Supplemental Materials for

**Acute depletion of METTL3 implicates $N^\epsilon$-methyladenosine in alternative intron/exon inclusion in the nascent transcriptome**

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Supplemental_Fig_S1

A

ChrRNA Bioanalyzer profile

25 50 100 150 200 250 300 350 400 450 500 550 600

SySy (n=10749)

Abcam (n=11726)

B

Transcriptome

SySy

Abcam

MeRIP_ChrRNA_Input

ChrRNA samples

nRNA samples

C

SySy_MeRIP_1

SySy_MeRIP_2

SySy_input_1

SySy_input_2

Abcam_MeRIP_1

Abcam_MeRIP_2

Abcam_input_1

Abcam_input_2

D

Abcam_MeRIP_1

Abcam_input_1

Abcam_MeRIP_2

Abcam_input_2

E

mRNA intensity

0.0 0.2 0.4 0.6 0.8 1.0

0.5 kb

-0.5 0.5 1.0
Supplemental Figure S1. Characterization of the nascent m\textsuperscript{6}A transcriptome by ChrMeRIP-seq, related to Figure 1.

(A) RNA Bioanalyzer traces of ChrRNA samples. Arrowheads indicate the two subpeaks after 18S and 28S rRNA representing pre-rRNA.

(B) The intronic percentage of ChrMeRIP-seq samples (purple) lined up with ChrRNA-seq samples from our previous study (Nesterova et al. 2019). The intronic proportion of the annotated transcriptome (mm10) and mRNA samples (orange) are displayed for comparison.

(C) Pie chart comparisons of the distribution of m\textsuperscript{6}A summits called using the SySy antibody (left) and Abcam antibody (right).

(D) Heatmap showing the signal for called m\textsuperscript{6}A peaks in each replicate of m\textsuperscript{6}A IP and input samples for all the confidence group. The centre of m\textsuperscript{6}A peak stands for the peak summit called by MACS2 and the flanking strand-specific 500nt regions were taken for calculating the signal and making plot. The color key is shown aside.

(E) Boxplots showing the distribution of m\textsuperscript{6}A peak intensities for each confidence group, calculated as averages over 4 replicates (2 SySy + 2 Abcam).
**Supplemental Figure S2.** Comparison of m^6^A peaks from ChrMeRIP-seq and standard MeRIP-seq, related to Figure 1.

(A) Pie charts showing the distribution of m^6^A peaks for Cfg3 group. Same as Fig. 1D.

(B) Pie charts showing m^6^A peak distributions from two previous studies with MeRIP (Batista et al. 2014; Geula et al. 2015) and one study with m^6^A-CLIP in mESCs (Ke et al. 2017).

(C,D) Same as Fig. 1F but for Cfg2 (C) and Cfg3 (D) groups.

(E) UCSC Genome Browser screenshot showing an example IncRNA (Norad) harboring m^6^A methylation. From top to bottom, tracks denote gene annotation, CAGE-seq and ChrMeRIP-seq (Abcam 2 replicates, SySy 2 replicates).
Supplemental Figure S3. Intrinsic m\(^6\)A methylation patterns, related to Figure 2.
(A) Same as Fig. 2A but for all intrinsic m\(^6\)A peaks from Cfg2 and Cfg3 groups.
(B) Schematic showing methodology used to calculate enrichment in the most 5' 25% of introns, used in Fig. 2B.
(C) Boxplots showing GC percentage of intrinsic m\(^6\)A peaks compared to matched controls for all confidence groups.
(D) Same as (C) but for length of introns hosting m\(^6\)A peaks. Controls are gene matched.
(E) Histogram showing intrinsic m\(^6\)A peaks relative to annotated transcription start sites, using representative isoforms from MaxORF_LongestNcRNA.
**Supplemental Figure S4. Performance of RBM15 irCLIP-seq, related to Figure 3.**

(A) Odyssey image showing successful transfer of UV crosslinked RNA-protein. The arrowhead indicates the shift caused by crosslinking of emGFP-RBM15 with its interacting RNAs. Materials from all 3 lanes were used for one replicate irCLIP-seq experiment.

(B) UCSC genome browser tracks showing the profile of m^6_A in Xist RNA, aligned with the two replicates of RBM15 binding. RT stops (i.e. the 5’-end of each sequencing read) were shown for the irCLIP-seq tracks. Landmark A- and E-repeats of Xist are also indicated.

(C) Metaprofile of RBM15 binding (CITS>=3 in two replicates) over noncoding RNA, calculated by RNAmpp analysis.

(D) Zoomed-in view of strand-specific RBM15 binding on loci of example genes: Spen intron 2 (top), Xist E-repeat (middle), and Ythdc1 intron 11 (bottom). RT stops are shown in the tracks. Direction of RNA transcription is also indicated.
Supplemental Figure S5. RBM15 binding correlates with m^6A modification, related to Figure 3.

(A) RBM15 binding (CITS) metaprofiles and heatmaps for exonic m^6A peaks of 3 different confidence groups (red, blue, and grey for Cfg1, Cfg2, and Cfg3 respectively). The color key is shown for all heatmaps. 0.5kb strand-specific flanking regions each side of m^6A peak summit are included for the plot.

(B) Same as (A) but for intergenic m^6A peaks.

(C-F) Same as Fig. 3E,F but for Cfg2 groups (C,D) and Cfg3 groups (E-F).
Supplemental Figure S6. H3K36me3 correlates with RBM15 binding and RNA m⁶A modification, related to Figure 3.

(A) H3K36me3 metaprofiles and heatmaps for m⁶A peaks from confidence groups Cfg1 (red), Cfg2 (blue) and Cfg3 (grey), ordered vertically (from top to bottom are Cfg1 to Cfg3) in the heatmaps. Exonic (left) and intronic m⁶A peaks (right) are separated. Color keys are shown as bellow. 5kb flanking regions each side of the m⁶A peak summit are included for the plot.

(B) Metagene profile of H3K36me3 at the intronic m⁶A sites as well as flanking 5 kb regions. The intronic m⁶A sites are more than 5 kb away toward the annotated transcription terminal site of MaxORF _LongestInRNA transcript by comprehensive GENCODE_vM24 annotation.

(C-E) Metaprofiles and heatmaps of H3K36me3 for Cfg1 (C), Cfg2 (D), and Cfg3 (E) m⁶A peaks with strong (red and green) and weak (grey) RBM15 binding. Red and green curves denote exonic and intronic m⁶A peaks respectively. 5kb regions flanking each m⁶A peak summit are included for the heatmaps, with color keys of H3K36me3 shown below.
Supplemental Figure S7. Validation of functional m^6A loss by dTAG METTL3 in mESC, related to Figure 4.

(A) Additional replicate of western blot analysis shown in Fig. 4C.

(B) Heatmap showing the m^6A peak signals from two METTL3_FKB12^F36V lines, C3 and H5. Both MeRIP and input samples are shown. The centre of m^6A peak stands for the peak summit called from SySy ChrMeRIP-seq by MACS2 and the flanking strand-specific 500nt regions were taken for calculating the signal and making plot. The color key is shown on the right.

(C) Genome browser tracks showing methylation changes on Xist RNA upon dTAG-13 treatment. Samples names are indicated. The two dominant m^6A peaks on Xist RNA lie proximally downstream of Xist A- and E-repeat regions.

(D) Same as Fig. 4E but using the conventional method of calculating peak intensity.
Supplemental Figure S8. Differentially expressed genes upon 3.5 hours of dTAG-13 treatment from 4sU-seq, related to Figure 5.

(A) Volcano plots show the differentially expressed genes (red dots) between control and dTAG-13 treatment samples from 3 independent replicates of METTL3_FKBP12<sup>F36V</sup> clone C3 (left) and METTL3_FKBP12<sup>F36V</sup> H5 clone H5 (right).

(B) Overlap of differentially expressed genes observed in the two clones.
Supplemental Figure S9. Profile of m^6A and RBM15 binding toward splicing changed 5' splice sites from A5SS and ALE, related to Figure 5 and 6.

(A) Same as Fig. 6B-D but for Abcam ChrMeRIP-seq.

(B) Same as Fig. 6B-D but for RBM15 binding. Two irCLIP-seq replicates are averaged (n=24 for ALE and n=25 for A5SS).

(C,D) Schematic (C) and scatter plot (D) showing the correlation between m^6A intensity and the effect size of splicing changes (deltaPSI) calculated from dTAG METTL3 4sU-seq experiment. The intensity was calculated from 1000nt upstream of 5'SS (left: SySy 2 replicates; right: Abcam 2 replicates). Fitted lines (blue) and correlation coefficients are indicated. The dot denoting Ythdc1 intron11 is also marked by red.
Supplemental Figure S10. Splicing choice score of short splicing form in m^6A-bearing alternative 5’ splice sites, related to Figure 5 and 6.

(A) Schematic showing m^6A-bearing alternative 5’ splice site. The splicing choice score is calculated as the proportion of short splicing form over all the splicing read numbers in this intron.

(B) The splicing direction for the m^6A-bearing short form upon acute depletion of METTL3 (red and blue lines denote METTL3_FKBP12^F38V clone C3 and H5 respectively from this study) or conditional Ythdc1 knockout (black line, GSE133585). “Ctrl” indicates no treatment in the corresponding experiments, while “KO” means treatment for knockout. Coordinates of alternative 5’ ss are shown above each panel and gene names are labelled at the bottom of each panel.
Supplemental_Fig_S11

**A** Ke et al. 2017

**B**

n=171 1.8
n=125 1.45
n=249 1.62
n=865 1.29

**C** Geula et al. 2015

**D**

n=26 1.86
n=68 1.58
n=254 1.49
n=1139 1.36

**E**

SAS  ES  pf_IR

SAS  n=3819  p=0.8919
ES  n=2496  p=0.04969
pf_IR  n=2567  p=0.9642

**F**

SAS  ES  pf_IR

SAS  n=2832  p=0.9346
ES  n=2023  p=2.832E-4
pf_IR  n=2023  p=0.2749

**G**

Ke et al  Geula et al

Splicing near to m6A sites

Splicing distant to m6A sites

Nascent transcriptome in this study

Nascent transcriptome in this study

221 73 125 748 228 566
25 16 14 41 14 244
115
28
Supplemental Figure S11. Splicing changes in constitutive Mettl3 KO mESCs, related to Figure 5 and 6.

(A-D) Same as Fig. 5B,C but using data from Ke et al. 2017, (A,B) and Geula et al. 2015, (C,D) generated from WT and Mettl3 KO mESCs.

(E-F) Same as Fig. 5E but using data from Ke et al. 2017 (E) and Geula et al. 2015 (F).

(G) Venn diagrams showing the intersection of splicing clusters among the three datasets. The left shows splicing clusters have or neighbor m⁶A sites, while right is for splicing cluster distant to m⁶A sites.
Supplemental_Fig_S12

A

Rep1

| Gene   | Rep1 | Rep2 | Rep3 |
|--------|------|------|------|
| C3_4sU30m |      |      |      |
| C3_dTAG3h_4sU30m |      |      |      |
| H5_4sU30m |      |      |      |
| H5_dTAG3h_4sU30m |      |      |      |

B

Ythdc1_Ctrl_Rep1
Ythdc1_Ctrl_Rep2
Ythdc1_cKO_Rep1
Ythdc1_cKO_Rep2

C

\[ \text{Ratio} = - \ln \left( \frac{n_{\text{intron3}}}{n_{\text{intron2}}} \right) \]

Both \((n_{\text{intron2}}, n_{\text{intron3}}) \geq 2\) spliced reads

D

| Condition | n= |
|-----------|----|
| WT        | 12 |
| Mettl3KO  | 6  |
| WtapKO    | 15 |
| Rbm15KO   | 15 |

GSE133585
Supplemental Figure S12. Expression profile of *Tor1aip2* gene, related to Figure 6.

(A,B) IGV genome browser view of *Tor1aip2* gene expression profiles. Sample names are indicated on the left, gene annotation is shown below. Arrowheads indicate the induced long isoform. (A) Samples in three replicates with and without dTAG-13 treatment are shown in red and blue, respectively. Data are from 4sU-seq generated in this study. (B) Samples in two replicates with *Ythdc1* control and conditional knockout which are shown in grey and red, respectively. Data are nuclear RNA-seq from GSE133585 (Liu et al, 2020).

(C) Schematic about how to calculate the splicing ratio of intron 3 and 2 as \(-\log(\text{Intron3}/\text{Intron2},e)\).

(D) Boxplots showing the splicing ratio as (C). ChrRNA-seq datasets in which components of m^6^A writer complex were perturbed (Nesterova et al. 2019). Samples were included only if at least 2 reads span each junction of intron2 and intron3.
Supplemental Figure S13. Selected genes related to m^6A pathways are methylated, related to Figure 7.

(A-E) UCSC Genome Browser tracks for m^6A-related genes. (A-C) Ythdf1/2/3, (D) Rbm15, (E) Virma, (F) Cbl1. From top to bottom, they denote ChrMeRIP-seq (Abcam 2 replicates, SySy 2 replicates), RBM15 irCLIP-seq (2 replicates), and H3K36me3 ChIP-seq. In (D), the CAGE-seq (Wei et al. 2020) and 4sU-seq data are included.
Supplemental Figure S14. m^6^-mediates alternative 5'-splicing occurring at Spen intron2, related to Figure 6 and 7.

(A) ChrMeRIP-seq shows that Spen intron2 m^6^-methylation as in Fig. 1G. Bottom shows the unannotated minor splicing form observed from 4sU-seq.

(B) IGV tracks showing that Spen intron2 RNA m^6^-methylaiton (arrowhead) is sensitive to acute depletion of METTL3. Samples are indicated by name on the left.

(C) Tables showing the splicing choice score for the minor splicing form of Spen intron2 from 4sU-seq datasets (this study) (top) and conditional Ythdc1 knockout nuclear RNA-seq from GSE133585 (bottom) (Liu et al. 2020).
Supplemental Figure S15. m\(^5\)A mediates splicing changes at Wtap intron6, related to Figure 7.

(A) Sashimi plot showing the splicing of Wtap alternative last exon in Ythdc1 control and conditional knockout of mESC nuclear RNA-seq (Liu et al. 2020). Top 2 tracks are 2 replicates of control samples while bottom 2 tracks are conditional knockout samples. The number of spliced reads is shown in the arcs. There are 3 main intron splicings as shown from left to right. Wtap is transcribed in an antisense manner. The left 2 splicings are increased upon loss of Ythdc1.

(B) Splicing of Wtap intron6 having m\(^5\)A modification. The pink dashed box indicates the intron having m\(^5\)A modification and arrowheads indicate the "partial" intron retention. The sample names are indicated by red. 4sU-seq for METTL3 dTAG study, and nuclear RNA for Ythdc1 cKO study.
Supplemental Methods

Cell culture
All mouse embryonic stem cells (mESCs), except E14 mESCs, were grown in feeder-dependent conditions on gelatinized plates at 37°C in a 5% CO₂ incubator. Mitomycin C-inactivated mouse fibroblasts were used as feeders. mESCs medium consists of Dulbecco’s Modified Eagle Medium (DMEM, ThermoFisher) supplemented with 10% foetal calf serum (Seralab), 2 mM L-glutamine (ThermoFisher), 1× non-essential amino acids (ThermoFisher), 50 µM β-mercaptoethanol (ThermoFisher), 50 g/mL penicillin/streptomycin (ThermoFisher), and 1 mL of Leukemia Inhibitory Factor (LIF)-conditioned medium made in-house. E14 cells were grown feeder-free in the same conditions and medium as above. Xist expression was induced by the addition of 1–1.5 µg/mL doxycycline (Dox) (Sigma-Aldrich, D9891) for 24 hours.

Molecular cloning and CRISPR-Cas9 mediated knockin
sgRNA targeting near Mettl3 stop-codon was designed by online tool CRISPOR (Haeussler et al. 2016) (http://crispor.tefor.net/) and oligos were synthesised from Invitrogen, then cloned into pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene #62988) backbone following instructions. A donor vector was built by Gibson assembly (NEB) of homology arms (~400 bp) PCR amplified from genomic DNA and the FKB12F36V sequence amplified from plasmid pLEX_305-N-dTAG (Addgene # 91797) (Nabet et al. 2018). The Cas9-sgRNA-containing plasmid (Addgene #165420) and donor vector (Addgene #165421) were co-transfected at a molar ratio of 1:6 into XX mESCs cells on a 6-well plate using Lipofectamine 2000 according to the manufacturer’s protocol (ThermoFisher). Transfected cells were passaged at different densities into three Petri dishes with feeders, 24h after transfection. The next day, cells were subjected to puromycin selection (3.5 µg/mL) for 48h and then grown in regular mESC medium until mESC colonies were ready to be picked and expanded. Western blot analysis was used to screen colonies for FKB12F36V knock-in as this results in slower mobility of the METTL3_FKB12F36V protein compared to the wild-type protein in an SDS-PAGE gel and disappearance of the wild-type METTL3 band on western blot. Selected clones were further characterized by PCR of genomic DNA and Sanger sequencing to confirm correct knock-in and homozygosity. The sensitivity of selected METTL3_FKB12F36V clones to dTAG-13 treatment was validated by western blot. The plasmids used in this study are deposited in Addgene.

Western blot
Total cell lysates were resolved on a polyacrylamide gel and transferred onto PVDF or nitrocellulose membrane by semi-dry transfer (15V for ~50 minutes) or quick transfer. Membranes were blocked by incubating them for 1 hour at room temperature in 10 mL PBS, 0.1% Tween (TBST) with 5% w/v Marvell milk powder. Blots were incubated overnight at 4°C with the primary antibody, washed 4 times for 10 minutes with PBST and incubated for 1 hour with secondary
antibody conjugated to horseradish peroxidase. After washing 5 times for 5 min with PBST, bands were visualised using ECL (GE Healthcare). TBP serves as loading control.

**Cell fractionation and Chromatin-associated RNA (ChrRNA) isolation**

ChrRNA was extracted from a confluent 15 cm dish of mESCs. Briefly, cells were trypsinised and washed twice in cold PBS, and then lysed on ice in RLB buffer [10 mM Tris pH 7.5, 10 mM KCl, 1.5 mM MgCl₂, and 0.1% NP40] for 5 minutes, and nuclei were purified by centrifugation through a sucrose cushion (24% sucrose in RLB). The supernatant was kept as cytosolic fraction. The nuclei pellet was resuspended in NUN1 [20 mM Tris pH 7.5, 75 mM NaCl, 0.5 mM EDTA, 50% glycerol], then lysed with NUN2 [20 mM Hepes pH 7.9, 300 mM, 7.5 mM MgCl₂, 0.2 mM EDTA, 1 M Urea]. Samples were incubated for 15 minutes on ice with occasional vortexing, then centrifuged for 10 minutes at 2800g to isolate the insoluble chromatin fraction. The upper phase was kept as nucleoplasm fraction. The insoluble chromatin pellet was resuspended in TRIzol by passing multiple times through a 23-gauge needle. The disrupted chromatin pellet was either frozen in -80°C or freshly purified through standard TRIzol/chloroform extraction followed by isopropanol precipitation. Samples were then treated with two rounds of Turbo DNaseI (AM1907, Life Technology) in order to eliminate any potential DNA contamination. Concentration and quality of RNA isolated from the chromatin pellet were determined by Nanodrop and Agilent RNA Bioanalyzer.

**4sU-RNA immunoprecipitation**

4sU-RNA was generated and isolated essentially as described in (Rabani et al. 2011; Pintacuda et al. 2017). In detail, 4-thiouridine (4sU, Sigma-Aldrich, T4509) was dissolved in sterile PBS and stored at -20°C. 4sU was thawed just before use and added to the cells in the growing media at a concentration of 500 μM. METTL3-FKBP12F36V expressing cells (C3 and H5 clones) were treated with or without dTAG-13 (100 nM) for 3 hours, then exposed to 4sU-supplemented medium for 30 minutes. Cell culture medium was rapidly removed from cells and 5 mL of TRIzol reagent (Life Technologies) was added. Total RNA was isolated, and any potential DNA contamination was removed using Ambion DNA-free DNase Treatment kit (Life Technologies) according to the manufacturer’s instructions. For each μg of total RNA, 2 μL of Biotin-HPDP (Pierce, 50 mg EZ-Link Biotin-HPDP), previously dissolved in DMF at a concentration of 1 mg/mL, and 1 μL of 10x Biotinylation buffer [100 mM Tris-HCl pH 7.4, 10 mM EDTA], was added. The reaction was incubated with rotation for 15 minutes at 25°C. RNA was transferred to Phase Lock Gel Heavy Tubes (Eppendorf), and an equal volume of chloroform was added. After vigorously mixing, tubes were left incubating for 3 minutes at 25°C and then centrifuged at 13,000 rpm for 5 minutes at 4°C. The upper phase was transferred to new Phase Lock Gel Heavy Tubes, and chloroform added again. After further centrifugation, the upper phase was transferred to a tube containing an equal volume of isopropanol and 1/10 volume of 5 M NaCl. After inversion, the tubes were centrifuged at 13,000 rpm for 20 minutes at 4°C. Supernatant was washed in 75% ethanol and resuspended in
water. Biotinylated 4sU-RNA was recovered using the μMacs Streptavidin Kit (Miltenyi), with a modified protocol. Per μg of recovered biotinylated 4sU-RNA, 0.5 μL of streptavidin beads were added, in a total volume of 200 μL. Samples were incubated with rotation for 15 minutes at 25°C. μMacs columns supplied with the μMacs Streptavidin Kit were equilibrated in 1 mL of washing buffer [100 mM Tris-HCl pH 7.5, 10 mM EDTA, 1 M NaCl, 0.1% Tween 20] at 65°C. Samples were added to the columns that were then washed 6 times with washing buffer, 3 times at 65°C and 3 times at 25°C. RNA was eluted in freshly prepared 100 mM DTT. RNA was further purified using the RNeasy MinElute Cleanup kit (Qiagen) according to the manufacturer's guidelines. 1 μL of 4sU-labelled RNA was quality-checked using the Agilent RNA 6000 Pico kit (Agilent technologies) according to the manufacturer's instructions and run on a 2100 Bioanalyzer Instrument (Agilent).

4sU-seq
Approximately 750 ng of 4sU-labelled RNA were subjected to sequencing library preparation by TruSeq Stranded total RNA LT Sample Prep with Ribozero gold (Cat. No. RS-122-2301) according to manufacturer's instructions. Libraries were purified with AmPure XP beads and quantified by Qubit. Equal moles of libraries with different barcodes were pooled together based on the KAPA quantification (Roche) and sequenced using Illumina NextSeq500 (FC-404-2002) in paired-end mode (2×80).

emGFP-RBM15 irCLIP
The emGFP-PreScission-RBM15 cell line was described above. irCLIP were performed as (Zarnegar et al. 2016) with few modifications. Briefly, cells were grown with feeder and harvested after 24 hours Dox treatment. 10 million mESCs were washed with ice-cold PBS irradiated once with 150 mJ/cm² in a Stratalinked 2400 at UVC 254 nm on ice. Snap frozen pellets were stored at -80°C until use. Cell pellets were resuspended in 1 mL lysis buffer [50 mM Tris-HCl pH 7.4, 100 mM NaCl, 1% IGEPIAL CA-630, 0.1% SDS, 0.5% sodium deoxycholate] supplemented with protease inhibitors, and transferred to a fresh 1.5 mL tube. Sonication was performed on a Bioruptor (Diagenode) for 10 cycles with alternating 30 secs on off at low intensity, and followed by RNase I (Thermo Scientific, EN0602) and Turbo DNase (Ambion, AM2238) treatment. Pre-washed antibody-conjugated beads were directly added to the RNase-treated lysate and rotated on a wheel overnight at 4°C. After discarding the supernatant, the beads were washed 2× with high-salt wash buffer [50 mM Tris-HCl pH 7.4, 1 M NaCl, 1 mM EDTA, 1% IGEPIAL CA-630, 0.1% SDS, 0.5% sodium deoxycholate]. 3’-end RNA dephosphorylation was performed on beads with PNK (NEB M0201L) for 20 min at 37°C. Beads were further washed with 1× PNK wash buffer [20 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 0.2% Tween-20], 1× high-salt wash buffer, and 1× PNK wash buffer. Washed beads were subjected to RNA ligation with pre-adenylated infrared adaptor L3-IP-App (/5Phos/AG ATC GGA AGA GCG GTT CAG AAA AAA AAA AAA /iAzideN/AA AAA AAA AAA AAA A/3Bio/, 1 µM) together with RNA ligase (M0204, NEB), and incubated overnight at 16°C in a thermomixer shaking at 1100 rpm. Beads were subsequently washed with 1× PNK
wash buffer, 1× high-salt wash buffer, 2× PNK wash buffer, and finally supplemented with 1× NuPAGE loading buffer. Supernatants were carefully collected and loaded on a 4-12% NuPAGE Bis-Tris gel (Invitrogen) according to the manufacturer’s instructions. The gel was run at 180V until dye front reached the bottom. The protein-RNA-L3IR complexes from the gel were transferred to a Protan BA85 Nitrocellulose Membrane (Whatman) using the Novex wet transfer apparatus according to the manufacturer's instructions (Invitrogen; transfer for 2 hours at 30V). The entire lane above the expected molecular size of the protein of interest (emGFP-PreScission-RBM15) was cut from the membrane, and mixed with proteinase K (Roche, 03115828001). RNA was isolated by Phenol:Chloroform:isoamyl Alcohol (Sigma-Aldrich P3803) using Phase Lock Gel Heavy tube (713-2536, VWR). The isolated RNAs were reverse transcribed by SuperScript IV with barcode primer (/5Phos/ WWW GTGGA NNNN AGATC GGAAG AGCGT CGTGAT /iSp18/ GGATCC /iSp18/ TACTG AACCG C). cDNA products were biotin captured with MyOne C1 SA-dynabeads, eluted, and subsequently circularised with CircLigase II (Epicentre). PCR amplification was obtained after 18-21 cycles. The amplified irCLIP libraries were purified using AmPure XP beads (Beckman Coulter) and subjected to size selection from TBE gel by cutting a 150-500 nt smear band. Library concentrations were quantified by Qubit and KAPA quantification kit (Roche), quality-checked by DNA Bioanalyzer (Agilent), and sequenced on an Illumina NextSeq500 machine using 75 bp single end sequencing.

Intronic patterns of m^6^A methylation
Classification of peak summits (single nucleotides) were intersected with the selected MaxORF_LongestNcRNA GENCODE vM24 isoform (intersectBed -a summits.bed -b *.GeneBed -s -wo). Constitutive or alternative intron location was determined by whether the peak summit locates in intron for all the annotated comprehensive GENCODE vM24 isoforms or subsets of the isoforms respectively. PhastCons scores for multiple alignments of 59 vertebrate genomes to the mouse genome (phastCon60way) from UCSC were used (Pollard et al. 2010). For calculating conservation and GC contents, control region for this intronic m^6^A methylation was chosen by randomly selecting the size-matched region from the same intron. For the relative intron length and position, random intron from the same transcript was chosen. Plots were generated by R package ggplot2. Scripts used for this analysis were attached.

Calibrated MeRIP-seq analysis
The raw reads from m^6^A immunoprecipitation were mapped to mouse (mm10) and Drosophila (dm6) concatenated genome by STAR (v2.5.2b) (Dobin et al. 2013). The resulted alignment files (BAM) were then split into mm10 and dm6 alignment files according to the chromosome name. The read counts mapping to the dm6 were counted (Li et al. 2009) and used for subsequent normalization (Supplemental Table S1). The raw reads from input samples were mapped to the mm10 genome by STAR (v2.5.2b) (Dobin et al. 2013). Alignment files for m^6^A IP and input
samples were also normalized in a conventional manner by 10 million mapped reads. The strand-specific bigwig files were generated and visualized by IGV (Robinson et al. 2011).

irCLIP-seq analysis
The irCLIP adaptors (sequence) from single-end sequencing reads were first cut by cutadapt (v1.12) (Martin 2011), and then collapsed by CTK toolkit to remove PCR duplication (Shah et al. 2017). The barcodes with UMI-containing 12 letters were moved to FASTQ header lines. The resulted single-end fastq reads, longer than 15 nt, were mapped to the mm10 genome by STAR (v2.5.2b) (Dobin et al. 2013) with key parameters (--outSAMattributes All --outFilterMultimapNmax 1 --outFilterMismatchNmax 2 --alignEndsType EndToEnd --seedSearchStartLmax 15 --outWigType bedGraph read1_5p). This setting is because RT stops were generated by reverse transcriptase around the UV crosslinked sites or crosslinking induced truncation sites (CITS), and RT-stops were main output when analysing the irCLIP-seq reads. The single nucleotide was considered as a CITS if there are no less than 3 stops (>=3) from independent strand-specific reads. In addition, crosslinking induced mutation sites (CIMS), particularly (deletion site) were also calculated. The single nucleotide was considered as a CIMS if this position has more than 2 deletions among total covered reads and the mutation rate is [0.01, 0.5], in order to avoid the potential contamination from the indels. Given that most of the RNA-binding proteins has 5-mer consensus motif, called CITSs or CIMSs from biological replicate 1 were kept if they were also called and within 5 nucleotides distance in biological replicate 2 (closestBed -a CITS_Rep1.bed -b CITS_Rep2.bed -s -d | awk '$13<=5'). The distribution of CITS and CIMS were analysed by the RNAmpp pipeline, described as above. Motifs around these CITSs or CIMSs were searched by Homer with key parameter (findMotifsGenome.pl CITS.bed mm10 MotifOutput -rna).

RBM15 binding, m\(^{6}\)A peak, and H3K36me3 modification
RBM15 CITS (>=3) called in 2 biological replicates were intersected with m\(^{6}\)A peaks from different confidence groups. Due to the strand-specificity, the closest distance between RBM15 binding and m\(^{6}\)A peak will be minus if RBM15 CITS located upstream of m\(^{6}\)A peak (closestBed -a RBM15_CITS.bed -b m6A_peaks.bed -s -D a) (Quinlan and Hall 2010). Strong RBM15 binding group was defined by no more than 1000 nt distance between RBM15 binding and m\(^{6}\)A peak. The density of RBM15 binding sites (CTIS>=3) 1000 bp flanking exonic m\(^{9}\)A peaks, intronic m\(^{6}\)A peaks, and intergenic m\(^{6}\)A peaks were plotted. BigWig files for H3K36me3 ChIP-seq data retrieved from ENCODE was generated in 10 bp bin size. The metagene profile and heatmap for RBM15 binding group and other groups were generated by deepTools (v3.4.3) (Ramirez et al. 2016). The matrix generating from negative strand bigWig files are combined with the one from positive strand, and with a brief modification to average two replicates, then subjected to plotHeatmap tool (Ramirez et al. 2016).

Differentially expressed genes analysis
4sU-seq were mapped to the mm10 genome by STAR (v2.5.2b) (Dobin et al. 2013) allowing multiple mapping (--outFilterMultimapNmax 100 --outFilterMismatchNmax 5 --alignEndsType EndToEnd --winAnchorMultimapNmax 100). TEtranscripts (Jin et al. 2015) was used to detect the differentially expressed genes and repeat elements.
Supplemental Table S1: Summary of ChrMeRIP-seq data, related to Figure 1.

| Samples       | total reads | mapped to mm10 | Normalization Factor |
|---------------|-------------|----------------|----------------------|
| SySy_IP_Rep1  | 171413985   | 75224035       | 7.5224035            |
| SySy_IP_Rep2  | 167773086   | 78103747       | 7.8103747            |
| SySy_input_Rep1 | 72543991   | 42643208       | 4.2643208            |
| SySy_input_Rep2 | 69979632   | 42790747       | 4.2790747            |
| Abcam_IP_Rep1 | 35247161    | 17359698       | 1.7359698            |
| Abcam_IP_Rep2 | 26431065    | 12922923       | 1.2922923            |
| Abcam_input_Rep1 | 18476488  | 12452285       | 1.2452285            |
| Abcam_input_Rep2 | 20645009  | 15864869       | 1.5864869            |
Supplemental Table S2, Sequencing depth for normalization, related to Figure 4.

| Samples           | mm10     | dm6     | ScalingFactor (Calibrate) | ScalingFactor (Convention) |
|-------------------|----------|---------|---------------------------|---------------------------|
| H5_input          | 8015137  | NA      | NA                        | 0.8015137                 |
| H5_dTAG_input     | 9702459  | NA      | NA                        | 0.9702459                 |
| C3_input          | 10305708 | NA      | NA                        | 1.0305708                 |
| C3_dTAG_input     | 10439183 | NA      | NA                        | 1.0439183                 |
| H5_m6A            | 32373084 | 2202971 | 11.01485                  | 3.2373084                 |
| H5_dTAG_m6A       | 23644313 | 1180777 | 5.90388                   | 2.3644313                 |
| C3_m6A            | 29186935 | 1133319 | 5.66659                   | 2.9186935                 |
| C3_dTAG_m6A       | 38241256 | 1364155 | 6.82077                   | 3.8241256                 |
Supplemental Table S3: Primers used in this study to clone METLL3_FKBP12F36V and PCR validation.
Primers used for sequencing the plasmid
>PQE30forward
CCCGAAAAGTGCCACCTG
>TNK78
GTTCAGGGGGAGGTGTTGAGTT

Primers used for sgRNA cloning
>Mettl3C_sgRNA_F
caccgTCTGAACGCTTAGCTTTGTA
>Mettl3C_sgRNA_R
aaacTACAAAGCTAAGCGTTCAGA

Primers used for PCR cloning genomic regions near to Mettl3 stop codon, and also check the insertion
>Mettl3_CF
AATTGACGTGGACTGGG
>Mettl3_CR
ATTGGGCTAGAAGGGAACGA

Primers used for Gibson Assembly
>Mettl3C_Frag1F
TCCGCGCAACTTTCCCCCGAAGGTGCCACCTGAGTA
>Mettl3C_Frag1R
catGCCTCCAACTCCACCTAAATTCTTAGGTTAGGATGATGCGCCGCTTG
>Mettl3C_Frag2F
TTGTAACAGCTGTGCTGCTAtttccagttttagctacagc
>Mettl3C_Frag2R
TGGCCTGTAGCCCATCTCTGAAAGCTTAGCTTTGTAAGGAGTGCGTCTG
>Mettl3C_Frag3F
gagctttcaaacAAGTAGACGCACCTTAGTTACAAAGCT
>Mettl3C_Frag3R
GATGCCCAGCGCTACGCGTAGCTGACTGACTAGTACAGACTATAAAACCACGCAAAGCTACATAGATAAGCA
>PAM2Mutation_F
ggaAAGTAGACGCACCTTAGTTACAAAGCTAAGGAGTGCGTCTGA
>PAM2Mutation_R
TGGCCTTAGCCCATCTCTGAAAGCTTAGCTTTGTAAGGAGTGCGTCTG
Supplemental Table S4: Antibodies used in this study.

| Antibody     | Company       | Catlog      |
|--------------|---------------|-------------|
| METTL3       | Abcam         | ab195352    |
| METLL4       | Sigma-Aldrich | HPA038002   |
| RBM15        | Proteintech   | 10587-1-AP  |
| WTAP         | Proteintech   | 10200-1-AP  |
| YTHDC1       | Sigma-Aldrich | HPA036462   |
| TOX4         | Invitrogen    | PA5-41558   |
| TBP          | Abcam         | ab51841     |
| EZH2         | Cell Signaling| mAb#5246    |
| m6A_Sysy     | Synaptic Systems | 202 003 |
| m6A_Abcam    | Abcam         | ab151230    |
| Anti-rabbit IgG, HPR, Donkey, WB (1:2000) | Amersham | cat#NA934V |
| Anti-mouse IgG, HRP, Sheep, WB (1:2000) | Amersham | cat#NXA931 |
Supplemental Data

**Supplemental Data S1: Confidence m^6A peaks characterized in this study.**

All the ChrMeRIP m^6A peaks (narrow peaks and peak summits) categorized in this study, based on genomic location and confidence group, are listed.

**Supplemental Data S2: Differentially expressed genes between control and dTAG-13 treatment.**

The differentially expressed genes and transposon elements called in C3 and H5 clones, separate and comment lists, are listed.

**Supplemental Data S3: Splicing changes calculated from LeafCutter.**

Splicing clusters, called by LeafCutter with p.value, between 6 untreated and 6 dTAG-13 treated samples, are listed.
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