SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell lines and antibodies

HeLa cells, HEK293T cells, and MEF cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). Antibodies used for western blot analysis were as follows: HSP70 (SPA-810) was purchased from Stressgen, anti-FTO (597-Fto) from Phosphosolutions or Abcam, RPS6 from Genetex, goat anti-GFP from Santa Cruz Biotechnology, mouse anti-β-actin from Genscript, rabbit anti-GAPDH from Abcam, Rabbit anti-YTHDF1 (Ptglabs 17479-1-AP), Rabbit anti-YTHDF2 (Ptglabs 24744-1-AP), Rabbit anti-YTHDF3 (Ptglabs 25537-1-AP), Rabbit anti-YTHDC1 (Ptglabs 14392-1-AP), Rabbit anti-YTHDC2 (Novus 17479-1-AP). Rabbit anti-m^6A was from Synaptic Systems.

Assembly and analysis of ribosomal complexes

Expression vectors for eIF1 and eIF1A (Pestova et al., 1998), eIF4A and eIF4B (Pestova et al., 1996), eIF4E (Volpon et al., 2006), and *E. coli* methionyl tRNA synthetase (Lomakin et al., 2006), as well as transcription vectors for MVHC-STOP mRNA (comprising the 54 nt-long 5' UTR of rabbit β-globin mRNA starting with two extra Gs, the coding sequence for an MVHC tetrapeptide, a UAA stop codon, and a ~150 nt 3′UTR consisting of the natural β-globin coding sequence) (Skabkin et al., 2013) and $tRNA_i^{Met}$ (Pestova and Hellen, 2001) have been described. The transcription vector for β-globin-2AUG mRNA was made by inserting an appropriate DNA fragment flanked by a T7 promoter and a *HindIII* restriction site into pUC57 (GenScript Corp.). Native 40S and 60S ribosomal subunits, eIF2, eIF3 eIF4F, eIF5B, eEF1H, eEF2 and total aa-tRNA synthetases, and recombinant eIF1, eIF1A, eIF4A, eIF4B, eIF5 and *E. coli* methionyl tRNA synthetase were purified as described (Lomakin et al., 2006; Pisarev et al., 2007). mRNAs and $tRNA_i^{Met}$ were transcribed using T7 RNA polymerase. Transcribed mRNAs were capped using the T7 mScript Standard mRNA Production System (Cellscript, Madison, WI, USA). *In vitro* transcribed $tRNA_i^{Met}$ and elongator tRNAs were aminoacylated using *E. coli* methionyl tRNA synthetase and native total tRNA synthetases, respectively, as described (Pisarev et al., 2007).

48S initiation complexes were assembled and analyzed by toe-printing essentially as described (Pisarev et al., 2007). Briefly, 60 nM mRNA was incubated with 40S subunits (90 nM), Met-$tRNA_i^{Met}$ (150 nM), and different combinations of eIF1 (450 nM), eIF1A (450 nM), eIF2 (160 nM),

eIF3 (150 nM), eIF4A (350 nM), eIF4B(350 nM), and eIF4F (150 nM) in 20 µl reaction mixtures containing buffer (20 mM Tris-HCl [pH 7.5], 100 mM KAc, 2.5 mM $MgCl_2$, 0.25 mM spermidine and 2 mM DTT) supplemented with 0.8 mM ATP and 0.4 mM GTP for 15 min at 37˚C. Assembled 48S complexes were then incubated with 120 nM 60S subunits, 300 nM eIF5 and 150 nM eIF5B for 10 min at 37˚C to allow formation of 80S initiation complexes. To form pre-termination complexes, 80S complexes were supplemented with 150 nM eEF1H, 150 nM eEF2 and appropriately aminoacylated native total tRNA (~200 nM each) and incubated for 10 min at 37˚C. After incubation, the assembled complexes were analyzed by primer extension using AMV reverse transcriptase and a [^{32}P]-labeled primer complementary to nucleotides 197-214 of *wt* β-globin mRNA. The resulting cDNAs were resolved by electrophoresis on a 6% acrylamide sequencing gel followed by autoradiography. The toeprint positions were determined by comparison with a DNA sequence obtained with the same primer.

Translation efficiency (TE) analysis

Ribosome profiling data from METTL3-depleted cells and Mettl3 knockout cells was previously described (Wang et al., 2015). TE measurements were analyzed for mRNAs containing m^{6}A within various regions of the transcript as identified by single nucleotide-resolution m^{6}A mapping (Linder et al., 2015). Cumulative distribution plots were generated for data visualization, and statistical analysis was done using the Mann-Whitney U test with a *P*-value cutoff of 0.05.

Heat shock induction of Hsp70

MEF cells with Fto knockdown or overexpression were incubated overnight and were 90% confluent prior to heat shock treatment. After incubation in a 42°C water bath for 1h, cells recovered in an incubator at 37°C and collected at the indicated time points.

Polysome Profiling

Sucrose solutions were prepared in polysome buffer (10 mM HEPES, pH 7.4, 100 mM KCl, 5 mM $MgCl_2$, 100 µg/ml of cycloheximide). A 15-45% (w/v) sucrose density gradients were prepared in SW41 ultracentrifuge tubes (Fisher) using a BioComp Gradient Master (BioComp Instruments). Cells with or without heat shock stress were treated with cycloheximide (100 µg/ml) for 3 min at 37°C and lysed in an ice-cold polysome buffer containing 2% Triton X-100. 500 µl supernatant of the cell lysate was loaded onto gradients, followed by centrifugation at 38,000 rpm at 4°C in a SW41 rotor for 2 h 28 min. Gradients were fractionated through Isco fractionation system and absorbance values at 254 nm were continually monitored.

Real-time quantitative PCR

Total RNA from whole cell lysate was isolated using TRIzol reagent (Invitrogen). RNA from fractions with spiked-in luciferase mRNA was extracted by TRIzol LS reagent (Invitrogen). Reverse transcription was performed using High Capacity cDNA Reverse Transcription Kit (Invitrogen). For HSP70-related experiments, Real-time PCR analysis was conducted with Power SYBR Green PCR Master Mix (Applied Biosystems). The reaction was performed on a LightCycler 480 Real-Time PCR System (Roche Applied Science). For luciferase *in vitro* translation and eIF3 pull-down experiments, qPCR was done using iQ SYBR Green Supermix (Bio-Rad) and an Eppendorf Mastercycler ep realplex thermocycler. Quantification for eIF3 target RNA immunoprecipitation was performed by calculating the C_t values of target RNAs in input and IP samples relative to *GAPDH* C_t values. Then IP/Input was computed for these values for each sample to determine target RNA enrichment in the IP samples. Primers used to amplify each target are as follows:

Gapdh: 5'-CAAGGAGTAAGAAACCCTGGAC-3', Fwd
5'- GGATGGAAATTGTGAGGGAGAT-3', Rev

Hsp70: 5'-TGGTGCAGTCCGACATGAAG-3', Fwd
5'-GCTGAGAGTCGTTGAAGTAGGC-3, Rev

Fluc: 5'-ATCCGGAAGCGACCAACGCC-3', Fwd
5'-GTCGGGAAGACCTGCCACGC-3', Rev

pGL4.34 Luc: 5'-TTCGTGAGCAAGAAAGGGCT-3', Fwd
5'-AGTCGTACTCGTTGAAGCCG-3', Rev

GAPDH: 5'- AAATCAAGTGGGGCGATGCT-3', Fwd
5'- CAAATGAGCCCCAGCCTTCT-3', Rev

SETD1A: 5'- AGCGGGCTATTCTCTCACTTG-3', Fwd
5'- GCTTTGCTTCTCTTCCCCGT-3', Rev

ITPRIPL2: 5'- AACACTTGAGCTGGGAGAGG-3', Fwd
5'- GAAGACGCGTAGATTGAGGGT-3', Rev

PNMA1: 5'- CTGGCTAGTCTCCCAAACGG-3', Fwd
5'- CATCTTGCGTCTGGGTCTGG-3', Rev

BMP6: 5'- GAGGGCCAGGAAGGGGAA-3', Fwd
5'- CGTGGAGCGGCGGAG-3', Rev

***In vitro* transcription**

In vitro transcription was performed using either the T7 Flash or Durascribe transcription kit (Epicentre) for making uncapped mRNAs or the mMessage mMachine SP6 transcription kit (Ambion) for making capped mRNAs. The luciferase mRNA sequence used for *in vitro* translation assays was amplified from the pGL4.34 [luc2P/SRF-RE/Hygro] vector (Promega). Unless otherwise specified, mRNAs were synthesized to contain 50% m^{6}A using *N*^{6}-methyladenosine 5' triphosphate. Synthesis of mRNAs with other modifications was performed by the addition of 50% of the indicated modified nucleotide: *N*^{1}-methyladenosine 5' triphosphate (TriLink), 2'-*O*- methyladenosine 5' triphosphate (TriLink), *N*^{6}-propargyladenosine 5' triphosphate (Jena Biosciences), 5-methyladenosine 5' triphosphate (TriLink), pseudouridine triphosphate (TriLink). *In vitro* transcription was stopped by addition of DNaseI and incubation for 20 minutes at 37°C. mRNAs were then purified using a Bio-Spin RNA purification column (BioRad). For mRNAs with a single 5' end A or m^{6}A, *in vitro* transcription was carried out using 20 mM adenosine 5'-monophosphate or *N*^{6}-methyladenosine 5'-monophosphate and 0.5 mM ATP, and using the T7 phi 2.5 promoter sequence (5'-TAATACGACTCACTATTA -3') (Huang et al., 2000). Of note, the T7 promoter incorporates both methylated (m^{6}AMP) and unmethylated (ATP) adenosines at the 5' ends of transcripts when using this promoter sequence; thus, the m^{6}A-containing reporter mRNA synthesized with this method (**Fig. 2E**) does not have m^{6}A incorporated in all transcripts. As a result, lower luciferase activity measurements are seen with this reporter than with other m^{6}A-containing reporter mRNAs in **Fig. 2**.

5'UTR sequences

The various 5'UTR sequences of the mRNAs used for *in vitro* translation assays are listed below. Although different 5'UTRs showed variability in the degree of cap-dependent translation for both methylated and unmethylated mRNAs, the presence of m^{6}A did not have a consistent enhancing or suppressing effect on this mode of translation. Conversely, the effect of m^{6}A was consistent and robust on cap-independent translation, regardless of the 5'UTR sequence used.

pGL4.34 5'UTR (Fig. 2A,C)*:*
5' GGAAGCUCGACUUCCAGCUUGGCAAUCCGGUACUGUUGGUAAAGCCACC 3'

β-globin (Fig. 2B,D,S1, Fig. 3D)*:*

5’ GACACUUGCUUUUGACACAACUGUGUUUACUUGCAAUCCCCCAAAACAGACAGA 3’

β-globin 3 As (Fig. 2D)*:*
5’ GACUCUUGCUUUUGCCUCUUCUGUGUUGCUUGACUUCCCCCUUGUUGAC 3’

β-globin 1 A 5’ end (Fig. 2D)*:*
5’ GACUCUUGCUUUUGCCUCUUCUGUGUUGCUUGCCUUCCCCCUUGUCUGC 3’

β-globin 1 A mid (Fig. 2D, Fig. S2F)*:*
5’ GGCUCUUGCUUUUGCCUCUUCUGUGUGACUUGCCUUCCCCCUUGUCUGC 3’

β-globin 1 A 3’ end (Fig. 2D)*:*
5’ GGCUCUUGCUUUUGCCUCUUCUGUGUUGCUUGCCUUCCCCCUUGUUGAC 3’

β-globin 0 As (Fig. 2D)*:*
5’ GGCUCUUGCUUUUGCCUCUUCUGUGUUGCUUGCCUUCCCCCUUGUCUGC 3’

HIST2H2BE (Fig. 2E)*:*
5’ ACUUCUUUUCUUGGCUAAGCCGCGUUUGUACUGUGUCUUACC 3’

β-globin hairpin (Fig. 3D):
5’GGGCCCCGCCCGGUGUCGGGCGGGGCCCGACACUUGCUUUUGACACAACUGUGUUU
ACUUGCAAUCCCCCAAAACAGACAGA 3’

β-globin 1 A mid UAC (Fig. S2F)*:*
5’ GGCUCUUGCUUUUGCCUCUUCUGUGUUACUUGCCUUCCCCCUUGUCUGC 3’

β-globin 1 A mid CAG Fig. S2F)*:*
5’ GGCUCUUGCUUUUGCCUCUUCUGUGUCAGUUGCCUUCCCCCUUGUCUGC 3’

The 5’UTR sequences use for *in vitro* ribosome complex assembly are as follows:

β-globin 2 AUG (Fig. 3A):

5’GACACUUGCUUUUAGAAUGGACAACUGUGUUUACUUGCAAUCCCCCAAAACAGACUGC
AUCUGUCCAGUGAGGAGAAGUCUGCGGAGAAUGG 3’

β-globin (Fig. 1A-C):
5’ GGACACUUGCUUUUGACACAACUGUGUUUACUUGCAAUCCCCCAAAACAGACAGA 3’

RNA decay analysis in HeLa lysates

[^{32}P]-CTP-labeled mRNAs encoding a short FLAG peptide were synthesized by *in vitro* transcription using the Epicentre T7 Flash kit and either 100% ATP or 50% ATP/50% N^6-meATP. mRNAs were purified using Bio-spin columns (Bio-Rad), and one microliter of mRNA was added to HeLa cell extracts (One-Step Human IVT Kit, Promega) and incubated at 30^{0}C for 30 min, followed by Trizol extraction. mRNAs were then separated on a 10% TBE-Urea gel and transferred to a nylon membrane. Radioactively labeled mRNA was detected with a phosphor screen and quantified using a PhosphoImager.

***Hsp70* pulldown and m^{6}A immunoblotting**

To isolate endogenous *Hsp70* mRNA, 400 pmol of biotin-labeled probe (5’-TTCATAACATATCTCTGTCTCTT -3') was incubated with 1 ml of M-280 Streptavidin Dynabeads (Life Technologies) in 1X B & W buffer (5 mM Tris-HCL pH 7.5, 0.5 mM EDTA and 1 M NaCl) at 4°C for 1 hr. 2 mg total RNA was denatured at 75°C for 2 min and added to the pre-treated Dynabeads for a further 2 hr incubation in 1X B & W buffer at 4°C. Captured RNA was eluted by heating beads 2 min at 90°C in 10mM EDTA with 95% formamide followed by Trizol LS isolation. Isolated RNA was quantified using NanoDrop ND-1000 UV-Vis Spectrophotometer and equal amounts were mixed with 2X RNA Loading Dye (Thermo Scientific) and denatured for 3 min at 70°C. *In vitro* transcribed mRNA containing 50% N^6-methyladenosine or 100% adenosine was used as positive and negative controls, respectively. These mRNAs were the corresponding 5’UTR sequence of mouse *Hsp70*. Samples were then run on a formaldehyde denaturing agarose gel and transferred to a positively charged nylon membrane by siphonage in transfer buffer (10 mM NaOH, 3 M NaCl) overnight at room temperature. After transfer, the membrane was washed 5 min in 2X SSC buffer and RNA was UV crosslinked to the membrane. Membrane was blocked for 1 hr in PBST containing 5% non-fat milk and 0.1 % Tween-20, followed by incubation with anti-m^{6}A antibody (1:1000 dilution) overnight at 4°C. After extensive washing with 0.1 % PBST 3 times, the membrane was

incubated with HRP-conjugated anti-rabbit IgG (1:5000 dilution) for 1 hr. Membrane was visualized by using enhanced chemiluminescence (ECL Plus, GE Healthcare).

Lentivirus knockdown and establishment of stable cell lines

The Fto knockdown cell line was established using the Lenti-X shRNA Expression System. The Fto target sequence (5'- GCTGAGGCAGTTCTGGTTTCA -3') and scramble control (5'-CCTAAGGTTAAGTCGCCCTCG -3') were inserted into the pRSI9-U6-(sh)-UbiC-TagRFP-2A-Puro empty vector (Cellecta, CA). shRNA lentivirus was packaged by Lenti-X 293T cells according to the manufacturer's instructions. MEF cells were infected by the shRNA lentivirus for 48 h before selection by puromycin at a dose of 1 µg/ml. Because the lentiviral-based overexpression often leads to a slightly stressed cellular state compared to stable cell selection, we often observe slight increases in Hsp70 levels following massive protein overexpression. Thus, there is a mild increase in the basal translation rate of Hsp70 following GFP overexpression compared to stable cells expressing a scramble control shRNA (**Fig. 6D,F**).

UV crosslinking of RNA probes

^{32}P-CTP labeled RNA probes were synthesized by *in vitro* transcription using MAXIscript® T7 Kit (Life Technologies). Probe sequences used were as follows: 5'-GGGACU$_{(14)}$-3', 5'-GGUACU$_{(14)}$-3', 5'-GGCAGU$_{(15)}$-3', 5'- GGGACU$_{(13)}$-3', and 5'-GU$_{(5)}$GGACU$_{(7)}$-3'. Probes were purified using RNA Clean & Concentrator™-5 columns (Zymo Research), and A- and m^{6}A-containing probes were ensured to have the same specific activity before performing the crosslinking assays. For identification of m^{6}A RNA binding proteins, protein was first preincubated in 1X Binding buffer (10 mM HEPES pH 7.4, 140 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 0.01% NP-40, 5% Glycerol and poly-uridylic acid (100 nM, average size 150 nt)) for 15 min at 25°C and then with either m^{6}A- or A-radiolabeled RNA probes (10^5 cpm, 1 nM) for another 15 min at 25°C. Equal amounts of protein were used for m^{6}A- or A-containing RNA reactions. Binding reactions were then irradiated twice with UV at 254 nm (150 kJ/cm^2) on a prechilled parafilm sheet on ice. Unbound RNA was digested with 50 Units of RNase If (NEB) at 37°C for 30 min. Samples were then mixed with NuPAGE LDS Sample Buffer (4X), heated at 95°C for 5 min, and electrophoresed on 4-12% NuPAGE Novex Bis-Tris Protein Gels in 1X MES-SDS buffer. To visualized proteins, gel was stained with silver using Silver stain kit (Pierce) and documented on a Biorad gel imager. Radioactive signal from protein-linked, RNase-protected RNA was captured on a phosphor screen and documented using a phosphor imager.

eIF3 protein/RNA immunoprecipitation

HEK293T cells were transfected with plasmids expressing GFP or FLAG-tagged Fto using FuGene HD (Promega). 24 hr later, cells were washed once with ice cold 1 X PBS and UV crosslinked in a Stratalinker at 254 nm (150 kJ/cm^2). Cells were then collected and spun down 500xg, 2.5min, 4°C. Pellets were collected and cells were lysed by addition of lysis buffer (50mM Tris HCl, pH 7.4, 100mM NaCl, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, Roche Protease Inhibitor added fresh) and then passed through a 21G needle ten times while maintaining the lysates on ice. 2µl of Turbo DNase and 10ul of RNase I (1:500 dilution in PBS) were then added, and lysates were incubated 3 min at 37C while shaking. Lysates were then placed back on ice and passed ten more times through a 21G needle and incubated 10 min on ice. Finally, lysates were clarified by centrifugation at 21,000xg at for 10 min. 10% of each supernatant was saved for RNA isolation from the input sample and the remainder was used for eIF3 immunoprecipitation.

For immunoprecipitation, 5 µg of rabbit anti-eIF3A antibody (Abcam) was incubated with 25 µl of Protein A/G magnetic beads (Pierce Biotechnology) for 45min at room temperature, rotating. Beads were then washed 3 times in lysis buffer and lysates were added. Immunoprecipitation was performed for 1h at room temperature and 1h at 4°C or overnight at 4°C. Beads were then washed 5 times in high salt buffer (50mM Tris HCl, pH 7.4, 1M NaCl, 1mM EDTA, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate) and RNA was eluted following 20 min incubation with Proteinase K at 37C and subsequent addition of Trizol LS for isolation of RNA. cDNA synthesis from IP and input RNA samples was performed using random hexamers and Superscript III reverse transcriptase according to the manufacturer's instructions (Life Technologies).

RNA crosslinking and immunoprecipitation

400 µg of Hela lysate (One-Step Human IVT Kit, Thermo Scientific) was crosslinked in 1X Binding buffer to A or m^{6}A RNA probes (5'-GGGACU$_{14}$-3', 3*10^5 cpm) in a 40 µl crosslinking reaction as above. Crosslinking reactions were diluted to 500 µl with ice-cold 1X PBS containing EDTA-free protease inhibitor (Roche). 2 µg of IgG control or anti-eIF3a, 3b, and ABCF1 were added and incubated at 4°C for 12 h on a shaker. Antigen-antibody complexes were bound to Protein AG beads (Thermo Scientific) and washed 3 times with WB500 buffer (20 mM HEPES pH 7.4, 500 mM NaCl, 0.1% NP-40) and 2 times with WB150 buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 0.1% NP-40) at room temperature. Beads were suspended in 1X Binding buffer

and subjected to RNase I digestion. Samples were denatured with NuPAGE® LDS Sample Buffer (4X), heated at 95°C for 5 min, and electrophoresed on 4-12% NuPAGE® Novex® Bis-Tris Protein Gels in 1X MES-SDS buffer. Proteins were transferred to pure nitrocellulose membrane in 1X transfer buffer containing 20% methanol by wet-transfer method. Membrane was washed twice in 1X TBS-T and exposed to a phosphor screen. Radioactive signal from protein-linked, RNase-protected RNA was captured on a phosphor screen and documented using a phosphor imager. Western blot analysis was performed on the same membrane for different components of the eIF3 complex and ABCF1. Antibodies used for IP and/or western blotting were: goat IgG (sc-2028, Santacruz Biotechnology), rabbit IgG (sc-2027, Santacruz Biotechnology), rabbit anti-ABCF1 (ab190798, Abcam), goat anti-eIF3a (sc-22375, Santacruz Biotechnology), goat anti-eIF3b (sc-16377, Santacruz Biotechnology), mouse anti-eIF3k (sc-393234, Santa Cruz Biotechnology). To visualize proteins, the membrane was stained with Amido black.

eIF3a/m^{6}A overlap analysis

eIF3a PAR-iCLIP site clusters were identified with Pyicoclip (Althammer et al., 2011; Raj et al., 2014) and pooled for replicate samples. Overlap between eIF3a PAR-iCLIP site clusters within 5'UTRs and m^{6}A sites within 5'UTRs was computed using a non-parametric test based on 100 random shufflings of eIF3a clusters. These random shufflings were performed within the same 5'UTR regions (as annotated using RefSeq genes) where the eIF3a PAR-iCLIP site clusters occur, so as to avoid any bias introduced by shuffling in regions outside of 5'UTRs. Single-nucleotide m^{6}A sites were obtained from Linder et al., 2015.

Distance analysis of eIF3a and m^{6}A sites

For eIF3A binding site and m^{6}A site distance plots, genomic coordinates (hg19) of single-nucleotide m^{6}A sites in 5'UTRs were expanded upstream and downstream by 200 nt and then divided into 10 nt bins. For each bin, a mean eIF3a read count was obtained which was derived from the eIF3a PAR-iCLIP clusters common to the two iCLIP biological replicates (clusters were called individually first for each replicate using pyicoclip ($p<0.0001$), and an intersection of the called clusters, representing high-confidence eIF3A RNA binding regions, was then used). The sum of mean read counts for each bin was then plotted. For the control (random As) dataset, a python script was used to generate the same number of random genomic coordinates as m^{6}A sites. These random coordinates were obtained from the same 5'UTR sequences that the m^{6}A sites were derived from. Bed files were processed using Bedtools, and the plot was plotted in R

using R Studio. Enrichment of mean read counts at 0+/-10 nt distance from m^{6}A sites was compared to the average of mean read counts generated from the randomly generated A sites (100 iterations) using a one sample t-test. Read coverage around one set of the randomly generated A sites is shown in the graph.

Comparison of eIF3 PAR-CLIP/iCLIP replicates and datasets

Correlation of mapped unique reads between the two eIF3A PAR-iCLIP replicates was determined for 25,000 randomly chosen genomic bins in 100 nt windows across the human genome. Mean read counts in RPM (reads per million uniquely mapped reads) for each window were plotted on a scatterplot for each replicate. Association between replicates was tested using Pearson's correlation test in R. Analysis of the correlation between replicates 2 and 3 (SRR1761289 and SRR1761290, respectively) of the eIF3 PAR-CLIP dataset published by Lee et al. (2015) was performed similarly, with 100 nt windowed bins including only those with RPM > 1 in order to analyze RNAs present in both replicates. Comparison of eIF3a PAR-iCLIP data to eIF3 PAR-CLIP data (Lee et al., 2015) was performed in the same manner as the comparison between the eIF3 PAR-CLIP replicates.

m^{6}A profiling and analysis

m^{6}A profiling was performed as described to detect m^{6}A sites at single nucleotide-resolution (Linder et al., 2015). Analysis of m^{6}A profiling datasets from Dominissini et al., 2012 was done using the MACS peak calling software. Annotation of bed files and metagene profiling was performed as described in Linder et al. (2015).

Gene Ontology (GO) analysis

GO functional annotation was performed using DAVID Bioinformatics Resources (Huang da et al., 2009) with a p-value threshold of 0.01. All m^{6}A-containing mRNAs were used as the background gene list.

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