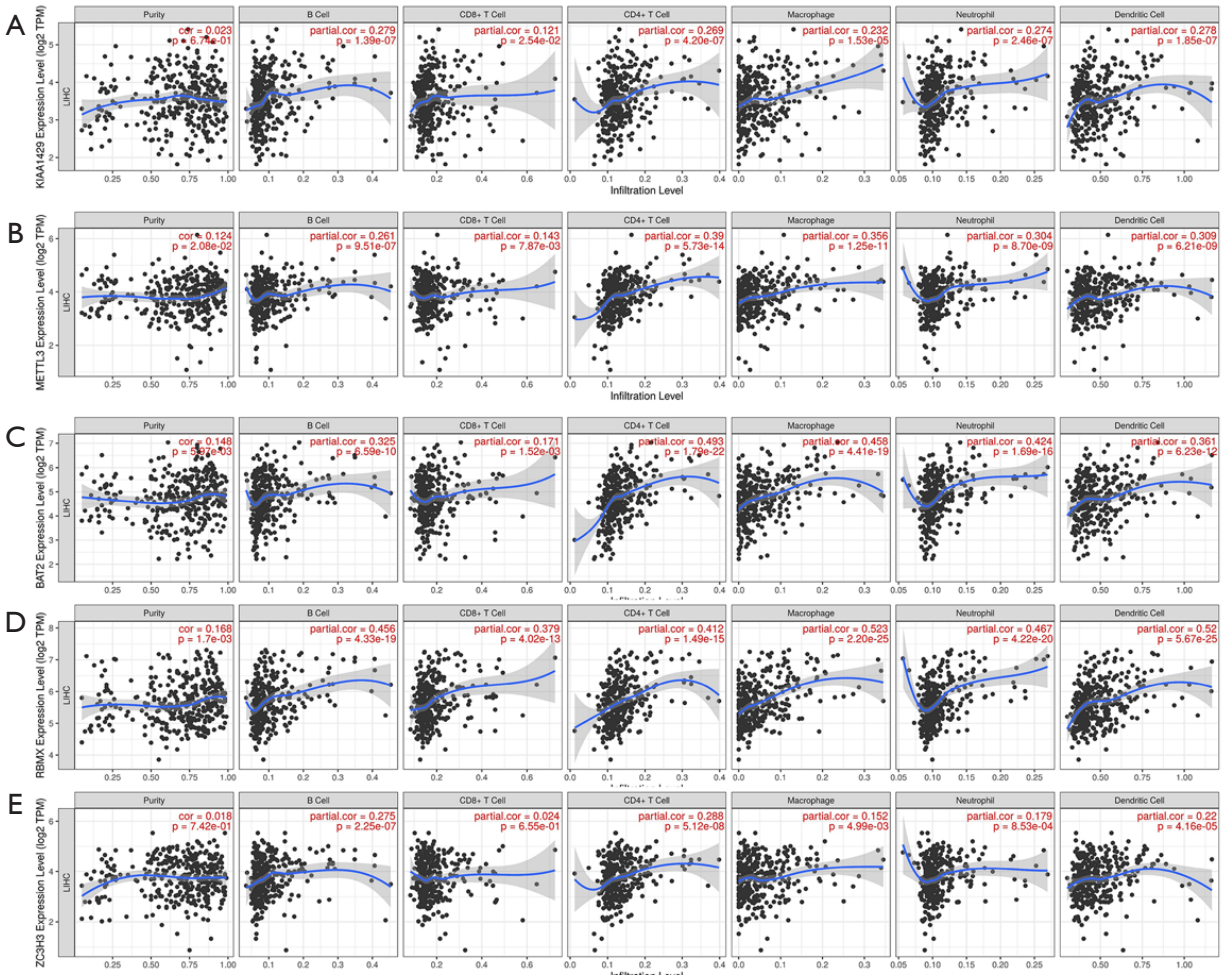

Figure S4 m6A regulators expression is correlated with immune infiltration levels in HCC. (A) KIAA1429 expression was correlated with infiltration levels of all six major immune cell types. (B,C) METTL3 and PRRC2A showed the strongest associations with $CD4^+$ T cells. (D) RBMX was correlation with macrophage infiltration. (E) ZC3H3 was also strongly associated with $CD4^+$ T cells.

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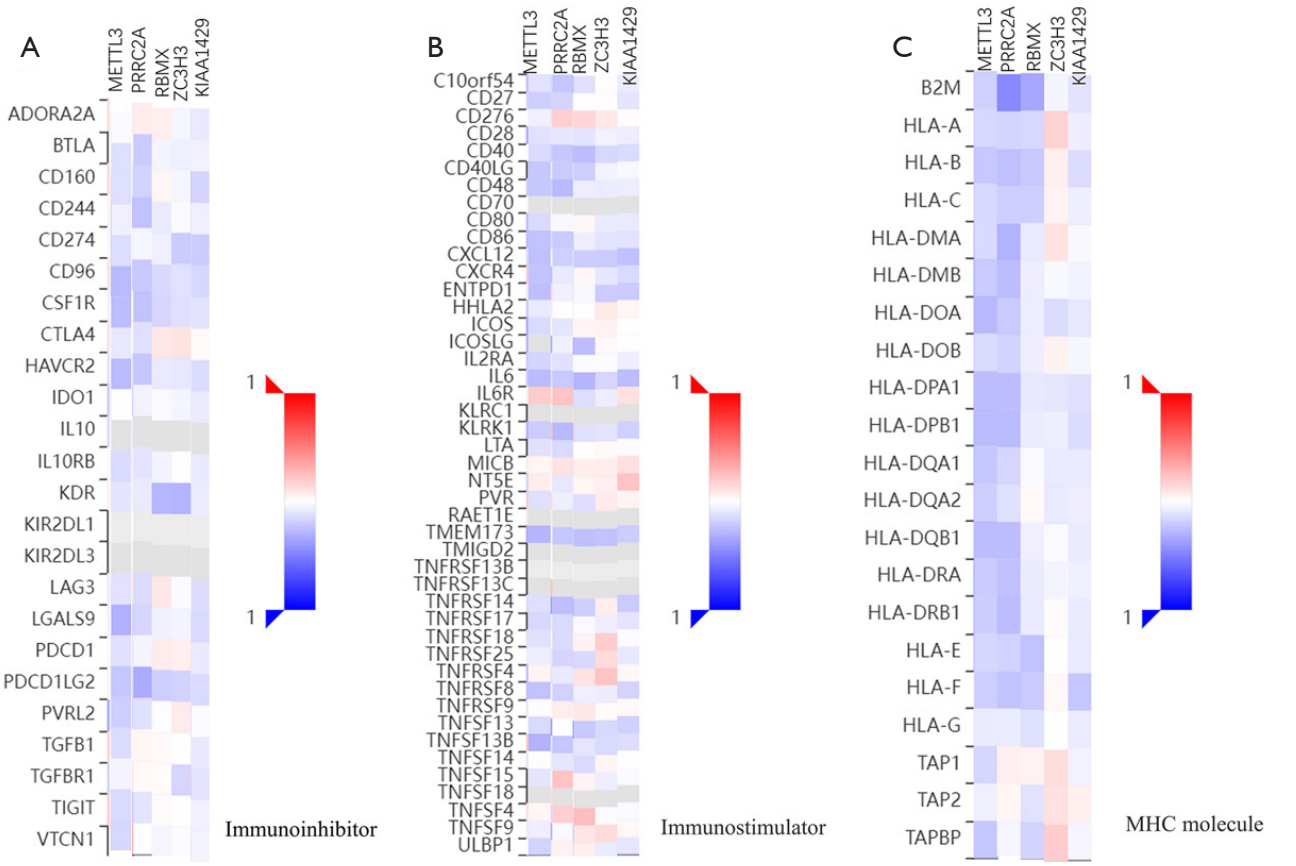

Figure S5 m6A regulators expression is correlated with the expression of immune regulators in HCC. (A,B) The expression levels of KIAA1429, METTL3, PRRC2A, RBMX, and ZC3H3 were negatively correlated with both immunoinhibitory and immunostimulatory factors. (C) ZC3H3 showed a mildly positive correlation with major histocompatibility complex (MHC) molecules, KIAA1429, METTL3, PRRC2A, and RBMX exhibited negative correlations.

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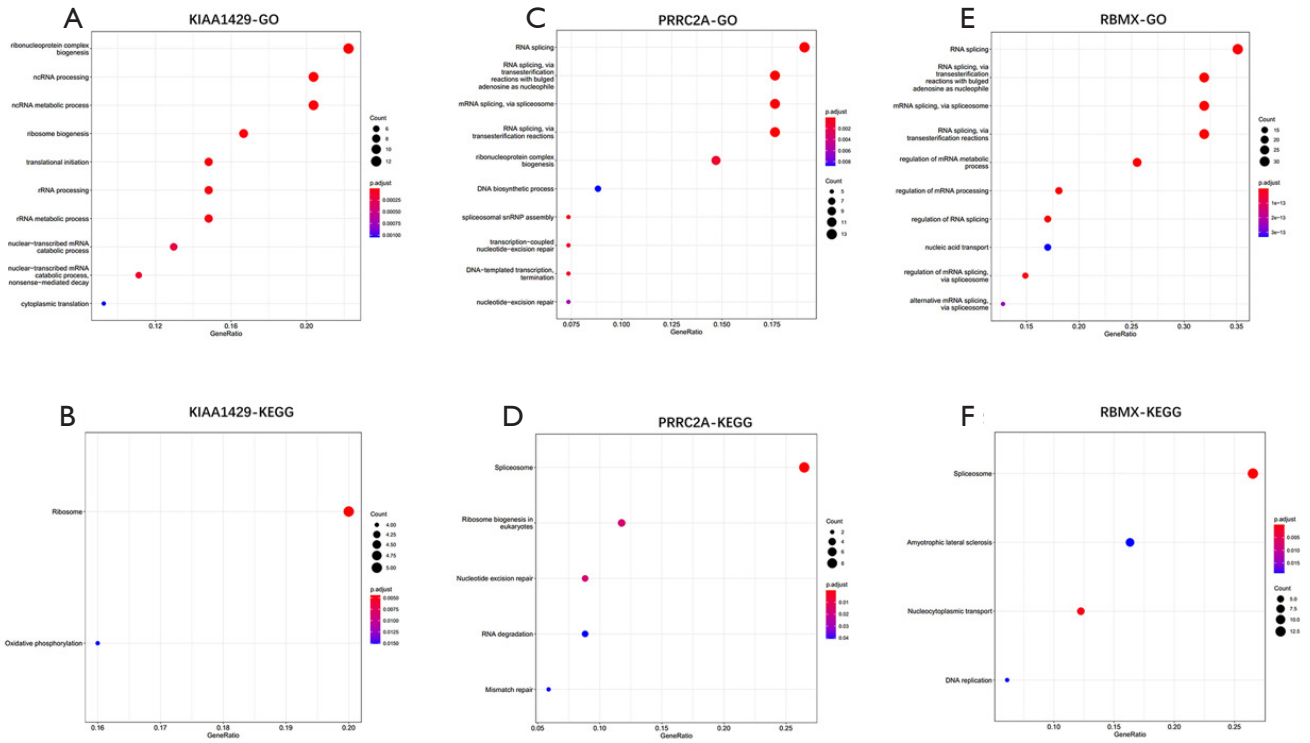

Figure S6 GO and KEGG enrichments. (A,B) KIAA1429-correlated genes were significantly enriched in 12 GO/KEGG pathways. (C-F) PRRC2A and RBMX were each associated with 15 enriched pathways.

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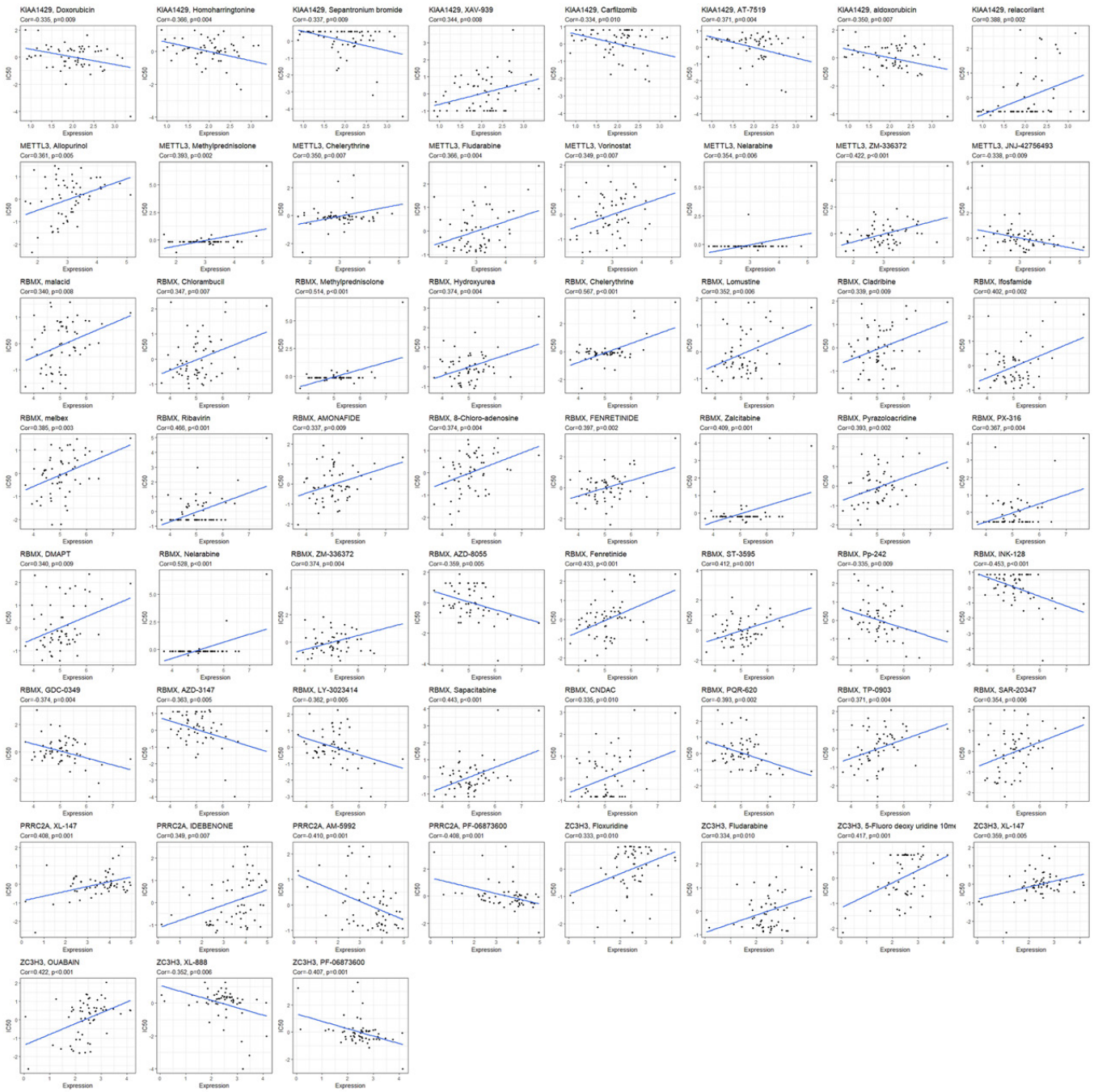

Figure S7 Correlation of m6A regulators and drug response.

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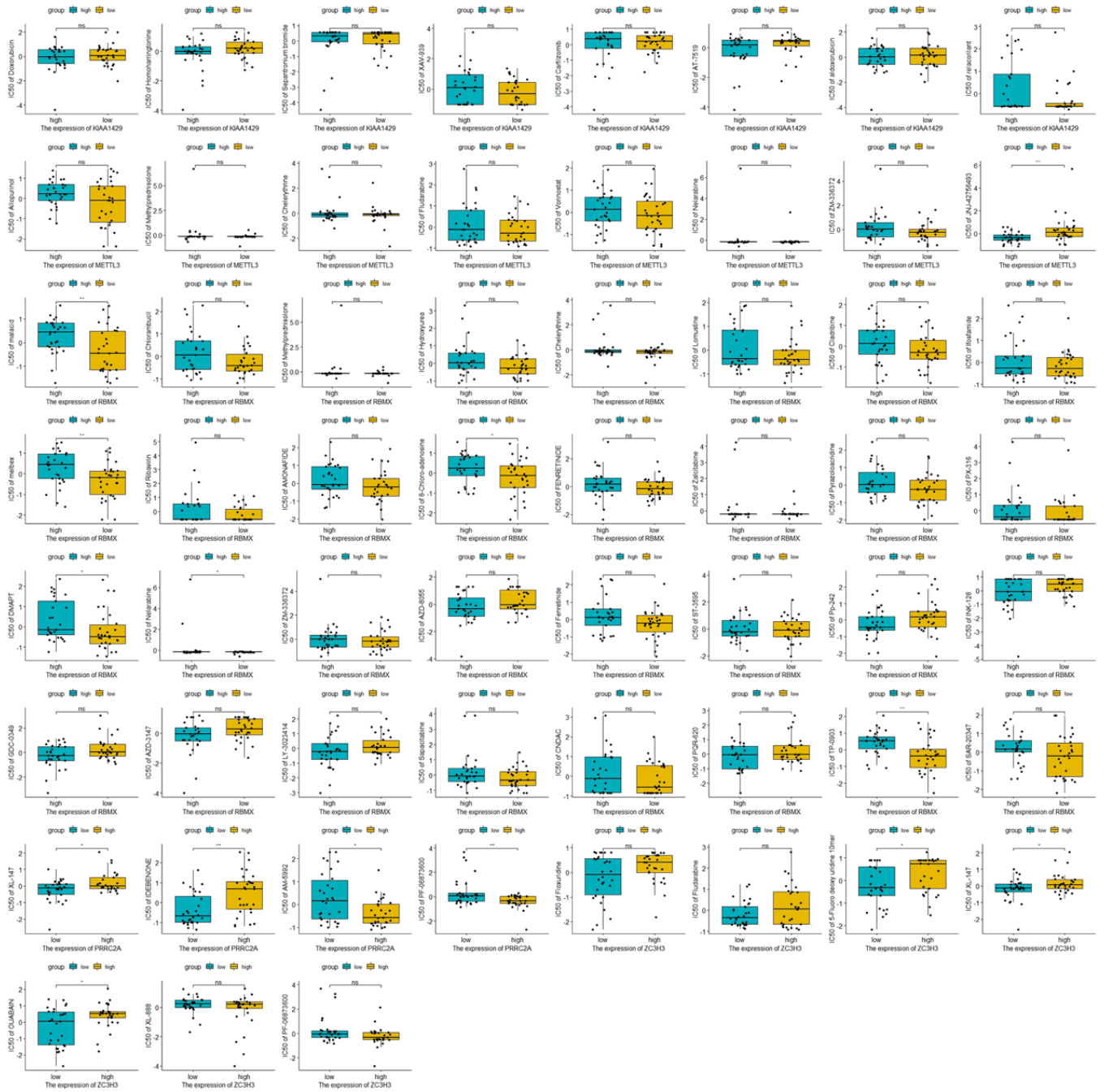

Figure S8 IC50 value.

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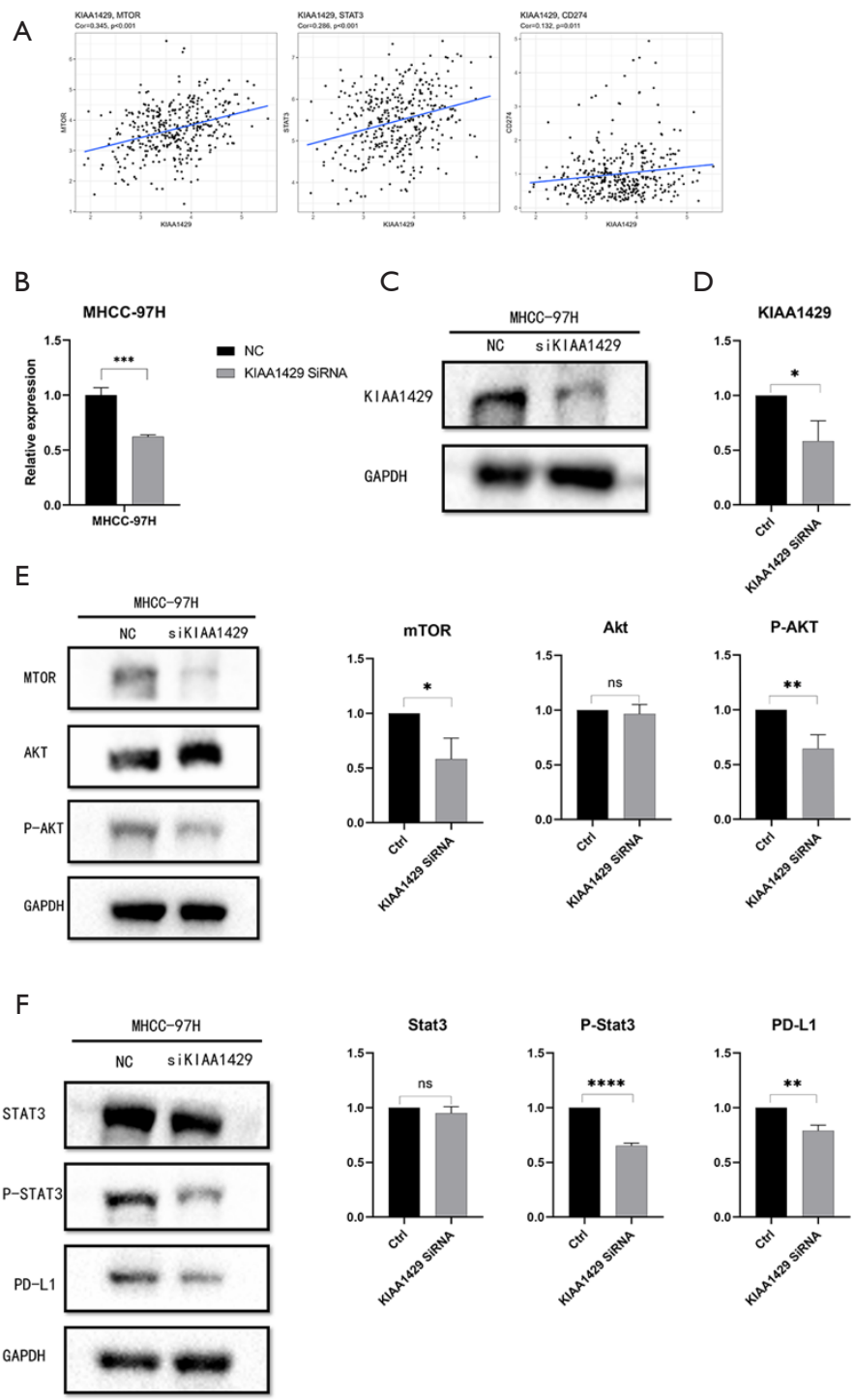

Figure S9 The validation of immune-related targets (ns: P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001 ; ****, P<0.0001). (A) The expression of KIAA1429 was associated with MTOR (r=0.345, P<0.001), STAT3 (r=0.286, P<0.001), and CD274 (r=0.132, P=0.011). (B-D) KIAA1429 was knocked by KIAA1429-specific siRNA. The PCR and western blotting results are shown in *Figure S9*. (E) P-AKT and MTOR protein levels decreased after KIAA1429 interference by KIAA1429-specific siRNA. (F) P-STAT3 and PD-L1 protein levels decreased when KIAA1429 was disrupted.

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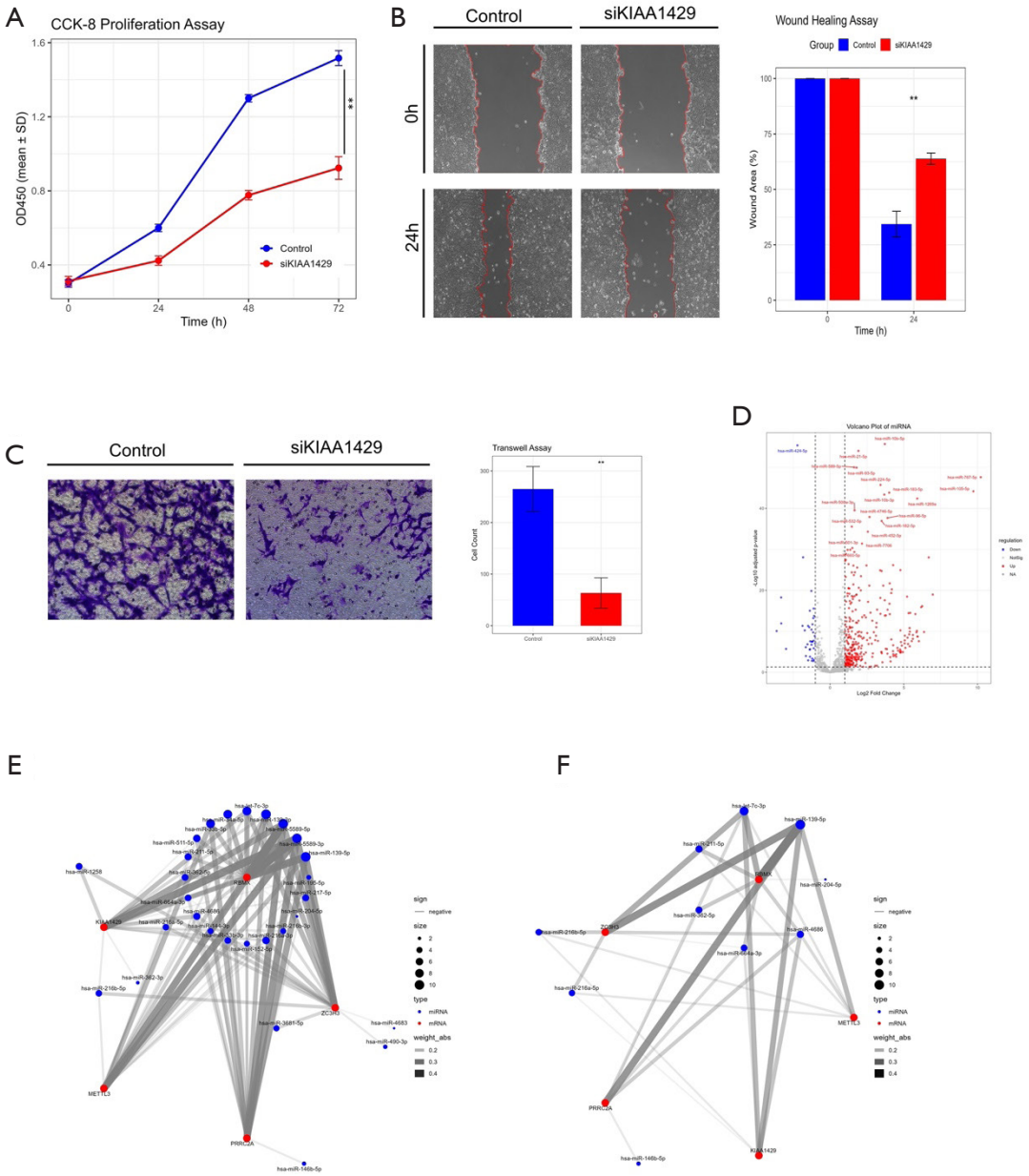

Figure S10 KIAA1429 silencing attenuates HCC cell proliferation, metastasis *in vitro* and DE-miRNA-mRNA interaction network. (A) Silencing KIAA1429 markedly suppressed the proliferation of MHCC-97H cells, as shown by significantly reduced OD450 values in the CCK-8 assay. (B) Wound-healing assay results after KIAA1429 was knocked down (P<0.01), observed under a microscope at 100× magnification. (C) Transwell assays also demonstrated reduced invasion capacity following KIAA1429 disruption invasion (P<0.01), stained with 0.1% crystal violet, and counted under a microscope at 200× magnification. (D) Differential expression analysis in the TCGA-LIHC cohort, 367 DE-miRNAs (329 upregulated and 38 downregulated). (E) Correlation analysis revealed 28 DE-miRNAs that were negatively associated with the expression of the five m6A regulators. (F) The 10 high-confidence regulatory miRNAs(predicted by the multiMiR package) (hsa-let-7c-3p, hsa-miR-139-5p, hsa-miR-146b-5p, hsa-miR-204-5p, hsa-miR-211-5p, hsa-miR-216a-5p, hsa-miR-216b-5p, hsa-miR-362-5p, hsa-miR-4686 and hsa-miR-664a-3p), which were subsequently used to construct a miRNA–mRNA interaction network.

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