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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	To analyze METTL3 or eIF3h expression level among TCGA tumors, RNA-Seq data for TCGA tumor types were downloaded from Genomic Data Commons Data Portal (GDC) of TCGA (http://cancergenome.nih.gov/) using R package TCGAbiolinks.
Data analysis	For high throughput sequencing data analysis, reads were aligned against the human hg19 (GRCh37) reference genome using Tophat2, rMATS v3.2.5 was used to detect the splicing events and significant splicing differences between METTL3 knockdown and control samples; MACS2.1.0 was used for the peak calling in m6A peak identification; mRNA half-life was calculated using the RPKM generated by HTSeq.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The m6A MeRIP-Seq and RNA-seq data have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE117299. Figures 2b, 2c, 2g, 4j, 4k, ED5b, ED6b, ED8j, ED8k, ED9b and ED9c have associated raw data.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For IHC staining, 75 lung adenocarcinoma and the adjacent control samples were used. For the mouse study, 5 mice (A549 cells) or 8 mice (NIH-3T3 cells) were used for each group. Based on Student T test, for comparison between two groups, a sample size of 5 is effencient to calculate the statistical significance.
Data exclusions	In Figure ED3d-f, we performed Mass spectrometry of FLAG-METTL3 containing complexes and found numerous translation factors including Ribosomal proteins and translation initiation factors. By doing independent IP, we confirmed several translation factors and ribosomal proteins. However, repeated data are already presented in our previous study (Lin et al., Mol Cell 2016) and current study. Therefore we decided to exclude the Western blot data of FLAG-METTL3 co-IP.
Replication	In the Figure legend, we stated how many number of replications were performed. Where it is applicable, Shapiro-Wilk test and Q-Q plotting (quantile-quantile plot,) were performed prior to Student's two-tailed t-test to assess if the data plausibly came from a normal distribution. Where indicated, Wilcoxon signed-rank test was used. All attempts at replication were successful.
Randomization	The mice were randomly divided into three groups for the xenograft study to determine the oncogenic role of METTL3 in vivo.
Blinding	The investigators were blinded to group allocation in the IHC staining.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	All antibodies used in the study are listed in Methods. Here we state in order: Antibody name; (Company; Catalogue number; Source; Reactivity ; Dilution) METTL3 (Proteintech; 15073-1-AP; Rabbit; Human, Mouse, Rat; 1:1000), (Abcam; ab195352; Rabbit; Mouse, Rat, Human; 1:1000) β-actin (Abcam; ab8227; Rabbit; Mouse, Rat, Sheep, Rabbit, Chicken, Guinea pig, Cow, Dog, Human, Pig, Xenopus laevis, Drosophila melanogaster, Fish, Monkey, Zebrafish, Rhesus monkey, Chinese hamster; 1:5000)

eIF3h (Abcam; ab60942; Mouse; Human; 1:1000)
CBP80 (Homemade; Rabbit; 1:5000)
CTIF (Homemade; Rabbit; 1:1000)
eIF4E (Cell Signaling Technology; #2067; Rabbit; Human, Mouse, Rat, Monkey; 1:1000)
eIF3b (Santa Cruz Biotechnology; sc-16377; Goat; Mouse, Rat, Human; 1:1000)
eIF4GI (Cell Signaling Technology; #2498; Rabbit; Human, Mouse, Rat, Monkey; 1:1000)
FLAG (Sigma; A8592; Mouse; FLAG-fusion protein; 1:5000)
BRD4 (Abcam; ab128874; Rabbit; Mouse, Rat, Human, 1:1000), (Cell signaling; #13440; Rabbit; Human; 1:1000)
CD9 (Cell Signaling Technology; #13174; Rabbit; Human; 1:1000)
MGMT (Cell Signaling Technology; #2739; Rabbit; Human, Monkey; 1:1000)
TIMP1 (Cell Signaling Technology; #8946; Rabbit; Human, Monkey; 1:1000)
FTO (Phosphosolution; 597-FTO; Moue; Rat, Mouse, Human; 1:1000)
Anti-Flag M2 Affinity Gel (Sigma-Aldrich; A2220; Mouse; FLAG-fusion protein; 1:200)
For PLA, Duolink® In Situ Red Starter Kit Mouse/Rabbit (Sigma-Aldrich; DUO92101; Mouse/Rabbit; Followed manufacturer's instruction)

Validation

The antibodies that have been validated by the supplyers for specific purposes (for example, western blot) were purchased for our experiment. In addition, for key essential antibodies such as METTL3, eIF3h antibodies, we further validated the antibodies by western blotting upon depletion of the proteins. For CBP80 and CTIF homemade antibodies, validation was performed in Choe et al., THE JOURNAL OF BIOLOGICAL CHEMISTRY 2012; VOL. 287, NO. 22, pp. 18500–18509.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

All cell lines used in this study, including human lung cancer cell lines (A549 and H1299), HEK293T, BJ, NIH-3T3, HeLa and MEFs were purchased from ATCC.

Authentication

Cell lines were authenticated with morphology, karyotyping, and PCR based approaches by ATCC

Mycoplasma contamination

The cell lines were tested for potential mycoplasma contamination and confirmed that they are mycoplasma negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

All research involving animals was complied with protocols approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee. 4-6 weeks old female NU/J (Nude) immunodeficient mice (Jackson Laboratory #002019) were used for subcutaneous injections.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Lung cancer tumor samples were isolated from four patients (Patient 1: Female, Age 73 mixed subtype, with acinar (10%), papillary (80%),and bronchioloalveolar (10%) patterns, moderately differentiated, 5.1cm; Patient 2: Male, Age 80, mixed subtype with 80% papillary, 14% acinar, 5% bronchioloalveolar, and 1% solid patterns, moderately differentiated, 8cm. Patient 3: Male, Age 79 with acinar (20%), papillary (60%), andbronchioloalveolar (20%) patterns, moderately differentiated, 5.0cm; Patient 4: Female, age 61, lung adenocarcinoma, micropapillary predominant, with solid, acinar and papillary areas, poorly differentiated, 10.5 cm).

Recruitment

lung adenocarcima samples from four patients (two males and two females) were used for the profiling of m6A modification in primary tumor tissues. Since this is a pilot study of m6A in primary lung cancer samples, we selected the patients with large size tumors so that we can get enough RNA samples for m6A profiling, there is no potential self-selection bias.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	METTL3 knockdown and control A549 cells were treated with 500nM JQ1 and then analyzed by Annexin V/PI staining using FACS.
Instrument	BD LSR II
Software	BD FACSDiva Software.
Cell population abundance	No sorting in this study.
Gating strategy	Gating was performed using three controls: Annexin V-/PI-, Annexin V+/PI-, Annexin V-/PI+.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.