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☐ 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 methods were used to determine sample size. The number of biological replicates are indicated in the figure legends.

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All replication attempts were successful.

4. Randomization

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

No randomization done for these experiments.

5. Blinding

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

Personnel handling the library preparation and sequencing of m6A/m-seq data were blinded.

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.	Microsoft Excel 2016 and Graphpad Prism 5 were used to generate graphs and statistical analysis. IGV was used to generate read and fold change tracks. http://software.broadinstitute.org/software/igv/ Tophat2 Aligner v2.0.6 was used for read alignment. 1. Kim, D. et al. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 14, R36 (2013). 2. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nat. Meth. 9, 357-359 (2012). exomePeak was used for m6A peak calling. 1. Meng, J. et al. A protocol for RNA methylation differential analysis with MeRIP-Seq data and exomePeak R/Bioconductor package. Methods 69, 274-281 (2014). 2. Meng, J., Cui, X., Rao, M. K., Chen, Y. & Huang, Y. Exome-based analysis for RNA epigenome sequencing data. Bioinformatics 29, 1565-1567 (2013). Cufflinks was used to calculate the half-lives of cellular transcripts. 1.Trapnell, Cole, et al. "Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks." Nature protocols 7.3 (2012): 562. Guitar package was used to plot m6A distribution. 1. Cui, X. et al. Guitar: an R/Bioconductor package for gene annotation guided transcriptomic analysis of RNA-related genomic features. BioMed Res. Int. 2016, 8367534 (2016). MEME was used for motif analysis. 1. Bailey, T. L. et al. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res. 37, W202-208 (2009).

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 non-commercially available materials are 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).

Rabbit anti-m6A antibody (202-003, Synaptic Systems, Goettingen, Germany) - validated previously in multiple publications for m6A-IP.
1. Meyer, K.D., et al., Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell, 2012. 149(7): p. 1635-46.
2. Dominissini, D., et al., Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature, 2012. 485(7397): p. 201-6.

Rabbit anti-human METTL3 antibody (A301-567A, Bethyl Laboratories, Inc., Montgomery, TX) - validated on manufacturer website for WB of human METTL3. We tested this antibody for rat in MM/KMM lysate and found bands of the correct size.

Rabbit anti-human WTAP antibody (NBP1-83040, Novus Biologicals, LLC, Littleton, CO) - validated on manufacturer website for WB of human and rat WTAP.

Rabbit anti-human YTHDF1 antibody (ab99080, Abcam, Cambridge, MA) - validated on manufacturer website for WB of human YTHDF1. We also validated this antibody with YTHDF1 knockdown cells. Tested to work for rat in MM/KMM lysate and found bands of the correct size.

Rabbit anti-human YTHDF3 antibody (ab103328, Abcam, Cambridge, MA); - validated on manufacturer website for WB of human YTHDF3. We also validated this antibody with YTHDF3 knockdown cells.

Goat anti-mouse YTHDF3 antibody (sc-87503, Santa Cruz Inc., Dallas, TX) - Tested to work for rat in MM/KMM lysate and found bands of the correct size.

Rabbit anti-human YTHDC1 antibody (ab133836, Abcam) - validated on manufacturer website for WB of human YTHDC1. We also validated this antibody with YTHDC1 knockdown cells.

Rabbit anti-human YTHDC2 antibody (ab176846, Abcam) - validated on manufacturer website for WB of human YTHDC2. We also validated this antibody with YTHDC2 knockdown cells.

Rat anti-LANA antibody (ab4103, Abcam, Cambridge, MA) - validated on manufacturer website for WB of LANA.

Rabbit anti-human YTHDF2 antibody (24744-1-AP, Proteintech Group, Rosemont, IL) - validated on manufacturer website for WB of human YTHDF2. We also validated this antibody with YTHDF2 knockdown cells. Tested to work for rat in MM/KMM lysate and found bands of the correct size.

Mouse anti-ORFK8 antibody (sc-57889, Santa Cruz Inc., Dallas, TX) - validated on manufacturer website for WB of K8.

Mouse anti-human β-actin antibody (sc-47778, Santa Cruz Inc., Dallas, TX) - validated on manufacturer website for WB of human and rat β-actin.

Rabbit anti-ORF57 antibody was generated by Sigma-Aldrich by immunizing a rabbit with the peptide IDGESPRFDDSIIP - validated in our laboratory with lysate of lytic KiSLK and BCBL1-R cells. A negative control of latent KiSLK and BCBL1-R cells produced no band.

Mouse anti-ORF65 was previously described.
Cheng, F. et al. Screening of the human kinome identifies MSK1/2-CREB1 as an essential pathway mediating Kaposi's sarcoma-associated herpesvirus lytic replication during primary infection. J. Virol. 89, 9262-9280 (2015).

Mouse anti-RTA was previously described.
Gao, S. J., Deng, J. H. & Zhou, F. C. Productive lytic replication of a recombinant Kaposi's sarcoma-associated herpesvirus in efficient primary infection of primary human endothelial cells. J. Virol. 77, 9738-9749 (2003).

10. Eukaryotic cell lines

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

iSLK - Myoung, J. and D. Ganem, Generation of a doxycycline-inducible KSHV producer cell line of endothelial origin: maintenance of tight latency with efficient reactivation upon induction. J Virol Methods, 2011. 174(1-2): p. 12-21.

KiSLK - Brulois, K.F., et al., Construction and manipulation of a new Kaposi's sarcoma-associated herpesvirus bacterial artificial chromosome clone. J Virol, 2012. 86(18): p. 9708-20.

BCBL1-R - Nakamura, H., et al., Global changes in Kaposi's sarcoma-associated virus gene expression patterns following expression of a tetracycline-inducible Rta transactivator. J Virol, 2003. 77(7): p. 4205-20.

KMM/MM - Jones, T., et al., Direct and efficient cellular transformation of primary rat mesenchymal precursor cells by KSHV. J Clin Invest, 2012. 122(3): p. 1076-81.

KMSC/MSC/KTIME/TIME - Lee, M.S., et al., Human Mesenchymal Stem Cells of Diverse Origins Support Persistent Infection with Kaposi's Sarcoma-Associated Herpesvirus and Manifest Distinct Angiogenic, Invasive, and Transforming Phenotypes. MBio, 2016. 7(1): p. e02109-15.

b. Describe the method of cell line authentication used.

Cell lines were obtained from investigators who generated them or generated in our lab. They are not further authenticated.

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

All cells used in this study were mycoplasma negative.

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.

SLK cells were isolated from a Kaposi's sarcoma lesion of an AIDS patient. However, they were found to be of renal cell carcinoma origin. Since SLK cells support efficient and robust KSHV lytic replication, they have been extensively used as a model for studying KSHV lytic replication. In this study, we employed SLK cells to study KSHV lytic replication.

Sturzl, M., et al., Kaposi's sarcoma-derived cell line SLK is not of endothelial origin, but is a contaminant from a known renal carcinoma cell line. Int J Cancer, 2013. 132(8): p. 1954-8.

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.

No human research participants.