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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.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒	A statement on 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 statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Deep sequencing data were collected using Illumina NextSeq Control Software 2.1.0.31 and Illumina RTA 2.4.11 & bcl2fastq 2.18 software. Confocal images were obtained using Zeiss LSM 710 Zen 2012 Black Edition Service pack 5 imaging software. hPSCs were sorted using Weasel version 3.0.2 (Walter and Eliza Hall Institute for Medical Research) and Beckman Coulter Kaluza Analyzis 2.1 software.
Data analysis	Deep sequencing data were processed using open source software tools. The following packages were used: Samtools version 1.3.1, Picard Tools Dedup version 2.5.0, MACS2.1.1, PhantomPeakQualTools version - Feb 13, 2012, Bedtools version 2.27.1, tximport, R 3.6, Bowtie2, IGV 2.4.0, Skewer version 0.2.2, BWA version 0.7.15, Deeptools version 2.5.7, awk version 4.0.2, Hisat2 version 2.1.0, StringTie version 1.2.3, Ballgown version 2.18.0, ChIPQC version 1.18.2, ggplot2 version 3.2.0, GenomicFeatures version 1.34.8, DESeq version 1.34.1, AnnotationDbi version 1.44.0, DiffBind version 2.10.0, Biobase version 2.42.0, GenomicRanges version 1.34.0, GenomeInfoDb version 1.18.2, BioVenn. The full pipeline including in-house scripts used for the analysis can be found in the following online repository (https://bitbucket.org/ADAC_UoN/adac0175-code/ Confocal Images were processed using Fiji Image J, Adobe Photoshop CS6 Version 13.0 x32, and Zeiss LSM 710 Zen 2012 Black Edition Service pack 5 imaging software. FACS data were analysed using Weasel V3.0.2 (Walter and Eliza Hall Institute for Medical Research) and Beckman Coulter Kaluza Analyzis 2.1 software. Signal intensity and qPCR data were plotted using GraphPad Prism 7.04.

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. 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 deep sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) with the Bioproject ID: PRJNA474076 (https://submit.ncbi.nlm.nih.gov/subs/sra/SUB4074125). The annotated bed files have been deposited to the following online repository (https://bitbucket.org/ADAC_UoN/adac1075-bed-files/src). The in-house scripts used for the analysis can be found in the following online repository (https://bitbucket.org/ADAC_UoN/adac0175-code/src).

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Samples size was chosen to ensure reproducibility of the results at affordable costs. Geneally, sample size was determined based on previosuly published studies and on experience and was equivalent to that is routinely used for any particular assay. Sample sizes are indicated separately for different experiments. At least 2 and typically 3 independent experiments were carried out for most of the assays. DRIP and DIP were performed in two and RNaseq in three biologically independent experiments. We observed generally good correlation between the replicates for different experiments suggesting that choosen samples size was sufficient. No statistical methods were used to predetermine sample sizes.
Data exclusions	In case of experimental mistakes or occasional loss of samples/reagents, the experiment was discarded and repeated. Otherwise, no data were excluded from the analysis.
Replication	All experiments were replicated independently. All attempts at replication were successful.
Randomization	No randomization was required as the study was based on molecular and cellular biology techniques and did not involve allocation of experimental units across different treatment groups, no human subjects were involved.
Blinding	Blinding was not relevant to the study as the study was based on molecular and cellular biology techniques, the results were derived from objective quantitative methods. No subjective measurements were taken.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

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

Methods

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

Antibodies

Antibodies used	Anti-m6A rabbit polyclonal (Synaptic systems, catalogue number 202003, Lot number 2-92, 1:200 dilution), anti-m1A mouse monoclonal (Diagenode catalogue number C15200235, 1:500 dilution), anti-5-methylcytosine (5mC) mouse monoclonal (clone 33D3, Diagenode, catalogue number C15200081-100, Lot number RD-002, 1:100 dilution), anti-phospho-Histone H3 (Ser10) mouse monoclonal (6G3, Cell Signalling, catalogue number 9706, Lot number 05636 JBW 301, 1:200 dilution), anti-YTHDF1 rabbit polyclonal (ProteinTech, catalogue number 17479-1-AP, Lot number 00040713, 1:500 dilution), anti-YTHDF2 rabbit

polyclonal (ProteinTech, catalogue number 24744-1-AP, Lot number 00052442, 1:500 dilution), anti-HNRNPA2B1 mouse monoclonal (Novus, catalogue number NB120-6102SS, clone number DP3B3, 1:200 dilution) and anti-γH2AX mouse monoclonal (Merck, catalogue number 05-636, clone JBW301, 1:200 dilution), Anti-DNA-RNA Hybrid S9.6 antibody (Merck Millipore, clone S9.6, catalogue number MABE1095, Lot numbers 3152139, 3172456, 1:200 dilution) primary antibodies, peroxidase-conjugated anti-rabbit secondary antibody (Thermofisher, catalogue number 65-6120, Lot number RL242746A, 1:400 dilution) and alexafluor 633-conjugated secondary antibody (Thermofisher, catalogue number A-21052, lot number 1848436, 1:400 dilution) were used for immunochemistry.
The western blots of RNA:DNA hybrid IP samples were probed with the following antibodies: Top1 (Abcam, catalogue number ab109374, Lot number GR49853-20, clone number EPR5375, dilution 1:2000), YTHDF1 (ProteinTech, catalogue number 17479-1-AP, Lot number 00040713, dilution 1:1000), YTHDF2 (ProteinTech, catalogue number 24744-1-AP, Lot number 00052442, dilution 1:500), METTL3 rabbit polyclonal (Bethyl Laboratories, catalogue number A301-567A, Lot number A301-567A-2, dilution 1:2000), HNRNPA2B1 (Novus, catalogue number NB120-6102SS, clone number DP3B3, dilution 1:500) and Lamin B1 rabbit polyclonal (Abcam, catalogue number ab16048, dilution 1:2000).
S9.6 antibody (Merck Millipore, catalogue number MABE1095, 5-10 µg/experimental point) and anti-m6A rabbit polyclonal antibody (Synaptic systems, catalogue number 202003, 6 µg/experimental point) were used for DRIP and DIP.

Validation

Anti-m6A rabbit polyclonal antibody (Synaptic systems, catalogue number 202003) has been validated for immunostaining on human cells in multiple studies (https://www.sysy.com/products/m6a/facts-202003.php; Xiang Y, et al. Nature (2017) 5437646: 573-576; Sobecki M, et al. Cell reports (2018) 2510: 2891-2903.e5.) and for DIP and immunocytochemistry in the current study (data provided in the manuscript).
Anti-5-methylcytosine (5mC) antibody (clone 33D3, Diagenode, catalogue number C15200081-100) is widely used in immunostaining experiments (https://www.diagenode.com/en/p/5-mc-monoclonal-antibody-33d3-premium-100-ug-50-ul) and was validated for immunocytochemistry on human cells by the vendor and in other studies (e.g. Wheldon, L. M. et al. (2014) Cell Rep. 7, 1353-1361; Abakir, A., et al. (2016) J Vis Exp. 114).
Anti-phospho-Histone H3 antibody (6G3, Cell Signalling, catalogue number 9706) is routinely used for immunostaining (https://www.cellsignal.co.uk/products/primary-antibodies/phospho-histone-h3-ser10-6g3-mouse-mab/9706) and was validated on human cells (Li J, et al. (2019) Cell Div. 14:5; Gookin S et al, (2017) PLoS Biol. 15(9):e2003268.).
Anti-m1A antibody (Diagenode catalogue number C15200235) has been validated for immunostaining on human (HeLa) cells by the vendor (https://www.diagenode.com/en/p/1-methyladenosine-monoclonal-antibody).
Anti-YTHDF1 (ProteinTech, catalogue number 17479-1-AP), anti-YTHDF2 (ProteinTech, catalogue number 24744-1-AP) antibodies have been validated for immunostaining and western blot on human cells by the vendor and in a number of previous studies (https://www.ptglab.com/products/YTHDF1-Antibody-17479-1-AP.htm#validation, Hesser CR, Karijolich J, Dominissini D, He C, Glaunsinger BA. (2018) PLoS Pathog. 14(4):e1006995, https://www.ptglab.com/products/YTHDF2-Antibody-24744-1-AP.htm#validation; Hao H, et al. (2019) Nucleic Acids Res. 47(1):362-374) and for ChIP in the present study (data provided in the manuscript).
anti-HNRNPA2B1 (Novus, catalogue number NB120-6102SS) antibody has been validated for immunostaining and western blot on human cells by the vendor (https://www.novusbio.com/products/hnrnp-a2b1-antibody-dp3b3_nb120-6102#reviews-publications) and for ChIP in the present study (data provided in the manuscript).
Anti-γH2AX antibody (Merck, catalogue number 05-636, clone JBW301) has been validated for immunostaining and ChIP on human and mouse cells/tissues by the vendor and in a number of studies (http://www.merckmillipore.com/GB/en/product/Anti-phospho-Histone-H2A.X-Ser139-Antibody-clone-JBW301,MM_NF-05-636#documentation; Sato S, et al. (201%) Nat Commun. 2015 6:7035. doi: 10.1038/ncomms8035; Kanu N, et al. (2015) Oncogene. 34(46):5699-708; Abdou I, Poirier GG, Hendzel MJ, Weinfeld M. (2015) Nucleic Acids Res. 2015 43(2):875-92).
S9.6 antibody (Merck Millipore, catalogue number MABE1095) is widely used for DRIP (Boguslawski, S.J., et al. (1986). J. Immunol Methods. 89(1):123-130, El Hage, A., et al. (2014). PLoS Genet. 10(10):e1004716). It was previsously validated for DRIP and DRIPseq on a number of species including human (Skourti-Stathaki, K., et al. (2011). Mol. Cell. 42(6):794-805; Ginno, P.A., et al. (2012). Mol. Cell. 45(6):814-825; Bhatia, V., et al. (2014). Nature. 511(7509):362-365; Cristini A, et al. (2019) Cell Rep. 28(12):3167-3181), for RNA:DNA hybrids and protein co-immunoprecipitation on human cells (Cristini, A., et al. (2018) Cell Rep. 23, 1891-1905) and for immunocytochemistry on human and mouse cells (Bhatia, V., et al. (2014). Nature. 511(7509):362-365; Ginno, P.A., et al. (2012). Mol. Cell. 45(6):814-825).
Top1 (Abcam, catalogue number ab109374), METTL3 (Bethyl Laboratories, catalogue number A301-567A) and Lamin B1 (Abcam, catalogue number ab16048) antibodies have been validated for Western blot on human and mouse cells previously (https://www.abcam.com/topoisomerase-i-antibody-epr5375-ab109374-references.html#top-200; Cristini A, et al. (2016) Nucleic Acids Res. 44(3):1161-78; e6; Cristini, A., et al. (2018) Cell Rep. 23, 1891-1905; https://www.bethyl.com/product/A301-567A/METTL3+MT-A70+Antibody; Takata MA, et al. (2017) Nature. 550(7674):124-127; https://www.abcam.com/lamin-b1-antibody-nuclear-envelope-marker-ab16048-references.html#top-500; Du Q, et al. (2019) Nat Commun. 10(1):416; Vadnais C, et al. (2018) Nat Commun. 9(1):1418; Dingar D, et al. (2018) Nat Commun. 9(1):3502) and used in a number of studies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

REBL-PAT hiPSCs, source: Chris Denning's lab, University of Nottingham, described in Mosqueira D, et al. Eur Heart J. 43, 3879-3892 (2018).
HUES7 hESC, source: Chris Denning's lab, University of Nottingham, derived in DA Melton's lab, described in Cowan CA, et al. N Engl J Med. 350, 1353-1356 (2004).
U87MG, LN-18 and HeLa cell lines, source: ATCC.
YTHDF2 KO HAP1 cells, source: Horizon Discovery (catalogue number HZGHC006678c001).
Wild type parental HAP1 cells, source: Horizon Discovery.

Authentication

REBL-PAT hiPSCs and HUES7 hESC were authenticated by karyotyping, gene expression profiling (using qPCR and immunochemistry), phenotypic assays and differentiation into 3 germ layers.
U87MG, LN-18 and HeLa cell lines were authenticated by vendor.
HeLa cells were authenticated by short tandem repeat (STR) analysis by the vendor.

	U87MG and LN-18 were authenticated by gene expression (using qPCR and immunostaining) and phenotypic profiling. YTHDF2 KO HAP1 cells were authenticated by immunostaining, PCR and sequencing. Both YTHDF2 KO and parental HAP1 cells were also authenticated by vendor.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	C57BL/6 wild-type and YTHDF2 targeted mouse embryos at embryonic day 14.5 were used. Sex of the embryos was not determined.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All mouse experiments were approved by the Norwegian Animal Research Authority by Norwegian Food Safety Authority and done in accordance with institutional guidelines at the Centre for Comparative Medicine at Oslo University Hospital. Animal work was conducted in accordance with the rules and regulations of the Federation of European Laboratory Animal Science Association's (FELASA).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	Reads are submitted to SRA (SUB4074125): Bioproject PRJNA474076 Bed files are available at: https://bitbucket.org/ADAC_UoN/adac1075-bed-files/src The datasets can be accessed at: https://www.ncbi.nlm.nih.gov/account/?back_url=https%3A%2F%2Fdataview.ncbi.nlm.nih.gov with user = thomgiles; pass= n0078022
Files in database submission	DRIP/DIP: K1_6mA_R2.fastq.gz (fastq) K1_6mA_R1.fastq.gz (fastq) K2_6mA_R2.fastq.gz (fastq) K2_6mA_R1.fastq.gz (fastq) K3_6mA_R2.fastq.gz (fastq) K3_6mA_R1.fastq.gz (fastq) K4_6mA_R2.fastq.gz (fastq) K4_6mA_R1.fastq.gz (fastq) K3_DNA-RNA_R2.fastq.gz (fastq) K3_DNA-RNA_R1.fastq.gz (fastq) K4_DNA-RNA_R2.fastq.gz (fastq) K4_DNA-RNA_R1.fastq.gz (fastq) K1_DNA-RNA_R2.fastq.gz (fastq) K1_DNA-RNA_R1.fastq.gz (fastq) K2_DNA-RNA_R2.fastq.gz (fastq) K2_DNA-RNA_R1.fastq.gz (fastq) Input_R2.fastq.gz (fastq) Input_R1.fastq.gz (fastq) SAMN12283116/ IGG1 IGG1.R1.fastq.gz IGG1.R2.fastq.gz SAMN12283117 / IGG2 IGG2.R1.fastq.gz IGG2.R2.fastq.gz SAMN12283118 / SiM3-S1 SiM3-S1.R1.fastq.gz SiM3-S1.R2.fastq.gz SAMN12283119 / SiM3-S2 SiM3-S2.R1.fastq.gz SiM3-S2.R2.fastq.gz RNAseq: E8_48.R2.fastq.gz (fastq) E8_48.R1.fastq.gz (fastq) E8_48_2.R2.fastq.gz (fastq) E8_48_2.R1.fastq.gz (fastq) E8_72.R2.fastq.gz (fastq) E8_72.R1.fastq.gz (fastq) E8_72_2.R2.fastq.gz (fastq)

E8_72_2.R1.fastq.gz (fastq)
SAMN12283120 / siCTL-S1-RNA siCTL-S1-RNA.R1.fastq.gz siCTL-S1-RNA.R2.fastq.gz
SAMN12283121 / siCTL-S2-RNA siCTL-S2-RNA.R1.fastq.gz siCTL-S2-RNA.R2.fastq.gz
SAMN12283122 / siCTL-S3-RNA siCTL-S3-RNA.R1.fastq.gz siCTL-S3-RNA.R2.fastq.gz
SAMN12283123 / SiM3-S1-RNA SiM3-S1-RNA.R1.fastq.gz SiM3-S1-RNA.R2.fastq.gz
SAMN12283124 / SiM3-S2-RNA SiM3-S2-RNA.R1.fastq.gz SiM3-S2-RNA.R2.fastq.gz
SAMN12283125 / SiM3-S3-RNA SiM3-S3-RNA.R1.fastq.gz SiM3-S3-RNA.R2.fastq.gz

Bed files:
K2 S9.6 DRIP RNase H.bed
K1 S9.6 DRIP RNase H.bed
K3 S9.6 DRIP.bed
K4 S9.6 DRIP.bed
K3 m6A DIP RNase H.bed
K4 m6A DIP RNase H.bed
K1 m6A DIP.bed
K2 m6A DIP.bed
SiM3-3.bed
SiM3-4.bed
SiM3.consensus.bed

Genome browser session
(e.g. UCSC)

As Ensembl (GRCh38.90) genome version was used for mapping and the downstream visualization analysis was performed using IGV, no web genome browser is available but an IGV session can be made available upon request.
The bed files have been deposited to the following online repository (https://bitbucket.org/ADAC_UoN/adac1075-bed-files/src).

Methodology

Replicates

2 Biological replicates were used for each DRIP experiment. 4 or 3 Biological replicates were used for RNAseq experiments. Replicates agreement is:

DRIPseq:
K2 S9.6 DRIP RNase H, Replicate 1
K1 S9.6 DRIP RNase H, Replicate 2
K3 S9.6 DRIP, Replicate 1
K4 S9.6 DRIP, Replicate 2
K3 m6A DIP RNase H, Replicate 1
K4 m6A DIP RNase H, Replicate 2
K1 m6A DIP, Replicate 1
K2 m6A DIP, Replicate 2
SiM3.S1 m6A DIP Replicate 1
SiM3.S2 m6A DIP Replicate 2

RNAseq:

Wild type hPSCs:
Replicate 1 E8_48.
Replicate 2 E8_48_2.
Replicate 3 E8_72.
Replicate 4 E8_72_2.

siCTL hPSCs:
SAMN12283120 / siCTL-S1-RNA siCTL-S1-RNA.R1.fastq.gz siCTL-S1-RNA.R2.fastq.gz
SAMN12283121 / siCTL-S2-RNA siCTL-S2-RNA.R1.fastq.gz siCTL-S2-RNA.R2.fastq.gz
SAMN12283122 / siCTL-S3-RNA siCTL-S3-RNA.R1.fastq.gz siCTL-S3-RNA.R2.fastq.gz

siMETTL3 hPSc:
SAMN12283123 / SiM3-S1-RNA SiM3-S1-RNA.R1.fastq.gz SiM3-S1-RNA.R2.fastq.gz
SAMN12283124 / SiM3-S2-RNA SiM3-S2-RNA.R1.fastq.gz SiM3-S2-RNA.R2.fastq.gz
SAMN12283125 / SiM3-S3-RNA SiM3-S3-RNA.R1.fastq.gz SiM3-S3-RNA.R2.fastq.gz

Sequencing depth

Paired end 150 bp Illumina sequencing, >30x depth in all DRIP-seq samples.

Sample	Total reads	Uniquely mapped reads
m6A DIP, Replicate 1	49842240	48015356
m6A DIP, Replicate 2	56332128	54533737
S9.6 DRIP, Replicate 1	72691406	41780898
S9.6 DRIP, Replicate 2	79614278	49333291
m6A DIP RNase H, Replicate 1	79112558	76866727
m6A DIP RNase H, Replicate 2	87247890	82861485
S9.6 DRIP RNase H, Replicate 1	89788100	82922838

S9.6 DRIP RNase H, Replicate 2	84008987	78787622
Input	80873230	79003776
m6A DIP siMETTL3 Replicate 1	86122630	85691828
m6A DIP siMETTL3 Replicate 2	102058012	101591246
IGG control 1	17485250	17295879
IGG control 2	15556552	15347398

No BAM alignments are present for the RNAseq because a pseudo-alignment method (Salmon) was used to quantify transcripts.

RNAseq sample	Total reads	Mapping rate
Replicate 1	90100862	61.9419 %
Replicate 2	101043100	59.7604 %
Replicate 3	92569432	59.9858 %
Replicate 4	78634548	61.3253 %

	Total reads	Mapped reads
siCTL Replicate 1	62962212	62960691
siCTL Replicate 2	65937856	65936349
siCTL Replicate 3	63894368	63892770
siMETTL3 Replicate 1	67781652	67763727
siMETTL3 Replicate 2	71526676	71487072
siMETTL3 Replicate 3	61918065	61884273

Antibodies

S9.6 antibody (Merck Millipore, catalogue number MABE1095) and anti-m6A rabbit polyclonal antibody (Synaptic systems, catalogue number 202003) were used for DRIP/DIP-seq and DRIP/DIP-qPCR.

Peak calling parameters

Peaks were called according to the recommended default MACS2 parameters described here:
https://github.com/taoliu/MACS/blob/master/README.md
The code is available online at https://bitbucket.org/ADAC_UoN/adac0175-code/src

Data quality

Reads quality was assessed directly by the sequencing center using fastQC, the impact of each of the pull-downs on read distribution was assessed using Phantompeakqualtools and ChipQC was employed for assessing the peaks' genomic distribution and the quality of the data. Consensus peaks were defined using the dba.peakset() function to select for peaks overlapping in both replicates. Peaks were called with a q value of 0.01. The numbers of narrow peaks were as follows:

m6A DIP Replicate 1, 58120;
m6A DIP Replicate 2, 26699;
m6A DIP RNase H Replicate 1, 1742;
m6A DIP RNase H Replicate 2, 1103;
S9.6 DRIP Replicate 1, 11236;
S9.6 DRIP Replicate 2, 8691;
S9.6 DRIP RNase H Replicate 1, 506;
S9.6 DRIP RNase H Replicate 2, 483.
m6A DIP siMETTL3 Replicate 1, 381150
m6A DIP siMETTL3 Replicate 2, 133698

Software

Deep sequencing data were collected using Illumina NextSeq Control Software 2.1.0.31 and Illumina RTA 2.4.11 & bcl2fastq 2.18 software. The 150 bp Illumina paired end reads were trimmed using Skewer to remove low quality sequences. Reads that passed filtering were aligned to the human Ensembl genome (build hg38.89) using BWA with default parameters. As each biological sample was split across multiple lanes of sequencing, the corresponding alignments were merged with Samtools and de-duplicated to remove PCR artefacts with picard-tools MarkDuplicates. The highly modified regions (HMRs, peaks) were identified using MACS2.1.1. High confidence consensus peaks were identified using the bioconductor package DiffBind. In each instance the replicate sample BAM/bed files along with the corresponding input samples were used as input. The Ensembl Genome GTF file (Homo_sapiens.GRCh38.89.gtf) was downloaded and processed to extract gene biotypes, annotated gene features, introns, intergenic regions and sequences 3kb up/downstream of genes. The Repeatmasker Library (db20140131) was downloaded from UCSC, converted to the ensembl gene build and processed to identify repeat masked regions. The Ensembl regulatory build (HgGRCh38.Regulatory_Build.regulatory_features.20161111) was downloaded and processed to identify regulatory features. The consensus and DMR bedfiles were annotated with this list of gene features, gene biotypes, repeat-masked sequences and regulatory regions using BedTools intersect.

The code is available Online at https://bitbucket.org/ADAC_UoN/adac0175-code/src

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	G0/G1, S and G2/M phases flow cytometry sorting was performed according to the previously described method (Pozarowski, P., Darzynkiewicz, Z. Analysis of cell cycle by flow cytometry. Methods Mol Biol. 281, 301-311 (2004)). Briefly, enzymatically dissociated hPSCs were washed in PBS and fixed in 70 % ethanol for 2 h, washed with PBS again and stained with 10 µg/ml propidium iodide (PI) (Sigma-Aldrich, catalogue number P3566) in PBS supplemented with 0.1 % Triton X-100 and 100 µg/ml RNase A (Qiagen, catalogue number 19101). PI treated hPSCs were sorted based on the DNA content into G0/G1, S and G2/M cells using Beckman Coulter Astrios EQ and Beckman Coulter Kaluza Analysis 2.1 software.
Instrument	Beckman Coulter Astrios EQ, serial number AW24025
Software	Weasel V3.0.2 (Walter and Eliza Hall Institute for Medical Research), Beckman Coulter Kaluza Analyzis 2.1
Cell population abundance	2,000,000 sorted cells were used for each experimental point. The FACS-sorted cell populations were 98 % pure. The purity was confirmed by re-sorting of the obtained cell populations following PI treatment.
Gating strategy	Cells were gated using a forward scatter vs side scatter plot to exclude any debris and apoptotic cells. Single cells were then gated for plotting the side scatter against area. From there G0, S phase and G2/M cell populations were gated on the PI (488-620/29) plot, with G2 showing a peak at double the fluorescence intensity of G0. Please see Methods and Supplementary Figures.

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