SUPPLEMENTARY FIGURE and TABLE LEGENDS

Figure S1. Knockdown efficiency of Ubc9 or Senp1 in HeLa cells was determined by Western Blotting.

Figure S2. (A) SUMOplot prediction of human METTL3 protein. SUMOylation of human METTL3 protein was predicated by the program of Abgent SUMOplot (http://www.abgent.com/SUMOplot). **(B)** HA-METTL3-WT and HA-METTL3-4KR with or without His-SUMO1/Flag-Ubc9 were co-transfected into 293T cells. Forty-eight hours after transfection, cell lysates were prepared for Ni^{2+}-NTA pull down, followed by Western blotting analysis with anti-HA and anti-METTL3 antibodies. **(C)** Amino acid sequence alignment of METTL3 sequences from different species as indicated. The conserved lysines are shown in red.

Figure S3. (A) HA-METTL3 with or without His-SUMO1/Flag-Ubc9 and EBG-Senp1 were co-transfected into 293T cells. 48 hrs after transfection, cells were lysed for immunoblotting with indicated antibodies**. (B)** METTL14 is not be SUMOylated in 293T cells. Flag-METTL14 with or without His-SUMO1, Flag-Ubc9 and EBG-Senp1 were transfected into 293T cells and the SUMOylation assay was performed with the method of Ni^{2+}-NTA resin pull down. **(C)** HA-METTL3-WT or HA-METTL3-4KR was transfected into 293T-shSenp1 cells respectively. 48 hrs after transfection, cells were lysed in RIPA buffer and immunoprecipitated with anti-HA

antibody, followed by Western blotting with anti-CBP80, anti-eIF4E and anti-eIF3b. One-tenth of lysates as input were immunoblotted with indicated antibodies.

Figure S4. Representative LC-MS/MS spectra and the standard curve for m^6A and A. **(A)** The A and m^6A standard curve was obtained from four standard samples with different amounts of A and pure m6A, respectively. **(B, C)** On a LC-MS/MS system, the quantities of nucleotides in H1299-sh*METTL3*-METTL3-WT **(B)** and H1299-*shMETTL3*-METTL3-4KR **(C)** cells were quantified by its integration area in the   corresponding chromatogram.

Figure S5. *In vitro* methylation activity assay of METTL3. (A) schematic diagram of RNA probes Seq1 and Seq2 for the *in vitro* methylation activity assay; **(B-C)** Purified METTL3/METTL14 complexes with or without the RNA probe Seq1 with the consensus sequence of 'GGACU' **(B)** or the RNA probe Seq2 with the point mutation sequence of 'GGAUU' **(C)** were subjected to *in vitro* methylation activity assay. The methylation of RNA-probe was measured by immunoblotting with the m^6A antibody.

Table S1. Summary of the MeRIP m^6A-seq and RNA-seq.

Table S2. List of 90 genes that exhibit a significant change between METTL3-WT and METTL3-4KR in both in m^6A peak levels and abundance of the corresponding mRNA transcripts.
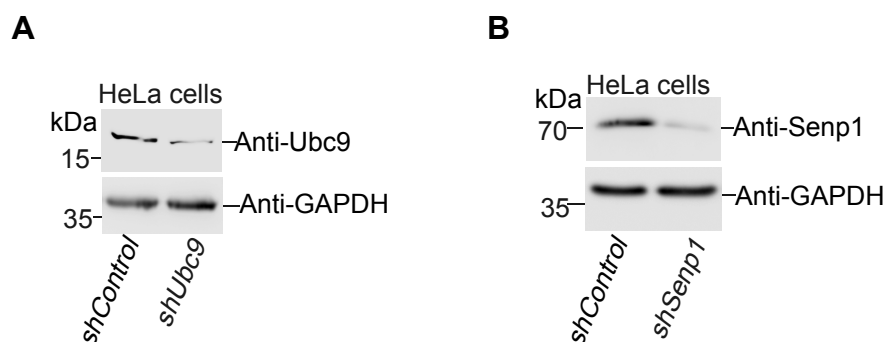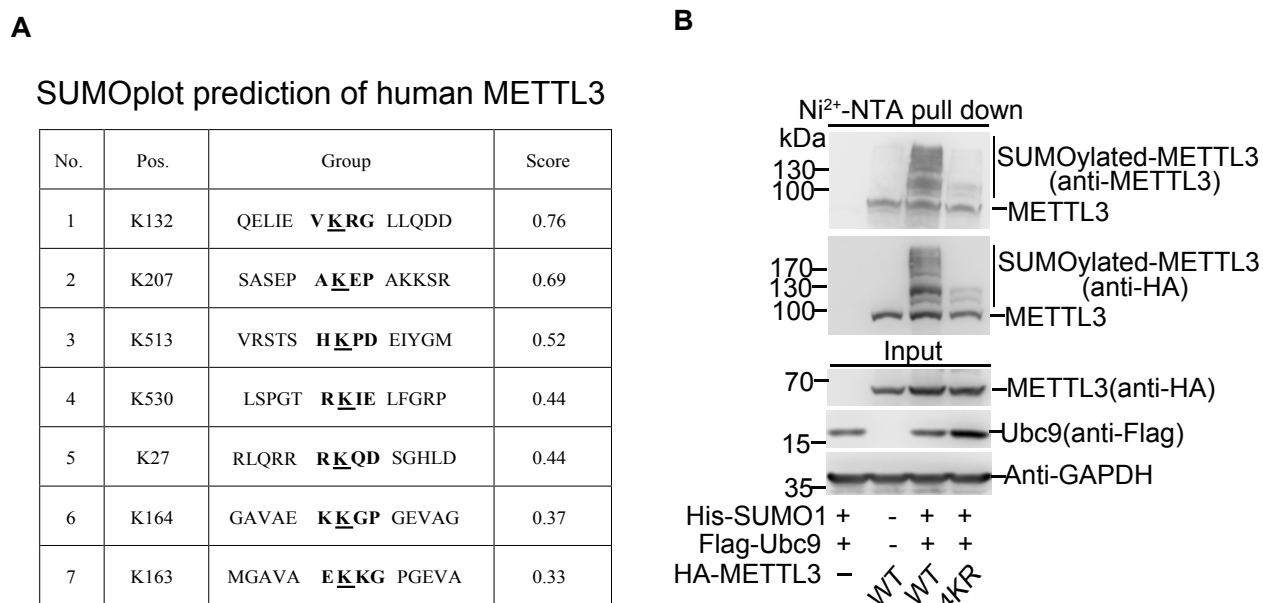
Figure S1

A

HeLa cells

kDa
15 —

—Anti-Ubc9

35 —

—Anti-GAPDH

shControl   shUbc9

B

HeLa cells

kDa
70 —

—Anti-Senp1

35 —

—Anti-GAPDH

shControl   shSenp1

Figure S2

A

SUMOplot prediction of human METTL3

No.	Pos.	Group	Score
1	K132	QELIE **V K RG** LLQDD	0.76
2	K207	SASEP **A K EP** AKKSR	0.69
3	K513	VRSTS **H K PD** EIYGM	0.52
4	K530	LSPGT **R K IE** LFGRP	0.44
5	K27	RLQRR **R K QD** SGHLD	0.44
6	K164	GAVAE **K K GP** GEVAG	0.37
7	K163	MGAVA **E K KG** PGEVA	0.33

B

Ni^{2+}-NTA pull down

kDa
130 —
100 —

SUMOylated-METTL3
(anti-METTL3)

—METTL3

170 —
130 —
100 —

SUMOylated-METTL3
(anti-HA)

—METTL3

Input

70 —

—METTL3(anti-HA)

15 —

—Ubc9(anti-Flag)

35 —

—Anti-GAPDH

His-SUMO1 +   -   +   +
Flag-Ubc9   +   -   +   +
HA-METTL3   –   WT WT 4KR

C

Homo sapiens   [170]AGTVTGQKRRAEQDSTTVAAFASSLVSGLNSSASEPAKEPAKKSRKHAASD[220]
Mouse   [170]AGT IAGQKRRAEQDLTTVTTFASSLASG LASSASEPAKEPAKKSRKHAASD[220]
Rattus norvegicus   [170]AGT ITGQKRRAEQD ITTVATFTSSLASG LGSSTSEPAKEPAKKSRKHAASD[220]
Bos taurus   [170]AGT IAGQKRRAEQDSTTAAAFTSSLASG LASSASEVAKEPTKKSRKHAASD[220]
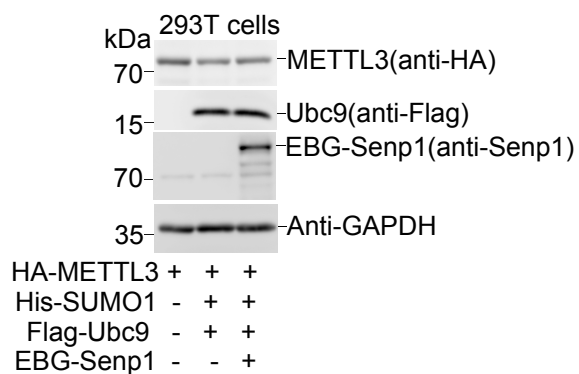Danio rerio   [162]ADD ITHQKRAPGSSPSIQA....LATASISQLTASSGGGGGGADKK.....KVQASH[225]
Pan troglodytes   [170]AGTVTGQKRRAEQDSTTVAAFASSLVSG LNSSASEPAKEPAKKSRKHAASD[220]
Xenopus tropicalis [168]AG.......AKKRRLQEADPSASSLSSS......SSASASGEKKASEPQKKARKNA..SH[213]
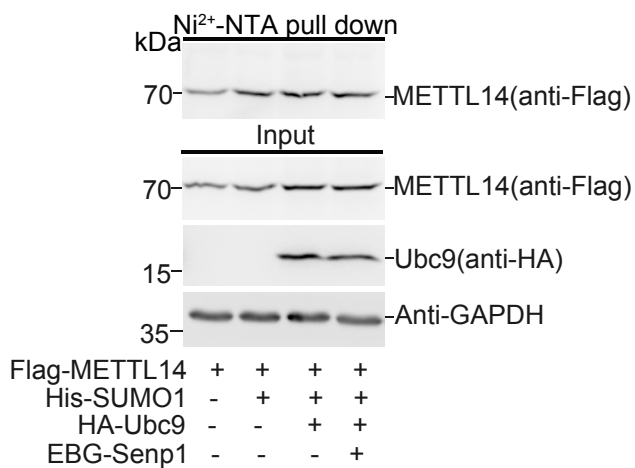Macaca mulatta   [170]AGTVTGQKRRAEQDSTTVAAFASSLA SG LSSSASEPAKEPAKKSRKHAASD[220]
capra hircus   [170]AGT IAGQKRRA EQDSTTAAAFTSSLASG LASSTSEVAKEPTKKSRKHAASD[220]
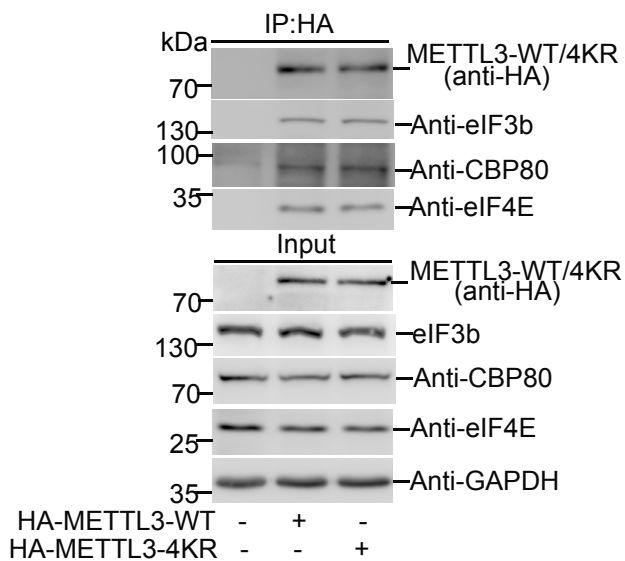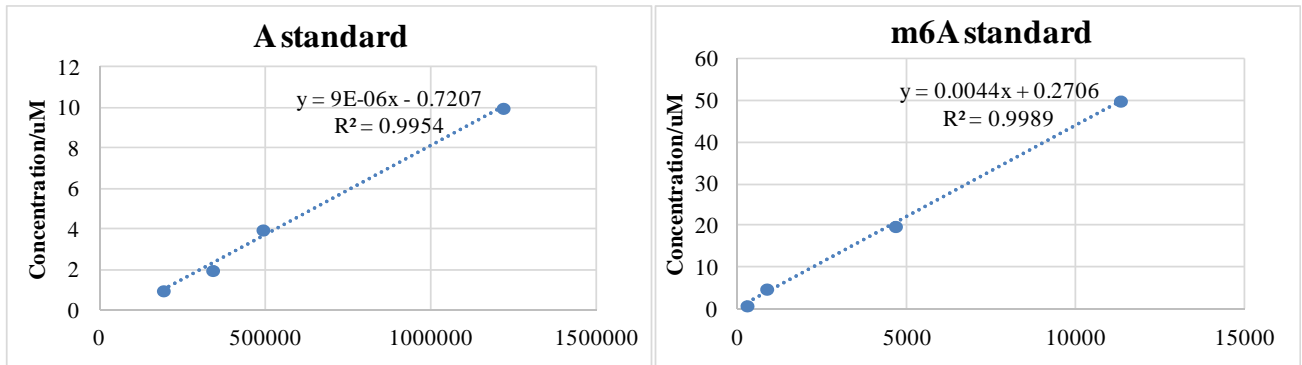
A

293T cells

kDa
70— ——— METTL3(anti-HA)

15— ——— Ubc9(anti-Flag)

——— EBG-Senp1(anti-Senp1)
70—

35— ——— Anti-GAPDH

HA-METTL3   + + +
His-SUMO1   - + +
Flag-Ubc9   - + +
EBG-Senp1   - - +

B

Ni²⁺-NTA pull down

kDa
70— ——— METTL14(anti-Flag)

Input
70— ——— METTL14(anti-Flag)

15— ——— Ubc9(anti-HA)

35— ——— Anti-GAPDH

Flag-METTL14   + + + +
His-SUMO1   - + + +
HA-Ubc9   - - + +
EBG-Senp1   - - - +

C

IP:HA

kDa
METTL3-WT/4KR
70— (anti-HA)

130— Anti-eIF3b
100—
Anti-CBP80
35— Anti-eIF4E

Input
METTL3-WT/4KR
70— (anti-HA)

130— eIF3b

70— Anti-CBP80

25— Anti-eIF4E

35— Anti-GAPDH

HA-METTL3-WT   - + -
HA-METTL3-4KR   - - +

A

A standard

$y = 9E\text{-}06x - 0.7207$
$R^2 = 0.9954$

m6A standard

$y = 0.0044x + 0.2706$
$R^2 = 0.9989$

Figure S4

B

H1299-sh*METTL3*-METTL3-WT

U

C

A

m⁶A

G

Figure S4

C

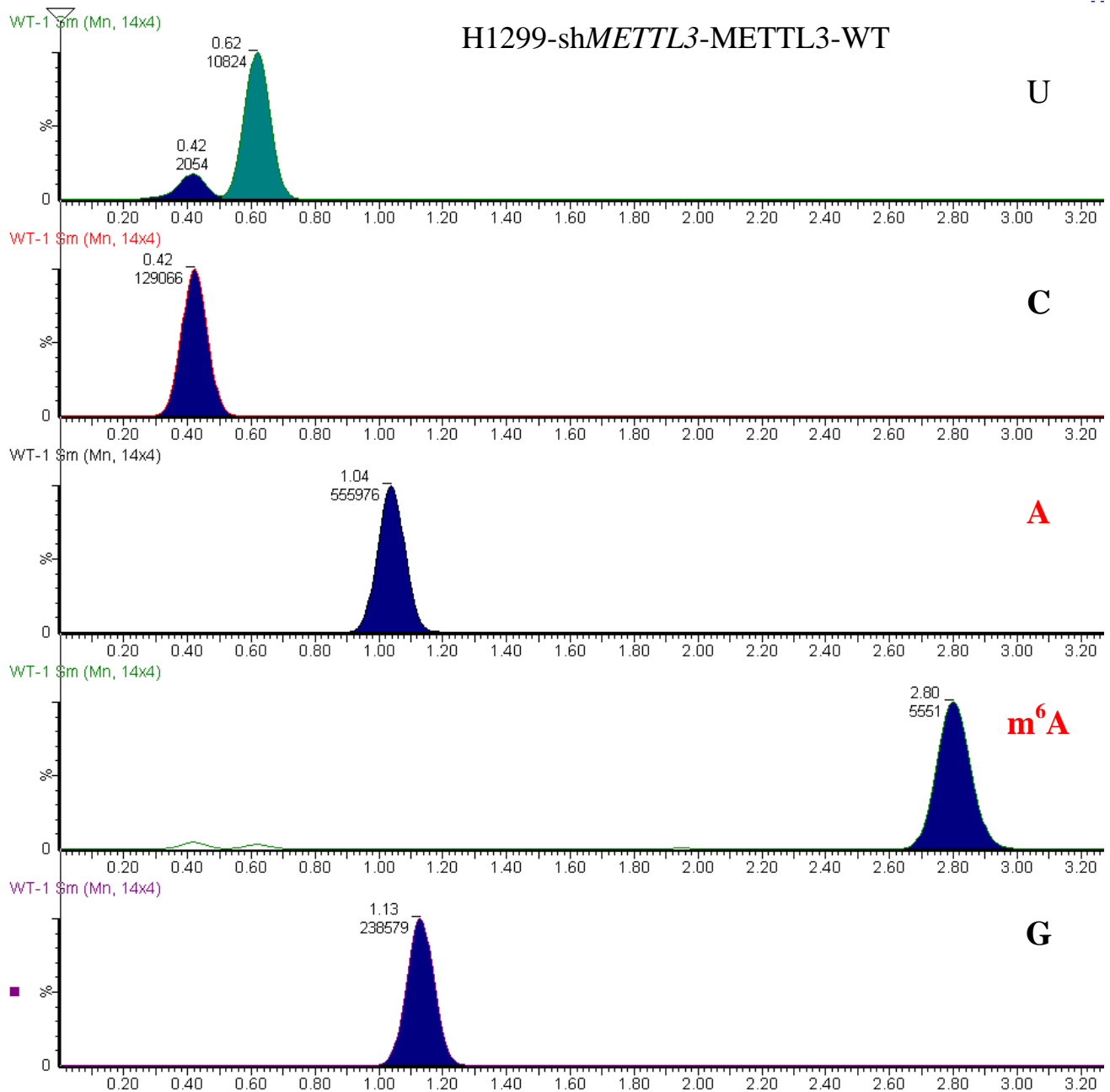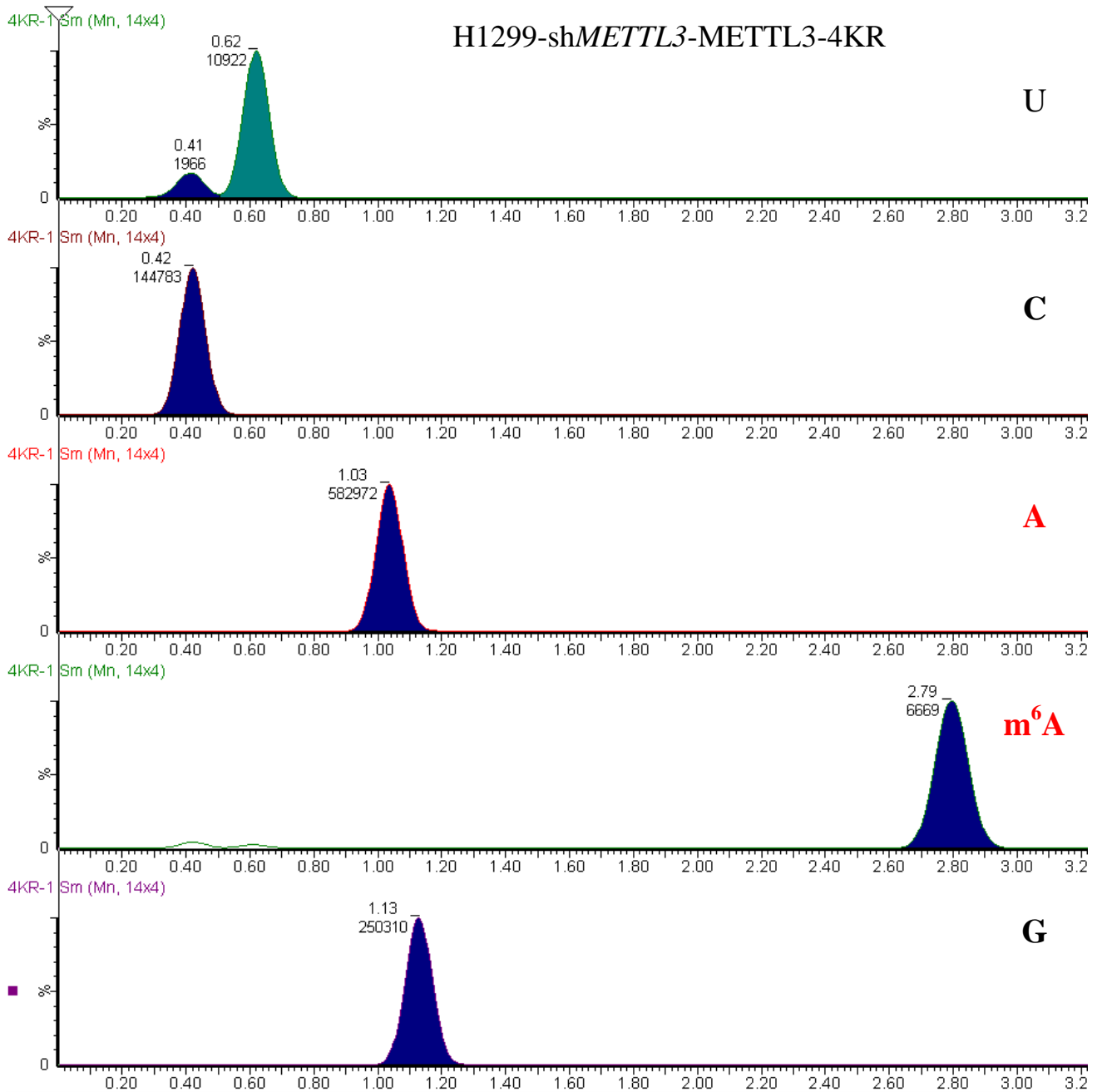H1299-sh*METTL3*-METTL3-4KR

U

C

A

m⁶A

G

Counts vs. Acquisition time(min)
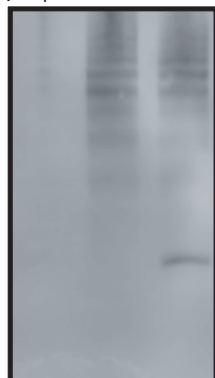
A

RNA probe Seq1: ACGAGUCCUGGACUGAAACGGACUUGC
Seq2: ACGAGUCCUGGAUUGAAACGGAUUUGC

B

Purified METTL3+METTL14   −   +   +
RNA probe seq1   +   −   +

←m6A-RNA probe

IB: anti-m6A

C

Purified METTL3+METTL14

Seq1 Seq2 Seq1 Seq2 Seq1 Seq2 Seq1 Seq2
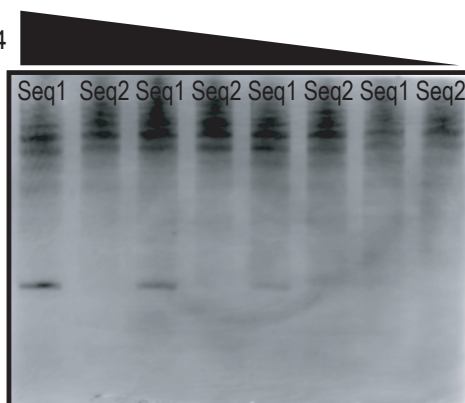
←m6A-RNA probe

IB: anti-m6A

Table S1 (Related to Figure 6). Summary of the m^{6}A-seq and RNA-seq reads

Sample	Seq Type	Total Reads	Aligned Reads
WT	m^{6}A-seq	40,896,078	40,542,449 (99.13%)
	RNA-seq	74,526,128	68,494,267(91.91%)
4KR	m^{6}A-seq	44,910,228	44,520,205 (99.13%)
	RNA-seq	73,850,942	68,607,238(92.90%)

Table S2. List of 90 genes that exhibited significant changes in both the m⁶A peak levels and the overall abundance of corresponding mRNA transcripts between METTL3-WT and METTL3-4KR re-expression.

Gene	Pattern	m⁶A level change						mRNA abundance change	
		peak 1			peak 2				
		Fold	p	Loc	Fold	p	Loc	Fold	p
HS3ST3A1	Hypo-down	7.031E-05	0	5UTR	0.0001563	0	CDS	0.3670427	5.00E-05
KIAA1211	Hypo-down	5.876E-05	0	CDS	0.0002103	0	CDS	0.6128063	1.71E-01
ENTPD1	Hypo-down	0.0002035	0	3UTR				0.0597048	1.00E+00
HIST2H2AA4	Hypo-down	6.152E-05	0	stopC				0.0751986	1.48E-01
JSRP1	Hypo-down	0.0002175	0	CDS				0.0983303	2.89E-01
HIST2H4B	Hypo-down	0.0001723	0	stopC				0.186282	3.73E-02
HIST2H2AA3	Hypo-down	0.0001159	0	stopC				0.2011606	1.86E-01
RBL1	Hypo-down	0.0002103	0	CDS				0.2507671	1.00E+00
ZBED6	Hypo-down	0.0001229	0	CDS				0.3324502	1.00E+00
DLG2	Hypo-down	0.0002089	0	stopC				0.3951192	1.76E-02
EFCAB6	Hypo-down	0.0001687	0	CDS				0.4535615	1.00E+00
COL11A1	Hypo-down	0.000216	0	CDS				0.4679382	1.00E+00
NAV3	Hypo-down	0.0001347	0	CDS				0.4734651	8.37E-02
AGAP8	Hypo-down	0.0001358	0	CDS				0.5003581	7.25E-02
HIST1H2AH	Hypo-down	8.098E-05	0	CDS				0.5106175	6.24E-01
AC096677.1	Hypo-down	9.703E-05	0	stopC				0.5858251	7.56E-01
PLEKHA3	Hypo-down	0.0001487	0	CDS				0.6308422	2.15E-01
AARD	Hypo-down	0.000219	0	stopC				0.6457291	2.71E-01
EXTL3	Hypo-down	0.0001733	0	3UTR				0.6654089	1.18E-01
FCGBP	Hypo-up	6.776E-05	0	CDS	0.0001196	0	CDS	1.5221463	3.99E-02
PLCXD1	Hypo-up	0.0001442	0	CDS	0.0001487	0	CDS	5.1047843	3.80E-02
HSPG2	Hypo-up	0.0001376	0	CDS				1.5190538	3.86E-02
ALMS1	Hypo-up	0.000113	0	CDS				1.5288162	2.22E-01
COL18A1	Hypo-up	0.0002107	0	CDS				1.5507456	5.65E-02
KLHL11	Hypo-up	0.0001876	0	CDS				1.5011623	1.00E+00
FBN1	Hypo-up	0.000152	0	CDS				1.6033907	3.59E-02
TMEM185A	Hypo-up	0.0001311	0	stopC				1.641506	1.71E-01
ABCA5	Hypo-up	6.001E-05	0	CDS				1.6493185	2.27E-01
ANKRD54	Hypo-up	0.0002044	0	CDS				1.6611404	4.99E-01
AGAP4	Hypo-up	0.0002023	0	CDS				1.7345639	4.05E-02
PCDHGB1	Hypo-up	0.0001555	0	CDS				1.7589202	1.00E+00
ZNF442	Hypo-up	0.0002112	0	5UTR				1.7611026	4.77E-02
BMP8A	Hypo-up	0.0002093	0	3UTR				2.0041215	1.00E+00
SHPK	Hypo-up	0.0001948	0	3UTR				2.0202726	1.20E-01
CACNA2D1	Hypo-up	0.0002195	0	3UTR				2.0496392	1.05E-01
GPR146	Hypo-up	0.0001186	0	CDS				2.0747823	6.52E-01
ATP6V1C2	Hypo-up	0.0002126	0	3UTR				2.1673317	6.83E-01
GAGE1	Hypo-up	0.0001832	0	CDS				2.2464392	5.00E-05
FREM2	Hypo-up	0.0002126	0	CDS				2.4535659	1.00E+00
RASA4B	Hyper-down	7639.7	0	5UTR				0.5724109	4.45E-01
TUBB3	Hyper-down	15774.8	0	stopC				0.6634511	7.55E-01
C5orf42	Hyper-down	5243	0	CDS	5176.2	0	CDS	0.5908714	9.45E-02

Gene	Type	Fold		Loc	Fold		Loc		p
GATSL2	Hyper-down	6455.7	0	stopC	4374.1	0	3UTR	0.6356687	4.47E-01
EGR1	Hyper-down	6904.4	0	stopC	5481.7	0	CDS	0.6645423	1.73E-02
GAGE2C	Hyper-down	8842.8	0	3UTR				0.1289318	5.00E-05
PLXNB3	Hyper-down	4374.1	0	CDS				0.2499012	1.00E+00
CNOT6L	Hyper-down	9864.4	0	CDS				0.3020732	1.50E-04
GAGE2E	Hyper-down	5147.5	0	CDS				0.3389654	1.00E+00
APC2	Hyper-down	6197.9	0	CDS				0.4183986	2.60E-01
TREX2	Hyper-down	6455.7	0	stopC				0.4683081	5.69E-01
ZNF649	Hyper-down	10045.8	0	CDS				0.5413704	1.43E-01
SOX4	Hyper-down	9864.4	0	3UTR				0.5811482	6.95E-03
PTPRU	Hyper-down	9644.8	0	CDS				0.5985846	1.00E+00
PRKG2	Hyper-down	5472.2	0	CDS				0.602919	1.00E+00
HIST1H2AI	Hyper-down	5424.5	0	stopC				0.6096635	4.12E-01
S1PR2	Hyper-down	14705.4	0	stopC				0.6154877	7.59E-01
PIANP	Hyper-down	4221.4	0	CDS				0.6252664	9.92E-02
POTEE	Hyper-down	3629.4	0	CDS				0.6279576	1.00E+00
CCDC142	Hyper-down	6217	0	CDS				0.6335481	4.05E-01
AKAP13	Hyper-down	5319.4	0	CDS				0.645927	2.25E-01
RASA4	Hyper-up	8078.9	0	5UTR				3.1345319	7.10E-02
GAGE2B	Hyper-up	16958.8	0	stopC	4822.9	0	CDS	1.5825916	1.38E-01
RGPD5	Hyper-up	3495.7	0	3UTR	3457.5	0	3UTR	6.516196	9.50E-04
GOLGA8J	Hyper-up	7945.2	0	3UTR				1.5023355	1.00E+00
PDP2	Hyper-up	4192.7	0	stopC				1.5307503	9.59E-02
ZBTB10	Hyper-up	10485.1	0	CDS				1.5419628	1.16E-02
TAP2	Hyper-up	11640.4	0	3UTR				1.5532155	6.08E-01
PDE4C	Hyper-up	12643	0	stopC				1.5638217	2.72E-01
VAX1	Hyper-up	4536.5	0	3UTR				1.5655125	2.28E-01
TNC	Hyper-up	12299.2	0	CDS				1.5694641	1.00E+00
KLF13	Hyper-up	3791.7	0	3UTR				1.6166216	2.27E-01
HIST4H4	Hyper-up	6121.5	0	stopC				1.6531997	4.29E-01
NEO1	Hyper-up	9215.1	0	3UTR				1.7734959	9.80E-02
ANK3	Hyper-up	3390.7	0	CDS				1.7910579	2.52E-01
GNRH2	Hyper-up	4479.2	0	CDS				2.2374105	1.00E+00
ZNF846	Hyper-up	5453.1	0	CDS				2.2731139	1.33E-02
PTPRM	Hyper-up	4851.6	0	CDS				2.4359086	1.00E+00
HIST1H1D	Hyper-up	11258.5	0	CDS				5.0422233	1.21E-01
NBPF8	Hyper-up	2970.5	0	CDS				5.0456146	1.00E+00
TXNDC2	Hyper-up	8098	0	CDS				5.4265302	6.47E-02
MEI1	Hyper-up	30326.5	0	CDS				12.833653	1.00E+00
PI16	Hyper-up	11764.5	0	3UTR				14.719533	1.00E+00

(a). Fold, fold change; Loc, location; 3UTR, 3' untranslated region; 5UTR, 5' untranslated region; CDS, coding region; StopC, stop codon; p, p value.

(b). Hypo-down and Hypo-up refer to the gene has a significant decrease in at least one m6A peak and a significant decrease and increase, respectively, in the abundance of the mRNA transcript in H1299-shMETTL3-overexpressing METTL3-4KR compared to METTL3-WT; Hyper-down and Hyper-up refer to the gene has a significant increase in at least one m6A peak and a significant decrease and increase, respectively, in the abundance of the mRNA transcript in H1299-shMETTL3-overexpressing METTL3-4KR compared to METTL3-WT.