Science Advances
AAAS

Supplementary Materials for

7SK methylation by METTL3 promotes transcriptional activity

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Sci. Adv. **9**, eade7500 (2023)
DOI: 10.1126/sciadv.ade7500

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Figs. S1 to S6

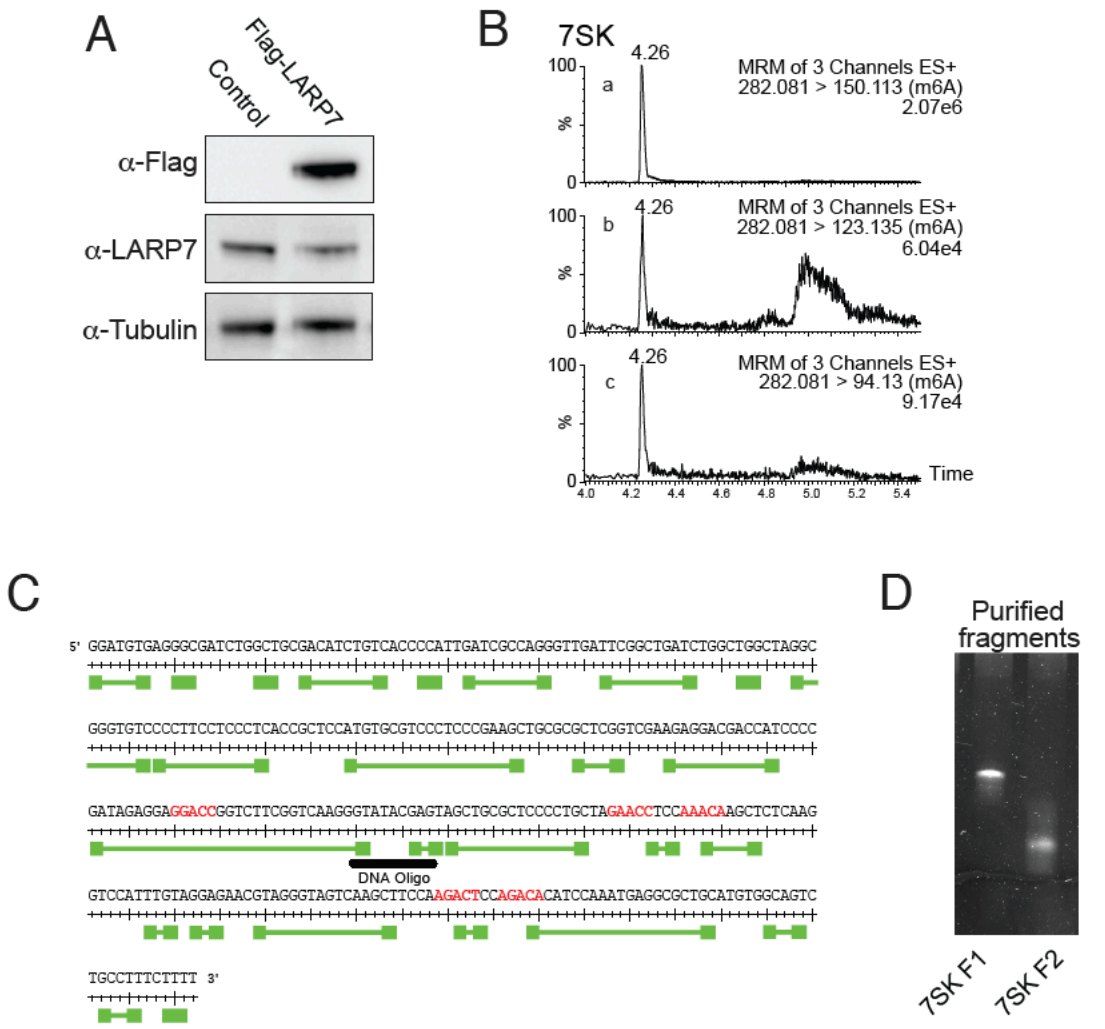

Figure S1. Mass spec analysis of endogenous 7SK m^{6}A methylation

(A) Total cell extracts from HeLa cells stably expressing Flag-tagged LARP7 and control cells analyzed by Western blot using the indicated antibodies. **(B)** Endogenous 7SK isolated from HeLa cells using immunoprecipitation of Flag-LARP7 was isolated and analyzed by mass spec. Extracted MRM chromatograms of m^{6}A (retention time, 4.26 min) from ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) of a representative sample of endogenous 7SK. Three MRM transitions (parent ion > daughter ion) were used to monitor m^{6}A, (a) m/z 282.08 > 150.11, (b) m/z 282.08 > 123.13, and (c) m/z 282.08 > 94.13. **(C)** 7SK full-length primary sequence depicting consensus m^{6}A methylation motifs in red and regions predicted to form hairpins (green). The DNA oligo required for the RNase H digestion is shown in black (DNA oligo). **(D)** RNase H digestion of endogenous 7SK. The fragments obtained from the digestion were purified from the gel and re-run to assess purity. Representative RNA gel of three biological repeats.

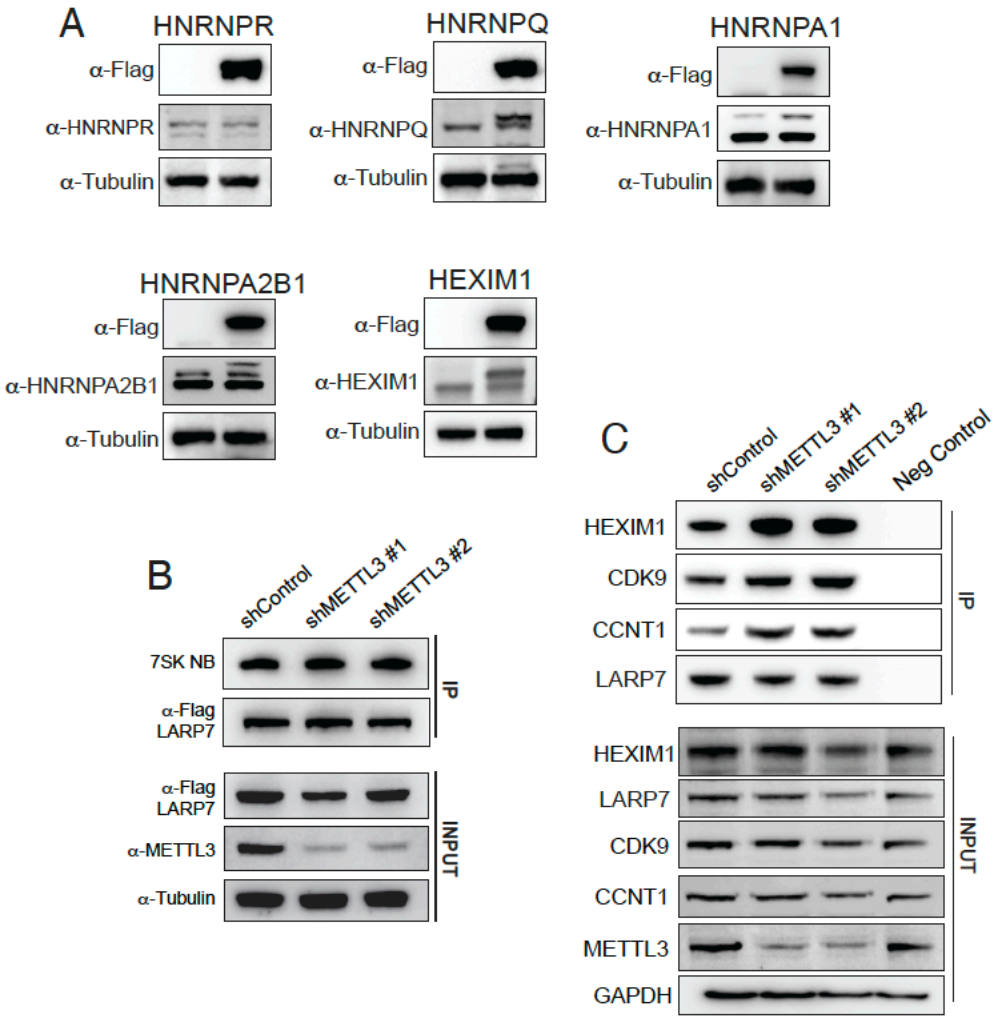

Figure S2. Stable expression of 7SK interacting proteins.

(A) HeLa cell lines stably expressing Flag-tagged proteins. Western blots show the levels of the proteins as indicated by the antibodies. Blots are representatives of biological duplicates. **(B)** Flag-LARP7 IP in controls and cells depleted of METTL3 using two independent shRNAs. Proteins detected using the indicated antibodies and 7SK detected by NB. Experimental design is similar to 2E. **(C)** Immunoprecipitation of endogenous LARP7 in control cells and cells depleted from METTL3 using two independent shRNAs. A negative control using no antibody was also used. The top panels show the IP and the bottom panels the inputs. Western blot using the indicated antibodies.

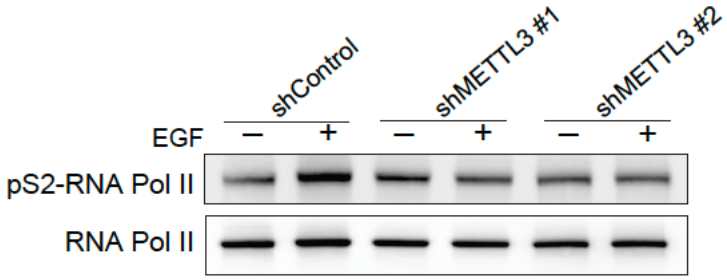

Figure S3. EGF-induced phosphorylation of RNA Pol II depends on METTL3.

Cells depleted of METTL3 using two independent shRNAs, or control cells were serum starved and then stimulated with EGF. Chromatin fraction was isolated and Western Blots testing the levels of the pS2 of the CTD of RNA Pol II were performed. Western blots were performed with the indicated antibodies.

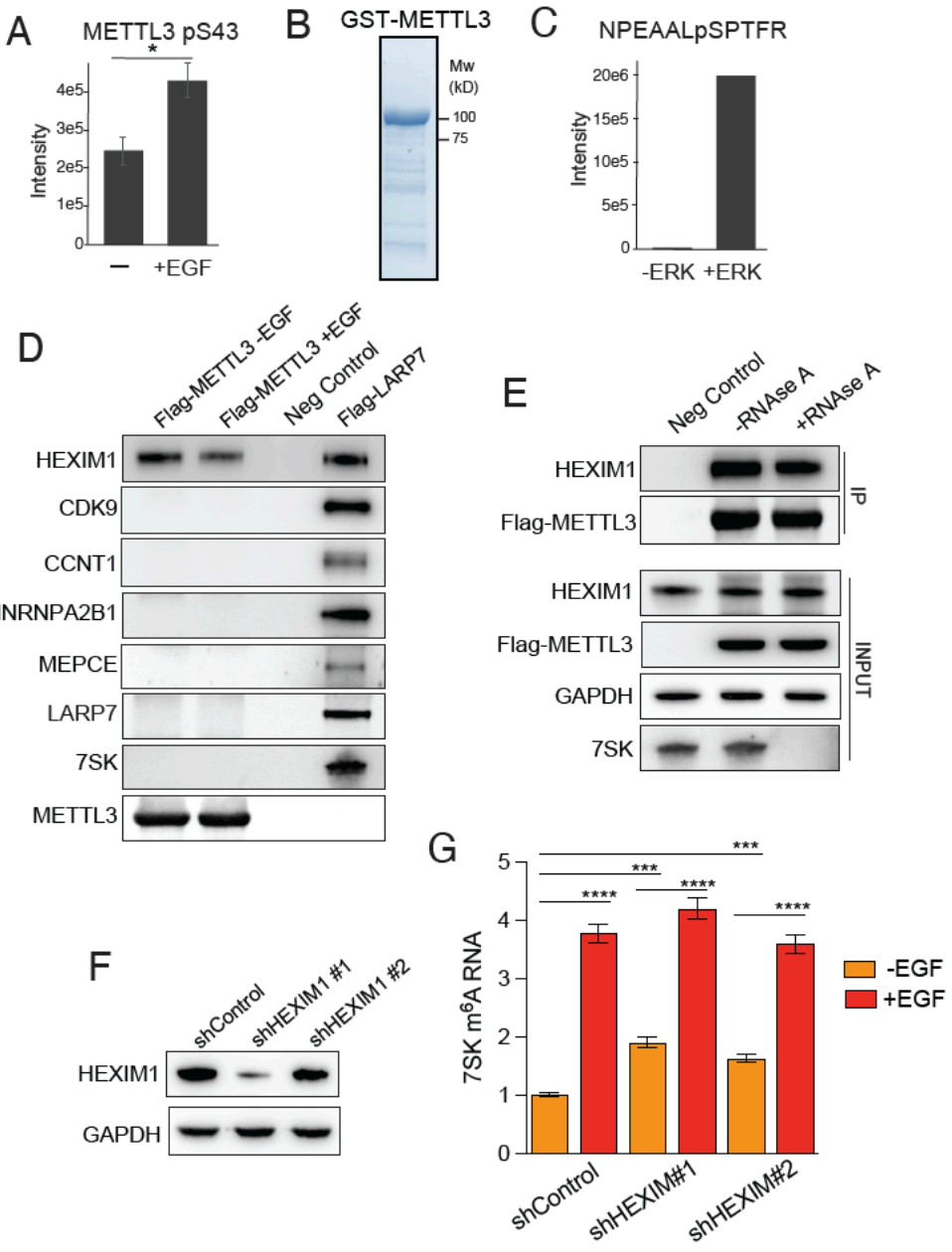

Figure S4. Mass spec analysis of pS43 METTL3

(A) Levels of pS43 METTL3 as measured by mass spec after EGF stimulation. The graph shows mean ± SD from two biological replicates. Student's 2-tailed t-test, $*P<5x10^{-2}$. **(B)** Purified GST-METTL3 fusion protein expressed in bacteria. Coomassie staining of the SDS-PAGE of the elution from the glutathione beads. **(C)** Phosphopeptide intensity levels of pS43 after ERK1 *in vitro* kinase assay on METTL3 as measured by phospho-mass spectrometry. **(D)** Immunoprecipitation of METTL3 or LARP7 was followed by WBs of the main 7SK interacting proteins as well as Northern blot for 7SK. Additionally, cells were serum starved and stimulated with EGF when indicated. **(E)** METTL3 interaction with HEXIM1 is RNA-independent. Immunoprecipitation of

METTL3 in the absence or presence of RNase A. The top panels show the co-IP of HEXIM1 and the bottom panels the input and the Northern Blot of 7SK. **(F-G)** Stable knock down of HEXIM1 was done using two independent shRNAs (**F**). Cells with decreased levels of HEXIM1 and control cells were serum starved and then stimulated with EGF. Nuclear RNA was extracted, and m^6A-IP followed by qPCR of 7SK was performed and normalized to total 7SK (**G**). The graph shows mean ± SD from three biological replicates. Two-way ANOVA, Tukey's post-test, ****P<1x10-4, ***P<1x10-3.

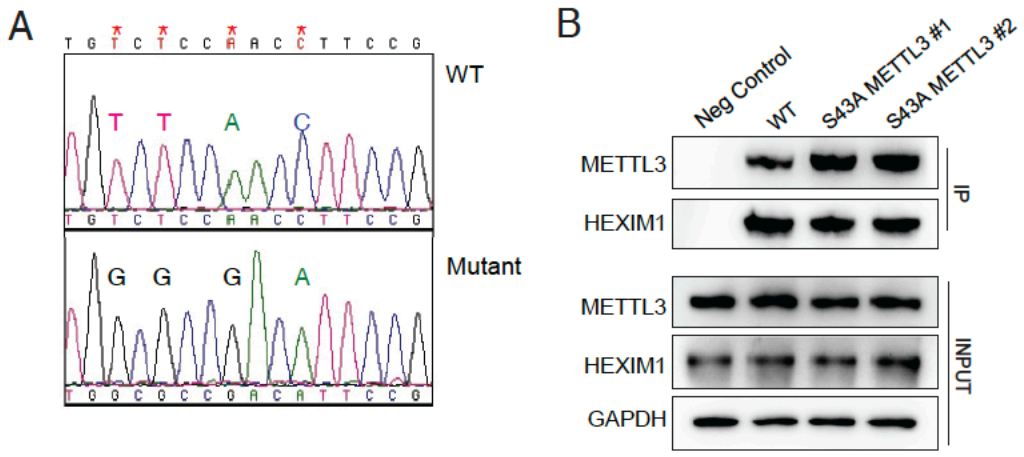

Figure S5. CRISPR/Cas9 mutation of S43A METTL3.

(A) Sanger sequencing of genomic DNA from HeLa cells mutagenized using the CRISPR/Cas9 technology. These samples represent examples of clones obtained after the CRISPR/Cas9 mutation of S43A. Four nucleotides were targeted for mutation. Those sites are required for the codon change (S/A), for silent mutations to achieve resistance to gRNA, and to create a novel restriction site for screening. The top sequence belongs to a wild-type clone. The bottom one shows complete mutagenesis of all the alleles of METTL3 in HeLa cells ($METTL3^{S43A}$). **(B)** Immunoprecipitation of endogenous HEXIM1 co-immunoprecipitates METTL3. This IP was performed in wild-type cells and cells genetically altered to carry the S43A mutation in METTL3. The top panels show the IP and the bottom the input fractions. Western Blots performed with the indicated antibodies.

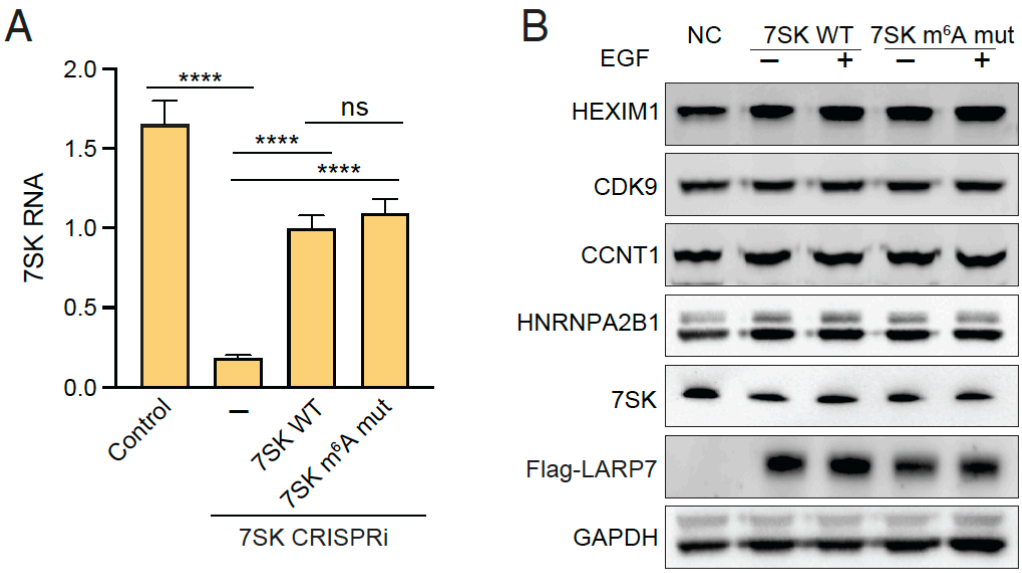

Figure S6. Mutation of m^{6}A sites on 7SK.

(A) Normalized 7SK levels as quantified by qRT-PCR. The bar graph depicts the effect of 7SK downregulation using CRISPRi and the re-introduction of exogenous wild-type or m^{6}A mutant 7SK. The bar graphs represent mean ± SD from a representative experiment out of two biological replicates and three technical replicates. One-way ANOVA, Tukey's post-test, ****P<1x10^{-4}. **(B)** Input protein levels before the IP shown in Fig. 6C. Total cell extracts were analyzed by Western blot using the indicated antibodies and by Northern blot using a probe against 7SK. Blots are representatives of at least two biological repeats.