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Corresponding author(s): Chengqi Yi

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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 statistical method was used to predetermine the sample size. A minimum of triplicates was chosen in our quantitative MS/MS and qPCR experiments, which is sufficient for us to perform statistical tests when needed. Two independent sample replicates were used in our high-throughput sequencing experiments, because larger sample size could provide very limited extra information but increase the costs.

2. Data exclusions

Describe any data exclusions.

No data were excluded from our qPCR, high-throughput sequencing and QQQ results.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experimental findings were reliably reproduced.

4. Randomization

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

When drugs (5FdUR) was used to treat the cells, the samples were equally divided into two parts and one was randomly selected for drug treatment. No other specific method was used to randomize the allocation of samples and all the experiments were performed in parallel.

5. Blinding

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

No animal subjects or human research participants were involved in this study, so blinding was not relevant. Uniform data processing was applied to all the samples in analysis of sequencing data regardless of whether they were control or pull-down samples. Since the results reported are either entirely quantitative (i.e., quantitative MS/MS results, enrichment quantified by qPCR) or unambiguous in nature (e.g., DNA agrose gel images), blinding was not necessary for the experiments performed.

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.

bowtie2 was used to reads mapping to corresponding sequences. Peaks were called using model-based analysis of ChIP-Seq (MACS2) using nonredundant reads. Reads visualization was done by IGV. Intersection between bed files was performed using BEDTools. Graphs were plotted with GraphPad Prism 7.

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 reagents and enzymes used were obtained from commercial suppliers. Plasmids and cell lines would be available upon request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Monoclonal rabbit anti-UNG antibody was purchased from abcam (ab109214), which was validated by western blot and immunohistochemistry by manufacturer (http://www.abcam.cn/ung-antibody-epr4371-ab109214.html).
Polyclonal rabbit anti-MBD4 antibody was purchased from abcam (ab191037), which was validated by western blot by manufacturer (http://www.abcam.cn/mbd4-antibody-c-terminal-ab191037.html).
Monoclonal rabbit anti-SMUG1 antibody was purchased from abcam (ab192240), which was validated by western blot by manufacturer (http://www.abcam.cn/smug1-antibody-epr15624-ab192240.html).
Polyclonal rabbit anti-TDG antibody was purchased from Sigma-Aldrich (HPA052263), which was validated by western blot, immunocytochemistry and immunocytochemistry by manufacturer (https://www.proteinatlas.org/ENSG00000139372-TDG/antibody#antibody_summary).
Monoclonal mouse anti-FLAG antibody was purchased from Sigma-Aldrich (F1804), which was validated by western blot by manufacturer (https://www.proteinatlas.org/ENSG00000139372-TDG/tissue).
Monoclonal mouse anti-β-actin antibody was purchased from CWBiotech (CW0096), which was validated by western blot by manufacturer (http://www.cwbiotech.com/upload/image/201709/97712f17-4ac7-4630-92c6-4370437679d8.pdf).
The secondary antibodies used were anti-mouse-IgG-HRP (CW0102; CWBiotech, http://www.cwbiotech.com/goods/content/201707/10118.html) and anti-rabbit-IgG-HRP (CW0103; CWBiotech, http://www.cwbiotech.com/goods/content/201707/10119.html).

10. Eukaryotic cell lines

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

HEK293T was purchased from China Infrastructure of Cell Line Resources (3111C0001CCC000212);
WPMY-1 was a gift from Dr. Min Fang.
Fang, M. et al. The ER UDPase ENTPD5 promotes protein N-glycosylation, the Warburg effect, and proliferation in the PTEN pathway. Cell 143, 711-724 (2010)
K562 was a gift from Prof. Zhengfan Jiang.
Sun, W. et al. ERIS, an endoplasmic reticulum IFN stimulator, activates innate immune signaling through dimerization. Proc Natl Acad Sci U S A. 106(21):8653-8. (2009)

b. Describe the method of cell line authentication used.

All the three cell lines used were authenticated by Short Tandem Repeat (STR) profiling methods.

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

Yes, mycoplasma contamination tests were performed routinely using GMyc-PCR Mycoplasma Test Kit from YEASEN (CAT #40601). See Cell culture part of Online Methods section.

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.

HEK293T cells were used because of their high transfection efficiency and easy accessibility to laboratories world-wide, facilitating replication of our experiments.
As a cancer cell line, K562 cells were widely used and these are many sequencing data sets available for further bioinformatics analysis. Thus we adopt this cell line in this study.
WPMY-1 cells have the same karyotype with normal human cells and were used as a control for HEK293T cells which are described as hypotriploid.

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 in this study.

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.

This study did not involve human research participants.