A programmable system to methylate and demethylate m\textsuperscript{6}A on specific RNA transcripts in mammalian cells

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**References**


Supplementary Figure 1

**A**

- US → CRNA
- NLS → GGSlinker → HA 2*NLS p2A → polyA
- CMV → dCas13a → METTL3 → mCherry

**B**

- Brightfield
- mCherry

**C** mCherry+ signal in untransfected cells

**D** mCherry+ signal in dCas13a-METTL3 transfected cells

**E** mCherry+ signal in dCas13a-FTO transfected cells

**F** Untransfected cells' cell cycle and cell death assay

**G** dCas13a-METTL3 transfected cells' cell cycle and cell death assay

**H** dCas13-FTO transfected cells' cell death and cell death assay
Supplementary Figure 1. Transfection of the dCas13a-fusions into 293T cells.

A. Schematic of the constructs made in this manuscript suitable for lipid or electroporation-based transfection. NLS=nuclear localization signal; HA=hemagglutinin tag; p2A=self-cleaving peptide sequence.

B. mCherry transfection images of the dCas13a-METTL3 or dCas13a-FTO constructs in 293T cells.

C. FACS analysis showing forward scatter (FSC-A) versus mCherry fluorescence, for untransfected cells.

D. As in panel C, but for dCas13a-METTL3 and crNT transfected cells.

E. As in panel E, but for dCas13a-FTO and crNT transfected cells.

F. FACS cell cycle and cell death analysis for untransfected cells.

G. As in panel F, but with dCas13a-METTL3/crNT transfection.

H. As in panel F, but with dCas13a-FTO/crNT transfection.
Supplementary Figure 2
Supplementary Figure 2. Circular RNA m^6A reporter structure.

A. Schematic of the circRNA construct. Sequences of the DRACH are indicated and the positions of specific A nucleotides used in the SELECT assay.

B. DRACH m^6A position weight matrix motif.

C. Western blot of GFP translated from circular RNA constructs containing 2, 1 or 0 DRACH motifs or a sequence with no A nucleotides. This experiment was performed once. Molecular weight markers (in kilo Daltons; kDa) are indicated on the right side for this and all subsequent Western blots.

D. Schematic of the zoomed in region between the IRES (in orange) and the ATG (at position +1) of GFP. The sequences of the 0DRACH and 2DRACH constructs are shown. Locations of the crRNAs are relative to the indicated nucleotide position numbers, numbered according to the location of the ATG (at nucleotide 1).
Supplementary Figure 3. Transfection of dCas13a-fusion proteins does not impact the levels of endogenous METTL3 or FTO.

A. Quantification of the Western blot result in Figure 1B. Quantitation is normalized to dCas13a-METTL3/crNT.

B. Quantification of the Western blot result in Figure 1C. Quantitation is normalized to dCas13a-FTO/crNT.

C. Western blot of GFP from 293T cells transfected with crNT, or crRNAs targeting specific sites around the ATG codon of the circular plasmid, and dCas13a-METTL3, or the catalytic dead dCas13a-METTL3_D395A. This experiment was repeated 3 times with similar results. Molecular weight markers (in kDa) are indicated on the left side for this and all subsequent Western blots.

D. Western blot of GFP from 293T cells transfected with crNT, or crRNAs targeting specific sites around the ATG codon of the circular plasmid, and dCas13a-FTO, or the catalytic dead dCas13a-FTO_Y180A. This experiment was repeated 3 times with similar results.

E. Western blot of endogenous FTO protein, dCas13a-FTO fusion and βACTIN (control) in 293T cells transfected with the indicated dCas13a-FTO fusions and various crRNAs.

F. Western blot of endogenous METTL3 protein, dCas13a-METTL3 fusion and beta-ACTIN (control) in 293T cells transfected with the indicated dCas13a-METTL3 fusions and various crRNAs.

G. Quantification of dCas13a-FTO from the Western blot result in panel E. Quantitation is normalized to endogenous FTO.

H. Quantification of dCas13a-METTL3 from the Western blot result in panel F. Quantitation is normalized to endogenous FTO.

I. Western blot of endogenous METTL3 protein and βACTIN (control) in 293T cells transfected with the indicated dCas13a-FTO fusions and various crRNAs.

J. Western blot of endogenous FTO protein and βACTIN (control) in 293T cells transfected with the indicated dCas13a-METTL3 fusions and various crRNAs.
Supplementary Figure 4
Supplementary Figure 4. Genome views of m$^6$A levels of transcripts targeted by the crRNAs in 293T cells.

A. Genome view (hg38 genome assembly) of m$^6$A RIP-seq data in 293T cells at the SGKI locus. The location of the crRNA is indicated with a dotted box. Red indicates m$^6$A enrichment data, grey tracks indicate the corresponding input data. Transcripts are from GENCODE v32. m$^6$A abundance data is from GSE129979 (1) (top two rows) or GSE29714 (2) (bottom two rows).

B. As in panel A, but showing MALAT1.
C. As in panel A, but showing HIF0.
D. As in panel A, but showing ID3.
A

SELECT assay for MALAT1

B

m^A RIP-qPCR: MALAT1

C

SELECT assay for MALAT1

D

m^A RIP-qPCR: MALAT1

E

SELECT assay for MALAT1 in crSGK1 transfected cells

F

SELECT assay for MALAT1 in crH1F0 transfected cells

G

shLUC cells' cell cycle and death rate assay

H

shMETTL3#1 cells' cell cycle and death rate assay
Supplementary Figure 5. m\textsuperscript{6}A editors can methylate and demethylate endogenous mRNA and long non-coding RNA transcripts.

A. SELECT assay for MALAT1, with dCas13a-MTD, dCas13a-METTL3 or the catalytic null, with a non-targeting crRNA or with a crRNA targeting MALAT1. Y axis indicates inverse normalized m\textsuperscript{6}A abundance normalized to dCas13a-METTL3/crNT sample. Dots indicate the mean for each biological replicate and the bar is the mean of all biological replicates, n=3 biological replicate with 3 technical replicates each.

B. MeRIP-qPCR for MALAT1 transcript, in cells transfected with dCas13a-METTL3, or dCas13a-MTD and non-targeting crRNA, or a crRNA targeting MALAT1. Dots indicate the mean for each biological replicate and the bar is the mean of all biological replicates, n=3 biological replicate with 3 technical replicates each. Data is normalized to dCas13a-METTL3/crNT sample and against the input.

C. SELECT assay for MALAT1, with dCas13a-FTO or the catalytic null form, with a non-targeting crRNA or with a crRNA targeting MALAT1. Y axis indicates inverse normalized m\textsuperscript{6}A abundance normalized to dCas13a-FTO/crNT sample. Dots indicate the mean for each biological replicate and the bar is the mean of all biological replicates, as n=3 biological replicate with 3 technical replicates each.

D. MeRIP-qPCR for MALAT1 transcript, in cells transfected with dCas13a-FTO, or dCas13a-MTD and non-targeting crRNA (crNT), or a crRNA targeting MALAT1. Dots indicate the mean for each biological replicate and the bar is the mean of all biological replicates, n=3 biological replicate with 3 technical replicates each.

E. ELISA for ratio of m\textsuperscript{6}A/A in the indicated cells transfected with dCas13a-MTD, or its catalytic null, with the indicated crRNAs. Dots indicate the mean for each biological replicate and the bar is the mean of all biological replicates, n=3 biological replicates with 3 technical replicates each. p-value is from a one-way ANOVA.

F. As in panel E, but using the dCas13a-FTO and its catalytic null.

G. SELECT assay for MALAT1 in crSGK1 transfected cells. Y axis indicates inverse normalized m\textsuperscript{6}A abundance normalized to dCas13a-METTL3 or dCas13a-FTO/crNT sample. Dots indicate the mean for each biological replicate and the bar is the mean of all biological replicates, n=3 biological replicate with 3 technical replicates each.

H. SELECT assay for MALAT1 in crH1FO transfected cells. Y axis indicates inverse normalized m\textsuperscript{6}A abundance normalized to dCas13a-METTL3 or dCas13a-FTO/crNT sample. Dots indicate the mean for each biological replicate and the bar is the mean of all biological replicates, n=3 biological replicate with 3 technical replicates each.

I. FACS cell cycle and cell death analysis for untransfected cells or cells transfected with an shRNA against METTL3.
Supplementary Figure 6
Supplementary Figure 6. Lentiviral vectors for editing m^6A in mESCs.

A. Schematic of the lentiviral vectors generated in this study. NLS=nuclear localization signal; HA=hemagglutinin tag; p2A= self-cleaving peptide sequence; LTR=long terminal repeat.

B. Western blot of METTL3 in Mettl3 knockout (KO) mESCs and wildtype (WT) mESCs.

C. Genome view (mm10) of m^6A RIP-seq data in mESCs cells at the Sox2 loci. The location of the crRNA is indicated with a blue dot. Red indicates m^6A enrichment data, grey indicates the corresponding input data. Data represents m^6A RIP-seq from Mettl3 KO and WT cells. Transcripts are from GENCODE M.v25. m^6A abundance data is from GSE52662 (3).

D. As in panel B, but for the Klf4 locus.

E. Half-life comparison of Klf4 between Mettl3 KO mESCs and WT mESCs. Data is normalized to their respective 0-hour time points.

F. Half-life comparison of Sox2 between Mettl3 KO mESCs and WT mESCs. Data is normalized to their respective 0-hour time points.