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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

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For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   No power calculations were performed. Sample size was determined depending on the experiment type based on what is standard practice in the field of pluripotent stem cell biology to statically examine a large effect within an in vitro system which experiences only limited biological variability (n=2-4, see Figure Legends).

2. Data exclusions

   Describe any data exclusions.

   No data points were excluded from any of the analyses presented.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   All of the presented experiments were successfully reproduced.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   No randomization was performed since this was not relevant to the study: all treatment/control experiments were performed on the same starting cell population (no covariates).

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   No blinding was performed. This was deemed unnecessary since none of the analyses reported involved procedures that could be influenced by investigator bias (such as manual counting/measuring and/or morphological assessments). Indeed, all analyses presented involved automated processing of data through experimental instrumentation and/or computing procedures (including counting of PLA signals presented in Fig. 2d, see the Methods)

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	All of the software used for the analyses presented is described in detail in the relevant Methods, which also describes the relevant parameters used when these were not the default. The software used was: MaxQuant, Perseus, Cytoscape, Enrichr, ImageJ, R/Bioconductor, Trimmomatic, TopHat 2.0.13, MetDiff, fCCAC v1.0.0, RSeQC-2.6, RCAS, DREME, DESeq2, Cufflinks, GraphPad Prism 6, Sickle, Samtools view, SeqMonk, timecourse (Bioconductor). All bioinformatic scripts have been deposited to GitHub (http://github.com/pmb59/neMeRIP-seq).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	All unique materials (cell lines and plasmids) are readily available from the authors

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	All of the antibodies used are detailed in Supplementary Table 5, which also reports the application for which they were used and the amount/dilution. The list includes: anti-CREBBP (Cell Signalling 7389); anti-EP300 (Santa Cruz sc-584); anti-FOXH1 (R&D BAF4248); anti-METTL14 (Sigma-Aldrich HPA038002); anti-METTL3 (Proteintech 15073-1-AP, and Bethyl Lab A301-567A); anti-NANOG (R&D AF1997, and Abcam ab21624); anti-OCT4/POU5F1 (Santa Cruz sc-5279); anti-SETDB1 (Cell Signalling 2196); anti-SMAD2/3 (R&D AF3797, and Cell Signalling 12470S); anti-SNON/SKIL (Santa Cruz sc-9595x); anti-SOX1 (R&D AF3369); anti-SOX17 (R&D AF1924); anti-TUB4A4 (Sigma-Aldrich T6199); anti-WTAP (Bethyl Lab A301-436A). All of the antibodies were validated to recognize the relevant human protein, and most of them were specifically validated for the relevant application, as specified on the relevant catalog pages on the suppliers' websites. In the few cases in which the antibody was not previously validated for a specific application, extensive testing in house with the appropriate negative controls was performed to confirm the specificity (see Methods and Figure legends). Such applications were: anti-METTL14 (IP and ChIP); anti-METTL3 (ChIP); anti-NANOG (flow cytometry and PLA); anti-SMAD2/3 (RIP and PLA); anti-SOX1 (flow-cytometry); anti-WTAP (PLA, RIP, and ChIP).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

The H9 hESC line was obtained from WiCell (Madison, Wisconsin). The A1ATR/R hiPSC line was obtained in house and previously described in Yusa et al 2011.

Yusa, K. et al. Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. Nature 478, 391-4 (2011).

b. Describe the method of cell line authentication used.

No authentication was performed on the H9 hESCs as they were used directly from the commercial supplier. The A1ATR/R was genotyped in house to confirm the presence of A1AT R/R allele. Both cell lines were routinely karyotyped by standard G-banding to confirm euploidy.

c. Report whether the cell lines were tested for mycoplasma contamination.

Yes, mycoplasma screening was performed every month.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals were used

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

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

☒ 2. 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).

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

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

Methodological details

5. Describe the sample preparation.	Biological samples: H9 hESCs (Extended Data Figure 7f), or hESC-derived neuroectoderm (Extended Data Figure 8b). Single cell suspensions were prepared by incubation in cell cell dissociation buffer (CDB; Gibco) for 10' at 37° followed by extensive pipetting. Cells were washed twice with PBS and fixed for 20' at 4°C with PBS 4% PFA. After three washes with PBS, cells were first permeabilized for 20' at RT with PBS 0.1% Triton X-100, then blocked for 30' at RT with PBS 10% donkey serum. Primary and secondary antibodies incubations (Supplementary Table 5) were performed for 1h each at RT in PBS 1% donkey serum 0.1% Triton X-100, and cells were washed three times with this same buffer after each incubation.
6. Identify the instrument used for data collection.	Cyan ADP flow cytometer (Beckman Coulter)
7. Describe the software used to collect and analyze the flow cytometry data.	Data collection: Summit software (Beckman Coulter). Data analysis: FlowJo X
8. Describe the abundance of the relevant cell populations within post-sort fractions.	No cell sorting was performed in the study
9. Describe the gating strategy used.	Cells were first gated on the basis of forward and side scatter properties, after which singlets were isolated on the basis of relationship between side scatter area peak area and width. A secondary-only negative control was used to determine the background fluorescence, and positive cells were quantified by setting a boundary so that less than 1% of the secondary-only control cells would be considered positive.

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