SUPPLEMENTARY DATA

The novel lysine specific methyltransferase METTL21B affects mRNA translation through inducible and dynamic methylation of Lys-165 in human eukaryotic elongation factor 1 alpha (eEF1A)

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Supplementary Figures

SUPPLEMENTARY FIGURE S1. Nucleotide dependency of METTL21B-mediated methylation of eEF1A from HeLa cells. Incorporation of [³H]-methyl into eEF1A from the HeLa 0.3S fraction incubated with [³H]AdoMet, METTL21B and increasing concentration of either GTP or ATP, was assessed by fluorography (upper panel) of Ponceau S-stained membrane (lower panel). Arrows indicate the position of eEF1A and METTL21B.

SUPPLEMENTARY FIGURE S2. Control experiments related to the folding/stability of eEF1A and its potential GTP dependence. A) GTP does not influence the binding of eEF1A from HeLa extracts to recombinant eEF1B. eEF1A from HeLa cells was pulled down by glutathione-Sepharose beads, using GST-eEF1B as bait, in the absence or presence of 1 mM GTP, and bound proteins were resolved by SDS PAGE and stained with Coomassie Blue. B) Presence of GTP has a marginal effect on N6AMT2-mediated methylation of eEF1A from HeLa cells. Incorporation of [³H]-methyl into eEF1A from the HeLa 0.3S fraction incubated with [³H]AdoMet and either METTL21B or N6AMT2, in the absence or presence of 1 mM GTP, was assessed by fluorography (upper panel). C) Recombinant eEF1A1 is efficiently methylated by N6AMT2. Incorporation of [³H]-methyl into recombinant eEF1A1...
incubated with $[^3]$H]AdoMet and either METTL21B or N6AMT2, was assessed by fluorography (upper panel).

SUPPLEMENTARY FIGURE S3. Up-concentration of cellular METTL21B-GFP in centrosomes. 
A) Partial co-localization of METTL21B-GFP with eEF1A and pericentrin. Dox-induced TRex-METTL21B-GFP cells were analyzed as in Figure 8C. Selected single z-stack images of cells, presented in Figure 8C, are shown, to visualize the intracellular localization of METTL21B-GFP (green), pericentrin (white), eEF1A (red) and Hoechst-stain (blue). 
B) Partial co-localization of METTL21B-GFP with pericentrin and gamma-tubulin. Dox-induced TRex-METTL21B-GFP cells were analyzed as in Figure 8D. Selected single z-stack images of cells are shown, to visualize the intracellular localization of METTL21B-GFP (green), pericentrin (white), gamma-tubulin (red) and Hoechst-stain (blue).
SUPPLEMENTARY FIGURE S4. Intracellular localization of vimentin and ubiquitin in cells expressing METTL21B-GFP. Dox-induced TRex-METTL21B-GFP cell were fixed with formaldehyde, and stained with appropriate antibodies and Hoechst. The intracellular localization of METTL21B-GFP signal (green), pericentrin (white), Hoechst-stain (blue), and either vimentin (A) or ubiquitin (B) (red) was visualized using fluorescence microscopy. Whole-cell projection images are shown.
SUPPLEMENTARY METHODS

**eEF1A pull-down using GST-eEF1B**

The assay was performed at 4 °C. HeLa cells were lysed in Lysis Buffer 1 supplemented with protease inhibitor cocktail (P8340; Sigma-Aldrich). Cell debris was removed by centrifugation, and the cell extract was additionally pre-cleared by rotating for 1 h with 100 µl of glutathione-Sepharose 4B beads. Pre-cleared lysate was split into 2 equal parts and rotated for 2 h with 50 µl of glutathione-Sepharose 4B beads, in the presence of 20 µg of GST-eEF1B, and in the absence or presence of 1 mM GTP. Beads were washed 3 times with 1 ml Lysis Buffer 1 (also in the absence/presence of 1 mM GTP) and bound proteins were analyzed by SDS-PAGE and stained with Coomassie Blue.

**RNA extraction, cDNA synthesis and quantitative PCR**

Total RNA was extracted from BALB/c3T3 mouse fibroblasts (1x10^6 cells) by using the RNeasy mini kit (Qiagen) according to the manufacturer’s protocol and stored at -80 °C until use. cDNA was synthesized by using SuperScriptIII First Strand Synthesis System (Thermo Fisher Scientific) according to manufacturer’s protocol. Briefly, 1 µg of total RNA, 200 ng of oligo(dT)18, and 50 U of reverse transcriptase was incubated in a total reaction volume of 20 µl at 42°C for 50 minutes. The reaction was then terminated by incubation at 70 °C for 15 minutes, and treated with 1 U of RNaseH (Invitrogen) to digest the RNA. For reverse transcriptase quantitative PCR (RT-qPCR), Brilliant III Ultra-Fast SYBR Green qPCR master mix (Agilent Technologies) was used, together with a RocheLightCycler 96. Twenty five microliters reactions were performed with 400 nM of each exon specific primer and reaction condition given in Supplementary Table S1. All the samples were run in triplicates using the following sequence of thermal cycling conditions, 95 °C for 180 s, 40 cycles of 95 °C for 5 s, 60 °C for 10 s and 65 °C for 5 s. To control for RNA input, GAPDH was selected as a reference gene, since this gene has been reported to show stable expression under various conditions in fibroblasts (1). Target mRNA expression was calculated by using the 2^-ΔΔCT method (2). The exon specific primers used in this paper were designed by using the GenScript primer design tool and are listed in Supplementary Table S2. MIQE standards were applied to our protocols (3).

The METTL21B (wild-type) mRNA expression in the complemented METTL21B-KO cells was confirmed by reverse transcription PCR, using primers Fwd2 and Rev2, and compared to GAPDH expression, using primers Fwd3 and Rev3 (see Supplementary Table S3). PCR reactions were run with Phusion DNA Polymerase HF and 1.5% DMSO, with the following sequence of thermal cycling conditions, 98°C for 30 s, 23-30 cycles of 98°C for 10 s, 68°C for 5 s and 72°C for 5 s.
**Supplementary Table S1:**

| Component                                                                 | Volume (µl) |
|---------------------------------------------------------------------------|-------------|
| Brilliant III Ultra-Fast SYBR Green qPCR master mix                       | 12.5        |
| Forward primer (10µM)                                                    | 1           |
| Reverse primer (10µM)                                                    | 1           |
| cDNA                                                                      | 1           |
| PCR grade water                                                           | 9.5         |
| **Total Volume**                                                          | **25**      |

96 well lightcycler plates (Sarstedt) were used

**Supplementary Table S2:**

Mouse primer sequences used for RT-qPCR:

| Primer name | Primer sequence | Ensembl gene ID | Ensembl ID of target exon | Amplicon length (bp) |
|-------------|-----------------|-----------------|----------------------------|----------------------|
| METTL21B Fwd | ATCCGGCTCTTCACAGACTC | ENSMUSG000000080115 | ENSMUSE0000705304 | 127                  |
| METTL21B Rev | TCAGAGCCGCATCCCACACGCG | | | ENSMUSE0000705303 |
| GAPDH Fwd   | ACAACTTTGGCATTGTGGAA | ENSMUSG00000007666 | ENSMUSE00001237509 | 133                  |
| GAPDH Rev   | GATGCAGGGGATGTGTCTG | | | ENSMUSE0000472146 |

**Supplementary Table S3:**

Human primer sequences used for RT-PCR in Figures 5A, B:

| Primer name | Primer sequence |
|-------------|-----------------|
| Fwd1        | ATGTGCTGACCATCAGCAGAA |
| Rev1        | GCCAGGGGCAGTCATGATG |
| Fwd2        | TGTCGGACGCGGCCCTGAG |
| Rev2        | TGCCAAGATCCCCACGATGCC |
| Fwd3        | GTATTGGGCGCCTGGTCACCAGGCTG |
| Rev3        | CATGACGAACATGGGGGCAATCAGCAGA |
References

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2. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. *Methods*, **25**, 402-408.

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