Supporting Information

Regulation of CRISPR-Cas12a system by methylation and demethylation of Guide RNA

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Table of Contents

Experimental Procedures 4
Experimental Materials 4
Experimental Instruments 4
Experimental Details 5
The map of dsDNA target sequence 10
Figure S1 10
M6A modifications in different region of gRNA 11
Figure S2 11
Site and number screening of m6A modifications in 5' handle of gRNA 12
Figure S3 12
The mechanism of m6A methylation inhibiting CRISPR-Cas12a activity 13
Figure S4 13
Figure S5 13
Site and number screening of m1A methylation in 5' handle of gRNA 15
Figure S6 15
The analysis of the interaction between adenine N1 site and surrounding molecules in 5' handle of gRNA 16
Figure S7 16
Re-activating m6A-modified CRISPR-Cas12a via FTO demethylase 17
Figure S8 17
Re-activating m1A-modified CRISPR-Cas12a via ALKBH3 demethylase 18
Figure S9 18
The concentrations optimization of $m6A_{345}$-gRNA and $m1A_{345}$-gRNA for sensitive demethylases detection 19
Figure S10 19
Figure S11 19
The detection of FTO demethylase 20
Figure S12 20
Characterization of dAsCas12a-VPR-RFP and TS-miniCMV-RFP plasmids 21
Figure S13 21
Figure S14 22

Confocal laser scanning microscopy (CLSM) real-time imaging demethylases in HEK293 cell lines **23**

Figure S15 ..... **23**

Western Blot analysis **24**

Figure S16 ..... **24**

The analysis of qRT-PCR **24**

Figure S17 ..... **24**

The dynamic fluorescence results of *trans*-cleavage with m1A modification **24**

Figure S18 ..... **26**

Table S1-S8 ..... **28**

Experimental Procedures

Experimental Materials

The oligo DNAs, unmodified RNAs and m6A modified RNAs were purchased from GenScript (Nanjing, China) and Sangon Biotech Co., Ltd. (Shanghai, China). And the gRNAs modified with m1A were obtained from Generay Biotech Co., Ltd. (Shanghai, China) and Accurate Biotechnology Co., Ltd. (Hunan,China). All of these were purified by high-performance liquid chromatography (HPLC, no RNase and no DNase) and the molecular weights were verified by MALDI-TOF-MS or ESI-MS. In experiments of CRISPR-Cas12a *cis* cleavage, the dsDNA target (Table S3) was bought from Tolo Biotech Co., Ltd. (Anhui, China). The siRNA of ALKBH5 was directly purchase from OriGene Technologies, Inc. (USA).

Purified Cas12a nuclease was purchased from GenScript (Nanjing, China) and Tolo Biotech Co., Ltd. (Anhui, China). 30% polyacrylamide gel electrophoresis (PAGE (29:1 Acrylamide-bisacrylamide)), ammonium persulphate ($(NH_4)_2S_2O_8$), *N,N,N′,N′*-Tetramethylethylenediamine (TEMED), agarose dry powder, Goldview stain solution, 10X TBE buffer, 50X TAE buffer and Loading buffer were gotten from Beijing BioDee Biotech. Co., Ltd. All inorganic and organic reagents, such as $MgCl_2$ and α-ketoglutaric acid were purchased from Sigma Aldrich Biotechnology (Shanghai, China). The Proteinase K (10 mg/mL) was gotten from Biorigin Biotech. Co,.Ltd. (Beijing, China). DEPC treated ddH_2O were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). 2×Hieff® HotStart PCR Genotyping Master Mix (With Dye) and Hieff Trans™ Liposomal Transfection Reagent were purchased from Yeasen Biotechnology Co., Ltd. (Shanghai, China). The RNA transfection reagent: RNATransMate were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). ALKBH5 and FTO demethylases were purchased from Abcam (USA), and the m1A demethylase ALKBH3 were purchased from Active Motif Biotech. Co., Ltd. (Shanghai, China). P1 nuclease, alkaline phosphatase (ALP), T4 Polynucleotide Kinase (T4-PNK), bovine serum albumin (BSA), glutathione (GSH), Glutathione (Oxidized) (GSSG) and 30% H_2O_2 were purchased from Beijing BioDee Biotech. Co., Ltd. Dulbecco′s Modified Eagle′s Medium (DMEM), fetal bovine serum (FBS), Dulbecco's Phosphate Buffered Saline (DPBS), 100 IU/mL penicillin-streptomycin and 0.25% Trypsin were purchased from Sigma Aldrich (Shanghai, China) and Corning Cellgro (NY, USA). The SYBR Gold Nucleic Acid Gel Stain, Opti-MEM medium and Lipofectamine RNAiMAX Transfection Reagent were obtained from Thermo Fisher Scientific (USA). Moreover, entacapone as FTO inhibitor and IOX-1 as ALKBH5 inhibitor were purchased from MedChemExpress (Monmouth Junction, NJ, USA). The antibody of β-actin, ALKBH3, ALKBH5 and FTO were purchased from PROTEINTECH (Wuhan, China). Anti-Mouse IgA + IgG + IgM (H+L) Antibody, Human Serum Adsorbed and Peroxidase-Labeled, and Anti-Rabbit IgG (H+L) Antibody, Peroxidase-Labeled were purchased from Kirkegaard & Perry Laboratories Inc. (KPL, USA). HEK293 cell line and MCF-7 cell line were obtained from Cell Resource Center, Peking Union Medical College (which is the headquarter of National Infrastructure of Cell Line Resource, NSTI) and Shanghai Enzyme Research Biotechnology Co. Ltd. (Shanghai, China).

Experimental Instruments

All nucleic acids were pretreated (95 ℃ 5 min and cooled down to room temperature) on RT-PCR instruments (Xi'an TianLong Co. Ltd.). And this machine was also employed to realize the polymerase chain reaction (PCR) in this work. The traditional agarose gel (2 %) was imaged by Ultra-Violet Products CCD camera (Bio-RAD ChemiDOCTM XRS+ imaging system). The fluorescence PAGE gel picture was taken on Typhoon™ FLA 9500 laser scanner (FAM channel, ex:488 nm, em: 525 nm). We have used the Hitachi F-7000 fluorescence spectrometer to record fluorescence spectra and intensity after CRISPR-Cas12a *trans* cleavage. Automatic microplate reader (Spectra Max M3 (Molecular Devices, USA)) was employed to record the time dynamic of CRISPR-Cas12a *trans* cleavage. The cells were cultured in a Panasonic MCO5AC CO_2 incubator. And the cell fluorescence imaging was conducted on confocal laser scanning microscope (Olympus FV1000 confocal laser scanning microscope) with 100 x oil objective lens. Besides, HPLC-MS analysis was performed by an ACQUITY UPLC H-Class System coupled with a 6500 plus QTrap mass spectrometer (AB SCIEX, USA) equipped with a heated electrospray ionization (HESI) probe in positive ion mode. A binary solvent system was used in which mobile phase A was H_2O doped with 0.1% formic acid and mobile phase B was methanol doped with 0.1% formic acid. The results of western blot were monitored by ClinxChemiScope 6000.

Experimental Details

The CRISPR-Cas12a *cis* and *trans* Cleavage

For *cis* cleavage, the 0.4 μg dsDNA Target (sequence in Table S3) was added into 200 μL centrifuge tube. And then, the normal gRNA or methylated gRNA (1 μM 0.8 μL), Cas12a protein (4 mg/mL, 0.15 μL) and Cas12a reaction buffer (10X: 500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 1 mg/mL BSA, pH = 7.9, 2μL) were joined into centrifuge tube. Added the DEPC treated water to 20 μL quickly. The mixture was shaken on a vortex mixer. Centrifuge the liquid on the tube wall to the bottom. The centrifuge tube was placed on PCR instrument and kept at 37 °C for 1 h for cleavage reaction. After the reaction, 2 μL 10 mg/mL Proteinase K was added in the above mixture and kept at 55°C for 30 min to digest the Cas protein and terminate the reaction. Finally, the cleavage products were characterized by agarose gel electrophoresis. It should be noted that the concentrations of Cas12a, gRNA and dsDNA Target need to be optimized for best experimental results.

For *trans* cleavage, the normal or methylated gRNA (100 μM) was dissolved into 10 nM and added 4 μL into the 200 μL centrifuge tube. After that, joined the 4 μL 10 μM ssDNA Target or dsDNA Target (sequences in table S3) into the gRNA solution. And mixed the Cas12a protein (1 μM 0.5 μL) and CRISPR Cas12a buffer (10X: 500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 1 mg/mL BSA, pH = 7.9, 2 μL) into above mixture as soon as possible. Then, the **FQ probe** (5' FAM, 3'BHQ-1, 1 μM) was added into the mixture and the DEPC treated water was added to 20 μL quickly. The mixture was shocked and centrifuged to the bottom of tube. For detection of fluorescence versus time, the mixture was placed

into a 384-well microtiter plate. The excitation wavelength was at 488 nm. The fluorescence intensity at 525 nm was scanned, and the monitoring was kept about 2 h. For fluorescence spectra and intensity detection, the mixture was placed on a PCR instrument kept at 37 °C for 1 h, and then heated at 95 °C for 10 min to terminate the cleavage reaction. The results were recorded on the Hitachi F-7000 fluorescence spectrometer. It should be noted that the concentrations of Cas12a, gRNA and dsDNA Target need to be optimized for best experimental results.

The blockage rate of *trans* cleavage is calculated by $(F_{normal}-F_{methylation})/F_{normal}$. F_{normal} represents the fluorescence of *trans* cleavage by normal gRNA and Cas12a; $F_{methylation}$ indicates the fluorescence of *trans* cleavage by methylation modified gRNA and Cas12a.

The reaction of demethylases

The m6A modified gRNA (100 nM 10 μL) was mixed with varying concentrations of ALKBH5 or FTO demethylases and RNasin (0.2 U/μl, Promega). 2 μL 10X ALKBH5 or FTO reaction buffer (500 mM HEPEs, 1000 μM α-ketoglutaric acid, 1000 μM ascorbic acid sodium salt, 500 μM $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ and 10 mM TCEP) was added in the solution. Added DEPC-treated H_2O to 20 μL and shook the mixture quickly. The mixture was incubated at 37℃ 2 ~ 4 h or 16°C for 12 h, and then quenched by heating at 85°C for10 min. As for the demethylation of m1A modified gRNA, 1 μM 5 μL m1A modified gRNA was mixed with varying concentrations of ALKBH3 and RNasin (0.2 U/μl, Promega). 2 μL 10X ALKBH5 or FTO reaction buffer (500 mM HEPEs, 500 μM $(NH_4)_2Fe(SO_4)_2$, 10 mM α-ketoglutaric acid, 20 mM ascorbic acid sodium salt, 10 mM TCEP) was added in the solution. DEPC-treated H_2O was added to 20 μL. After that, the steps of reaction were same as the demethylation of m6A-gRNA. It should be noted the buffers of demethylases should not be sterilized by hot temperature.

Agarose gel electrophoresis analysis

Firstly, weighed 1.0 mg dry agarose powder into a beaker, and added 50 mL 1X TAE buffer and 15 μL Goldview staining solution. Heated the solution until the agarose completely dissolving, and then cooled to room temperature. The samples were mixed with 1X Loading buffer and electrophoresed in 1X TAE buffer at 120 V for 40 min. The electrophoresis results were imaged by an ultraviolet imaging device.

Electrophoretic mobility shift assay (EMSA)

In this work, the binding between normal/methylated gRNA and Cas12a was verified by gel shift assay following some published literatures[1-2].

5 mL 40% (37.5:1 Acr/bis), 5 mM 10X TBE and 39.4 mL DEPC-treated H_2O were mixed. 300 μg $Na_2S_2O_3$ were dissolved into 1 mL DEPC-treated H_2O and further take 166 μL into above mixture. After

that, joined into 20 μL TEMED (0.4 μl/mL PAGE) quickly. After 40 min, the PAGE gel was pre electrophoresed at 150 V in 1X TBE cooling bath about 20 min.

Mixed the FAM labeled normal/methylated gRNA (sequences listed in table S2 and S1, 20 nM), a series of concentrations of Cas protein and 1X binding buffer (150 mM NaCl, 5 mM $MgCl_2$, 20 mM HEPEs, 5% v/v Glycerol, 2 mM DTT) into 9 μL DEPC-treated H_2O. And then, this mixture was incubated about 45 min in 37 ℃ to form RNA and protein complex (RNP). 3 μL above RNPs were loaded with 5X Loading buffer (2.5X TBE, 40% v/v Glycerol) on gel. Finally, in cooling bath, the gel was conducted at 150 V in 0.5X TBE about 50 min and the results of electrophoresis were pictured through the Typhoon™ FLA 9500 laser scanner.

Enzymatically-digested process of gRNA for HPLC-MS analysis

First of all, 1 μM 5 μL methylated gRNA were demethylated by demethylases as previously mentioned steps. After the reaction, the mixture was heated at 95 ℃ and then cooled to room temperature. The pH of above solution was adjusted to 5.2 by acetic acid buffer (3 M, pH 5.0). After that, added 2 μL P1 Nuclease (1 U/μL) which degraded RNA into single nucleotide acid and kept the solution at 37 ℃ for 4 h. And then, added the 4 μL alkaline phosphatase and 5μL reaction buffer (10 X 500 mM potassium acetate 200 mM Tris-acetate, 100 mM magnesium acetate, 1 mg/ml BSA, pH 7.9). The mixture was further incubated at 37 ℃ for 1 h and heated at 95 ℃ for 10 min to inactivate alkaline phosphatase. Meanwhile, the gRNA in solution was completely degraded into mononucleotide. The nucleotide was extracted two times by chloroform and water mixture (1:1). The extracted solution was lyophilized and redissolved by adding 50 μL biomolecular water. The above solution was diluted 11 times, and 1 μL was taken into HPLC-MS.

dAsCas12a-VPR-RFP, TS-miniCMV-RFP and ALKBH3-GFP Plasmids construction

The ALKBH3-GFP overexpressed plasmid (sequence see table S9) was commercially available in VectorBuilder Inc. (Guangzhou, China). The dAsCas12a-VPR-RFP and TS-miniCMV-BFP plasmids were built briefly as following[3]:

The sequence of dAsCas12a referred to Addgene plasmid (#86209, D908A)[4] and the sequence of VPR protein (hybrid fusion of VP64, p65 and the Epstein-Barr virus Rta domains) referred to Addgene plasmid (#114078)[5]. They were listed into Table S6 and Table S7. The pRP[Exp]-Target Site -miniCMV>TagBFP and pRP[Exp]-mCherry/Puro-EF1A>(dAsCas12a/VPR) were built by two-step construction of LR reactions of Gibson assembly and Gateway Cloning. As for Target Site-miniCMV-BFP plasmid, the Target Site DNA fragment (including eight tandem repeats for the gRNA target site with PAM (TTTG)) was chemical synthesized by GenScript (Nanjing, China) and the miniCMV:TagBFP DNA fragment was synthesized by polymerase chain reaction. Through the Gibson Assembly® Master Mix (NEB, 50℃ incubating in 15 min), the two DNA fragments were grafted on

different carrier backbones. After that, the Gibson reaction products were respectively transformed into Ultra Stable competent bacteria (Kanamycin Resistance and 37 ℃ overnight) and further got intermediate carriers pUp-Target Site and pDown-miniCMV:TagBFP (the sequences were verified by PCR and sanger sequences). Finally, two intermediate carriers were spliced on pRP[Exp] backbone vector by Gateway™ LR Clonase™ II enzyme (Thermofisher, incubating at 25°C for 3 h, then adding proteinase K and keeping at 37°C for 15 min). The LR reaction products were transformed into UltraStable competent bacteria, and the monoclonal plaque was picked for PCR and enzyme digestion to verify sequences. After that, the final vector pRP[Exp]-Target Site1-miniCMV>TagBFP was obtained. As for the pRP[Exp]-RFP/Puro-EF1A>(dAsCas12a/VPR) vector, the building process was same as above steps. EF1A DNA fragment and dAsCas12a-VPR DNA fragment were obtained by PCR reaction and the LR reaction used backbone vector pRP[Exp]-RFP/Puro.

Cell culturing

HEK293 cell line and MCF-7 cell line were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 100 IU/mL PS (penicillin-streptomycin) at 37 °C under 5% CO_2. The growth rate of MCF-7 cell line was similar to HEK293 cell line, and they were split 1/4 once 2 days in T25 culture flasks.

Plasmid transfection

When the cells grew to 90% area of T25 culture flasks, digested by 0.25% trypsin (1 mL) and centrifuged. Added 2 mL DMEM medium to resuspend the cells. After that, took 200 μL above solution to glass bottom 12-well plate or a 35 mm glass bottom petri dish with DMEM medium (10% fetal bovine serum and 100 IU/mL PS). The cells were cultured at 37°C to 60% ~70% area of petri dish for plasmid transfection. The dAsCas12a-VPR-RFP and TS-miniCMV-BFP plasmids were co-transfected to cells by Hieff Trans™ Liposomal Transfection Reagent. The process of transfection referred to synopsis and reported protocols[3] with little modification. As for HEK293 cell line, 1.0 μg dAsCas12a-VPR-RFP and 500 ng TS-miniCMV-BFP plasmids were pre mixed with 4.5 μL transfection reagent for 20 min in 100 μL Opti-MEM medium. The mixture was added to 1 mL Opti-MEM medium to culture the HEK293 cells lasting about 6 h. After that, the Opti-MEM was changed to 1 mL DMEM medium (10% fetal bovine serum, 100 IU/mL PS) and incubated 18 h. Then, the optimized normal/methylated gRNA (60 pmol) with RNA Transmate (2.5 μL) was added into cell. The transfecting reaction lasted about 6 hours in cell incubator. After that, the original transfection medium was replaced with fresh complete medium. As for MCF-7, the transfected reagents should be optimized and in this work 3 μL Hieff Trans™ Liposomal Transfection Reagent was benefit for plasmid transfection. As for overexpression of ALKBH3, the overexpressed plasmid was pre transfected into HEK293 cell before joining the dAsCas12a-VPR-RFP and TS-miniCMV-BFP plasmids.

Measurement of confocal laser scanning microscope

The cells with plasmids and gRNA were firstly washed three times by DMEM medium (10% fetal bovine serum, 100 IU/mL PS). After that, the cell fluorescence imaging was taken by Olympus FV1000 confocal laser scanning microscope using a 100x objective lens. The GFP fluorescence was monitored by green channel (ex: 488 nm and em: 500 - 550 nm). The BFP channel was monitored by blue channel (ex: 405 nm and em: 425 - 475 nm). The RFP channel was monitored by red channel (ex: 559 nm and em: 575 - 625 nm). For researching the time response of methylation masked CRISPR-Cas12a in cells, the fluorescence pictures were acquired at different time intervals. For demethylase inhibitors experiment, the entacapone (80 μM) and IOX-1 (60 μM) were co-incubated with HEK293 cells before plasmid transfection to inhibit the activity of ALKBH5 and FTO demethylases.

Western Blot

We used the western blot to investigate the demethylases in HEK 293 cell lines and MCF-7 cell lines. Firstly, wash the cells 3 times by DPBS and add 200 μL 0.25% trypsin digestion (2 min). After centrifugation, resuspend cells in DPBS. Add 10 μL PMSF (100 mM) to 1 ml lysis buffer and placed on ice. The proteins of cytoplasmic and nuclear fractions were obtained by PARIS Kit^{TM} (Thermo Fisher, USA). According to the number of cells, add 100 - 500 μL PMSF-containing lysis buffer to each tube, and lyse them on ice for 30 min. The concentration of protein was monitored by BCA assay Kit (Beyotime Biotechnology, China). The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, ALKBH3: 12%; ALKBH5: 10%; FTO:10%) was used to separate the protein samples. After that, the samples were transferred into PVDF membrane (Merck Millipore, USA). Fully immerse the PVDF membrane in 5% milk-PBST overnight at 4 ℃ for blockage. The Blots were treated with the relevant primary antibodies in 5% BSA-PBST buffer overnight at 4°C. These membranes were then stained with secondary antibody (Peroxidase-Labeled) for 1 hour at room temperature and washed 3 times with PBST for 5 minutes each. The membranes were then incubated with BeyoECL Plus Kit (Beyotime Biotechnology, China) and then assayed using ClinxChemiScope 6000.

Results and Discussion

The map of dsDNA target sequence

Figure S1. The sequence of dsDNA target. The red feature represents spacer sequence of gRNA. The yellow feature represents PAM sequence.

M6A modifications in different region of gRNA

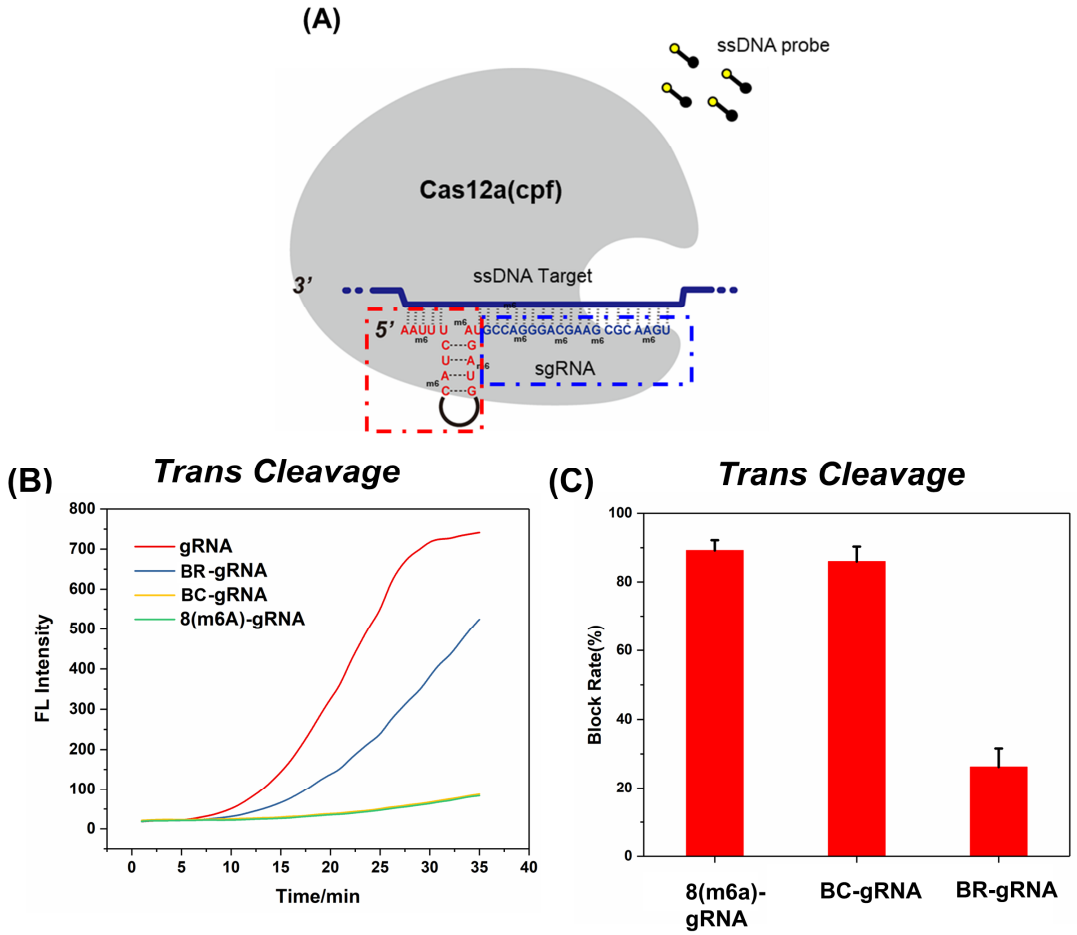

Figure S2. (A) The scheme of m6A modifications in gRNA inhibiting Cas12a-gRNA-ssDNA *trans* cleavage. Red Box represents the sequence of 5'handle of gRNA. Blue Box represents the sequence of recognized region of gRNA. "m6" in scheme represents the *N6*-Me-rA modification. (B) The dynamic fluorescence results of Cas12a with different gRNAs *trans* cleaving FQ probe. (C) The Inhibition rate of CRISPR-Cas12a *trans*-cleavage activity. The position and number of m6A modifications on 8(m6A)-gRNA, BC-gRNA and BR-gRNA are listed into Table S1. The error bars are the standard deviation of three parallel measurements.

Site and number screening of m6A modifications in 5' handle of gRNA

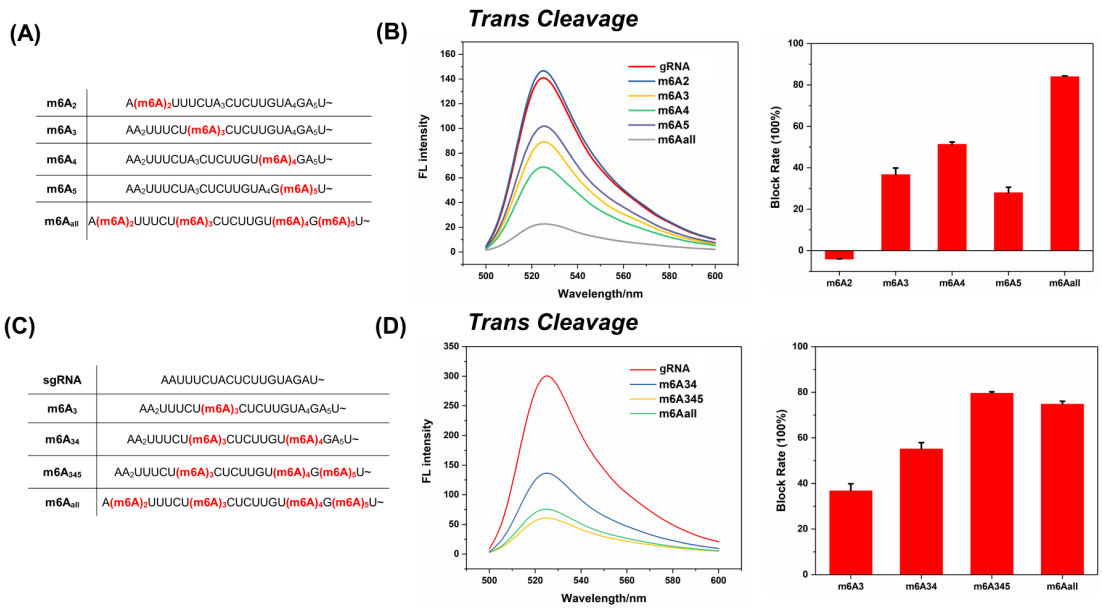

Figure S3. When the ssDNA is as target, screening the position and number of m6A modifications in the 5' handle of gRNA for effectivly inhibiting the *trans*-cleavage activity of CRISPR-Cas12a. (A) the position and (C) number of m6A modifications in the 5' handle of gRNA. (B) and (D) left graphs: the fluorescence spectra results of Cas12a with different gRNAs *trans* cleaving FQ probe. (B) and (D) right graphs: inhibition rate of CRISPR-Cas12a *trans*-cleavage activity. The error bars are the standard deviation of three parallel measurements.

The mechanism of m6A methylation inhibiting CRISPR-Cas12a activity

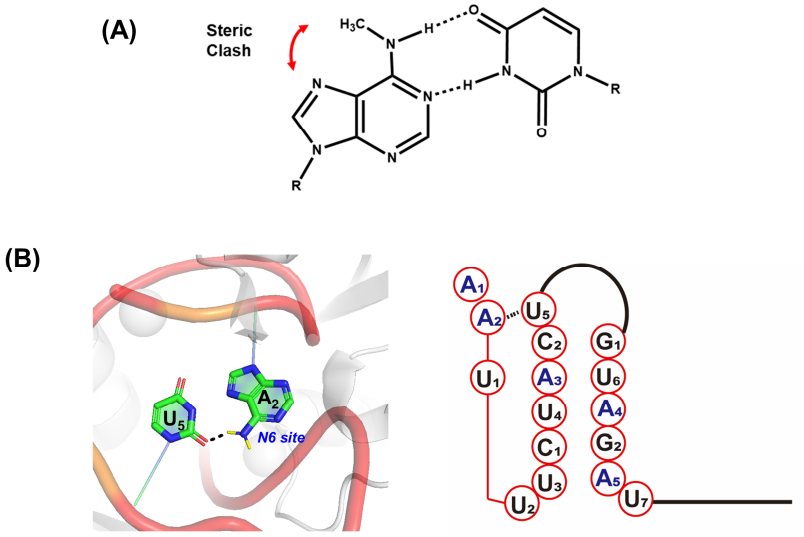

Figure S4. The effect of m6A modification on the hydrogen bonding in 5'handle of gRNA. (A) methyl group orientation in paired adenine base (left) and uracil base (right)[6]. (B) the hydrogen bond interaction between the A_2-N_6 site and surrounding molecules in conserved region of gRNA. In crystal structure graph (left), carbon atoms are in green; nitrogen atoms are in blue; oxygen atoms are in red; yellow rods represent hydrogen atoms; Black dotted line represents hydrogen bond. The serial numbers of different bases in 5'handle of gRNA is listed into right model graph. (PDB ID: 5ID6)

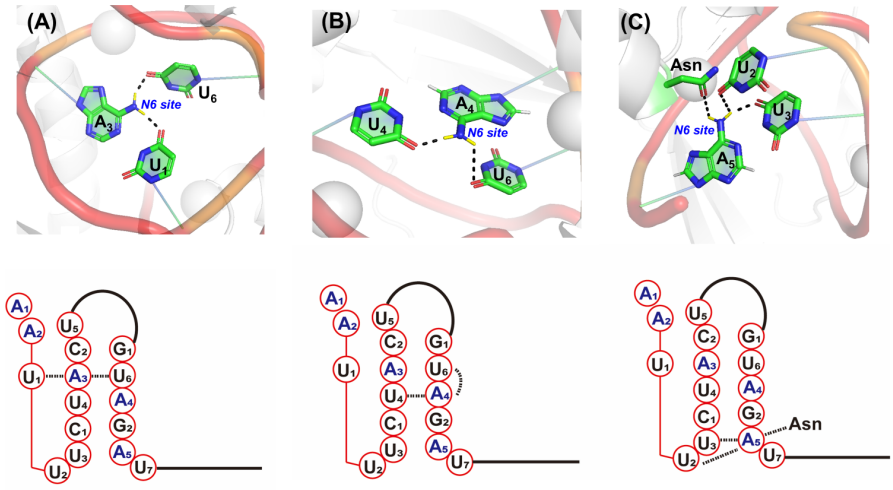

Figure S5. In the 5' handle of gRNA, the hydrogen bonds between the N6 site of adenine and surrounding molecules. As shown in scheme, all hydrogen atoms on A3, A4, A5-*N6* site could be involved in the formation of hydrogen bonds. Two hydrogen atoms of A_3-N_6 site form hydrogen bonds with U_6 and U_1. The two hydrogen atoms of A_4 could form hydrogen bonds with surrounding U_4 and U_8. As for A_5-N_6 site, in addition to pairing with surrounding uracils, it could also pair with asparagine (Asn) in Cas protein. In structure graph, carbon atoms are in green; nitrogen atoms are in blue; oxygen atoms are in red; gray rods represent hydrogen atoms; yellow dotted line represents hydrogen bond.

According to the reported structural analysis, although the N_6 site on adenine is not directly involved in the interaction between gRNA and protein, it helps in the maintenance of the secondary structure of the gRNA molecule[7-8]. Firstly, two hydrogen atoms at the N_6 site of A_2 only form a single hydrogen bond with the distal U_5, and this hydrogen bond cannot be affected by m6A which could adopt a low-energy *syn* conformation (Figure S4). However, the N_6 hydrogen atoms of A_3, A_4 and A_5 are all implicated in the formation of the pseudoknot structure. Once the hydrogen atom at the N_6 site of above adenines is methylated, the methyl group will directly destruct the hydrogen bonds in the 5' handle, hence preventing the formation of pseudoknot structure (Figure S5). In principle, gRNA without pseudoknot structure cannot form a functional complex with Cas12a. Overall, these structural observations are consistent with previous biochemical results, and these m6A modifications prevent the RNPs formation and further inhibit the cleavage activity of CRISPR-Cas12a.

Site and number screening of m1A methylation in 5' handle of gRNA

(A)

gRNA	AAUUUCUACUCUUGUAGAU~
$m1A_2$	A$(m1A)_2$UUUCUA_3CUCUUGUA_4GA_5U~
$m1A_3$	AA_2UUUCU$(m1A)_3$CUCUUGUA_4GA_5U~
$m1A_4$	AA_2UUUCUA_3CUCUUGU$(m1A)_4$GA_5U~
$m1A_5$	AA_2UUUCUA_3CUCUUGUA_4G$(m1A)_5$U~
$m1A_{34}$	AA_2UUUCU$(m1A)_3$CUCUUGU$(m1A)_4$GA_5U~
$m1A_{345}$	AA_2UUUCU$(m1A)_3$CUCUUGU$(m1A)_4$G$(m1A)_5$U~

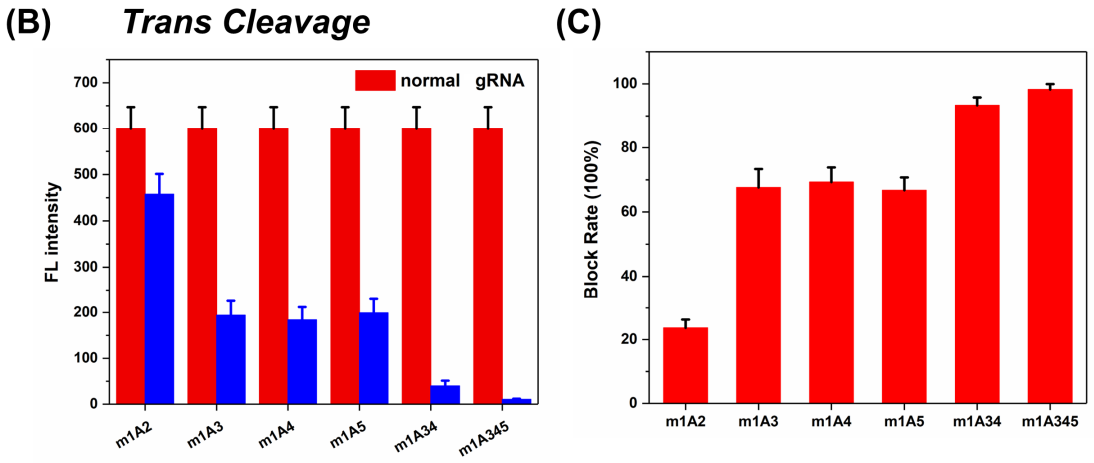

Figure S6. When the ssDNA is as target, screening the position and number of m1A modifications in 5' handle of gRNA for effectivly inhibiting the *trans*-cleavage activity of CRISPR-Cas12a. (A) the position and number of m1A modifications in the 5' handle of gRNA. (B) the fluorescence intensity at 525 nm after CRISPR-Cas12a with different gRNAs trans cleaving FQ probe. The red histograms represent the results of normal gRNA. The blue histograms are the results of methylated gRNAs. (B) inhibition rate of CRISPR-Cas12a *trans*-cleavage activity. The standard deviation of three parallel measurements are error bars.

The analysis of the interaction between adenine N1 site and surrounding molecules in 5' handle of gRNA

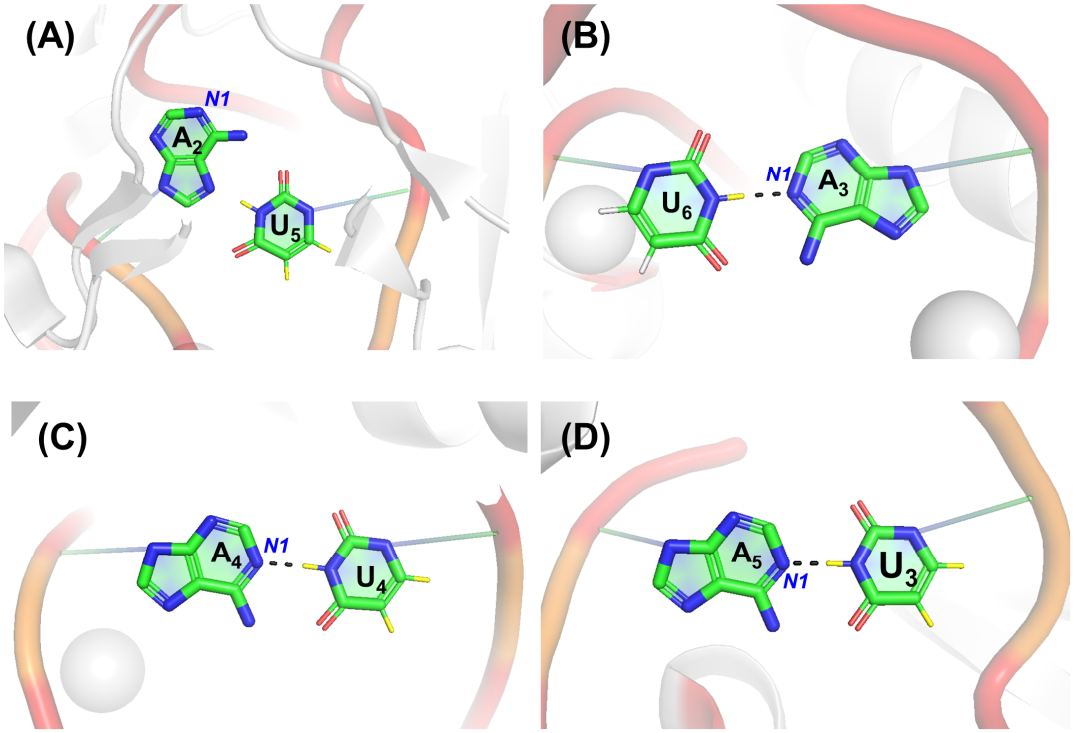

Figure S7 Overall interaction between adenine *N1* site and surrounding molecules. (A) There is no hydrogen bond between *N1* site on A_2 and surrounding molecules. (B) ~ (D) A_3 ~ A_4 *N1* sites in gRNA could directly form stronger hydrogen bonds with vicinal U bases according to Watson–Crick Pairing. Carbon atoms are in green; nitrogen atoms are in blue; oxygen atoms are in red; yellow rods represent hydrogen atoms; black dotted line represents hydrogen bonding. The serial number of bases in these graphs is same as Figure S4 and S5.

Re-activating m6A-modified CRISPR-Cas12a via FTO demethylase

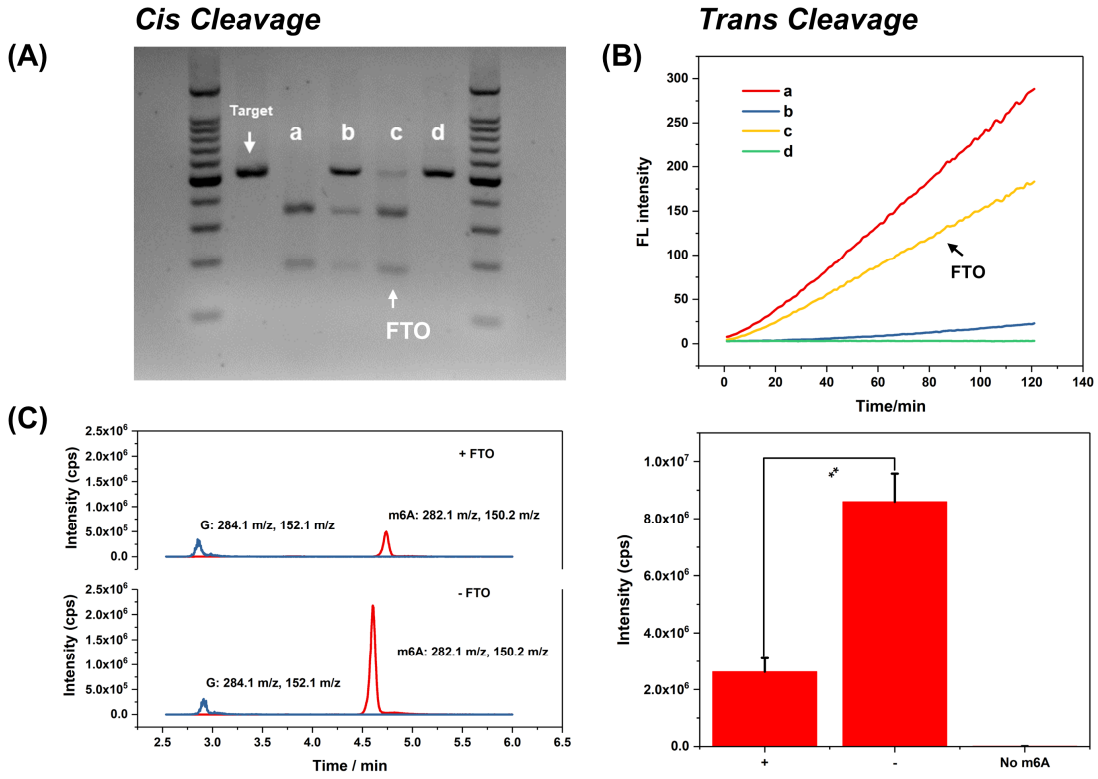

Figure S8 FTO-mediated re-activation. (A) Activation of CRISPR-Cas12a *cis* cleavage. Lane 1: only dsDNA Target (sequence in Table S3 and Figure S1); Lane a: normal gRNA + Cas12a + dsDNA; Lane b: m6A$_{345}$-gRNA (sequence in Table S1) + Cas12a + dsDNA Target; Lane c: FTO demethylase + m6A$_{345}$-gRNA + Cas12a + dsDNA Target; Lane d: FTO demethylase + Cas12a + dsDNA Target. (B) Activation of CRISPR-Cas12a *trans* cleavage. The experimental conditions of (a) ~ (d) are consistent with (A) and the FQ probe was added in these groups respectively. (C) the results of HPLC-MS/MS. In right graph, "+/-" represents with or without FTO demethylase. The molecular mass of mother ion of G nucleoside is 284.1 *m/z* and its special fragment ion molecular mass is 152.1 m/z. And the molecular mass of mother ion and special fragment ion of m6A nucleoside are *282.1* m/z and 150.2 m/z respectively. The error bars show standard deviation of three parallel measurements. $^{**}P < 0.01$ were obtained by Student's t test.

Re-activating m1A-modified CRISPR-Cas12a via ALKBH3 demethylase

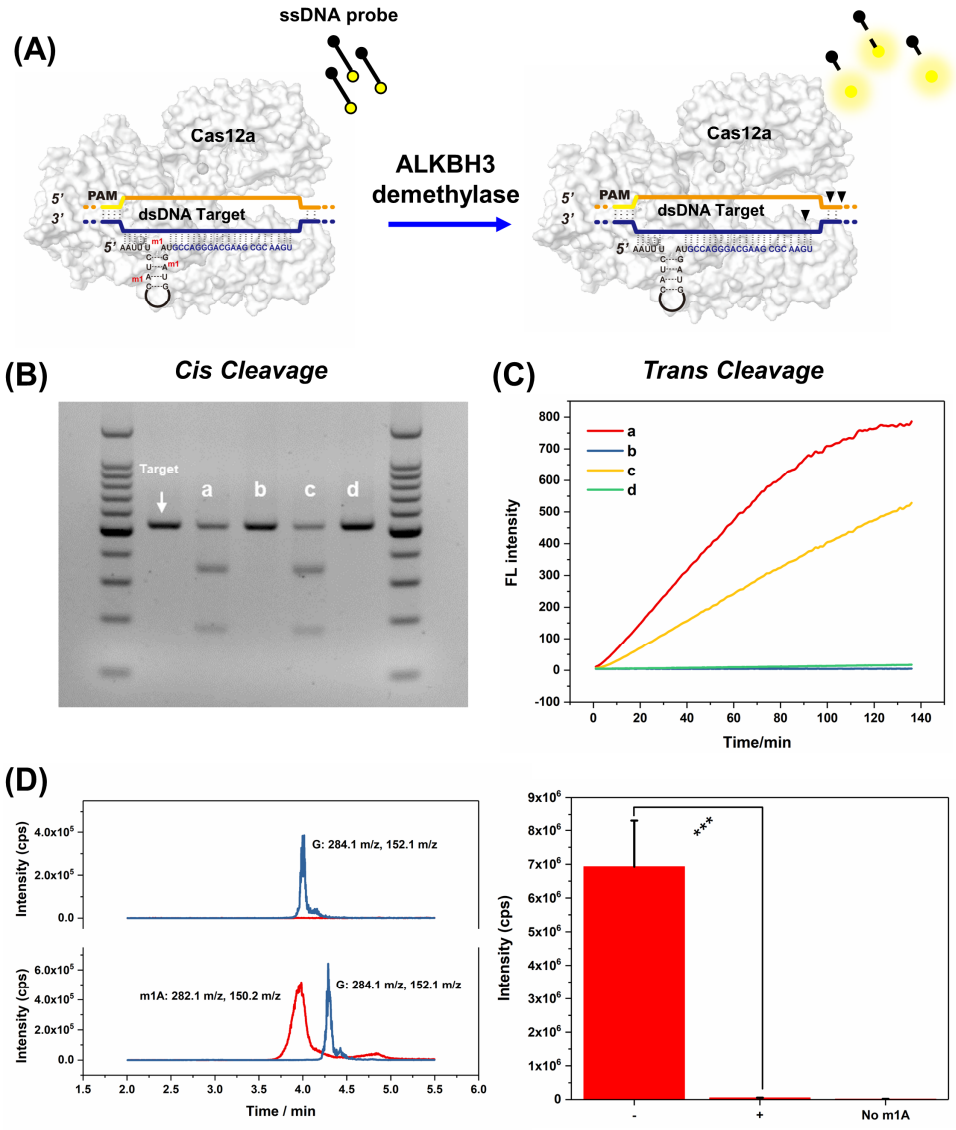

Figure S9 ALKBH3-mediated re-activation. (A) Schematic illustration. m1A$_{345}$-gRNA can be demethylated to normal gRNA by ALKBH3 and the normal gRNA further activate the *cis*- and *trans*-cleavage ability of CRISPR-Cas12a. (B) Activation of CRISPR-Cas12a *cis* cleavage. Lane 1: only dsDNA Target (sequence in Table S3); Lane a: normal gRNA + Cas12a + dsDNA; Lane b: m1A$_{345}$-gRNA (sequence in table S2) + Cas12a + dsDNA Target; Lane c: ALKBH3 demethylase + m1A$_{345}$-gRNA + Cas12a + dsDNA Target; Lane d: ALKBH3 demethylase + Cas12a + dsDNA Target. (C) Activation of CRISPR-Cas12a *trans* cleavage. The experimental conditions of (a) ~ (d) is consistent with (B) and the FQ probe was added in these groups respectively. (D) the results of HPLC-MS/MS, and in right graph, "+/-" represent with or without ALKBH3 demethylase. The mother ion of m1A nucleoside molecular mass is same as m6A because they are isomers. The error bars represent the standard deviation (three times). *** $P < 0.001$ was obtained by Student's t test.

The concentrations optimization of m6A$_{345}$-gRNA and m1A$_{345}$-gRNA for sensitive demethylases detection

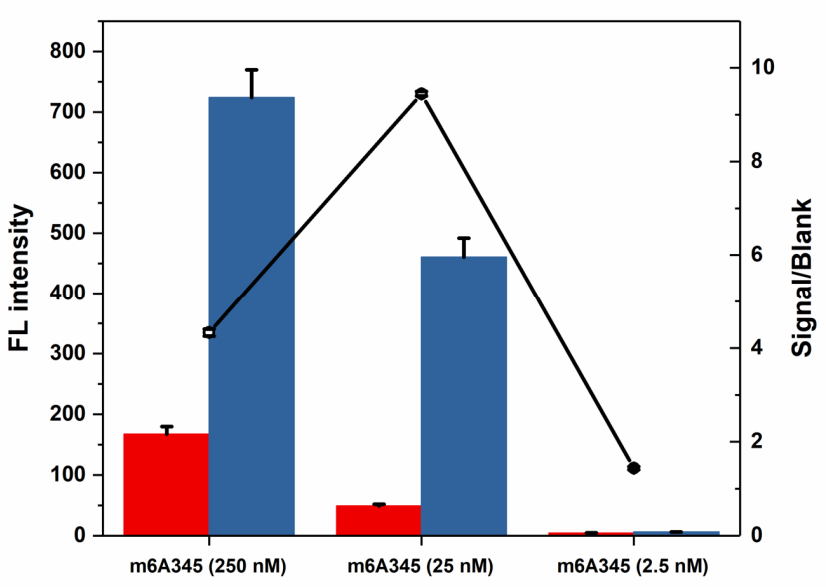

Figure S10 The concentrations optimization of m6A$_{345}$-gRNA for sensitive detection of ALKBH5 demethylase. The red columns represent the m6A$_{345}$-gRNA triggering *trans* cleavage without ALKBH5. The blue columns represent the m6A$_{345}$-gRNA triggering the *trans* cleavage with ALKBH5. The black dots (○) are signal/blank (fluorescence of blue column/red column).

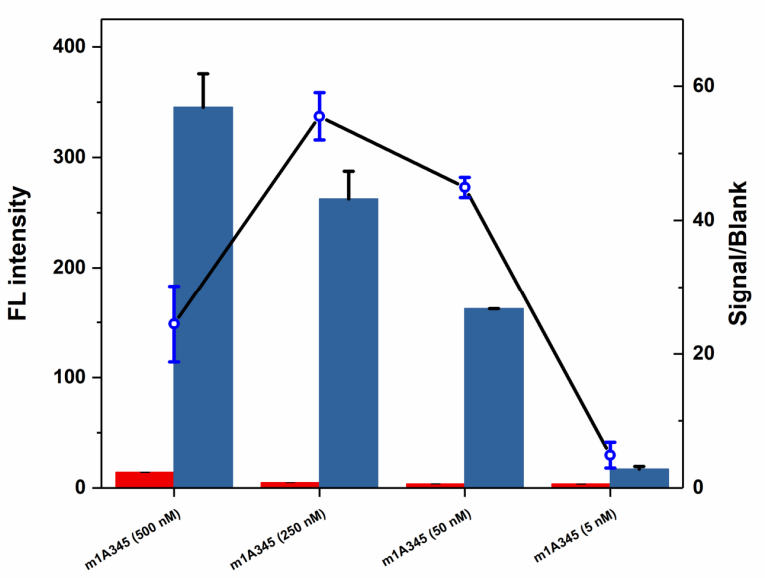

Figure S11 The concentrations optimization of m1A$_{345}$-gRNA for sensitive detection of ALKBH3 demethylase. The red columns represent the m1A$_{345}$-gRNA triggering the *trans* cleavage without ALKBH3. The blue columns represent the m1A$_{345}$-gRNA triggering the *trans* cleavage with ALKBH3. The blue dots (○) are signal/blank (fluorescence intensity of blue column/red column).

The detection of FTO demethylase

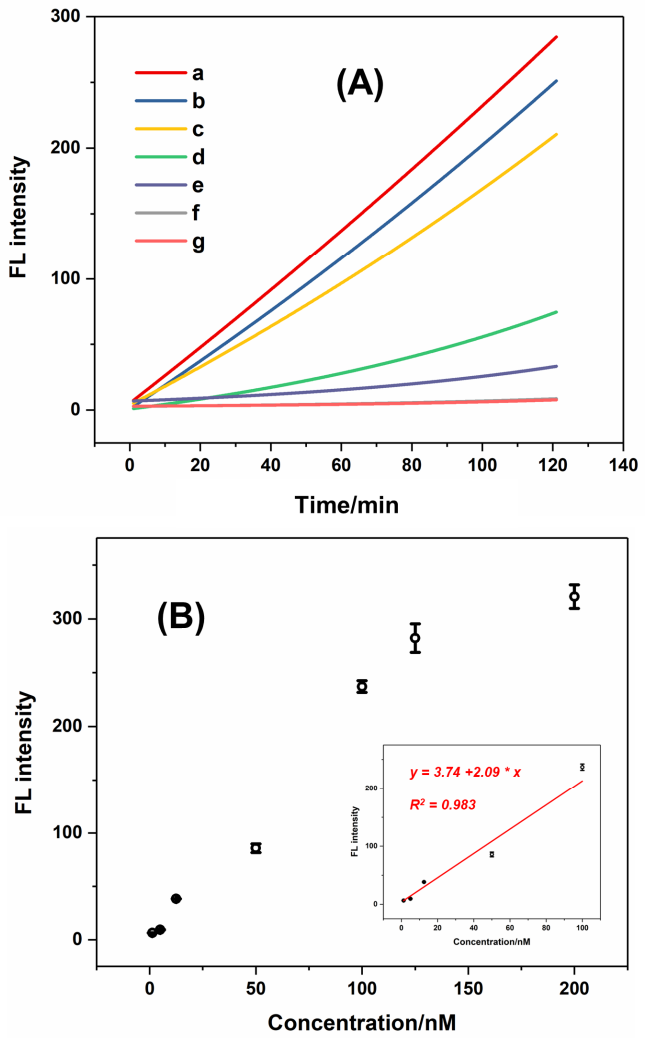

Figure S12 CRISPR-Cas12a *trans* cleavage guided FTO demethylase detection. (A) Fluorescence versus time curve with different concentrations of FTO demethylase, ranging from 250 nM (a), 200 nM (b), 125 nM (c), 50 nM (d), 12.5 nM (e), 5 nM (f), 0 nM (g). (B) The calibration curve of the fluorescence intensity at 525 nm versus FTO concentrations (250 nM, 200 nM, 125 nM, 50 nM, 12.5 nM, 5 nM, 0 nM).

Characterization of dAsCas12a-VPR-RFP and TS-miniCMV-RFP plasmids

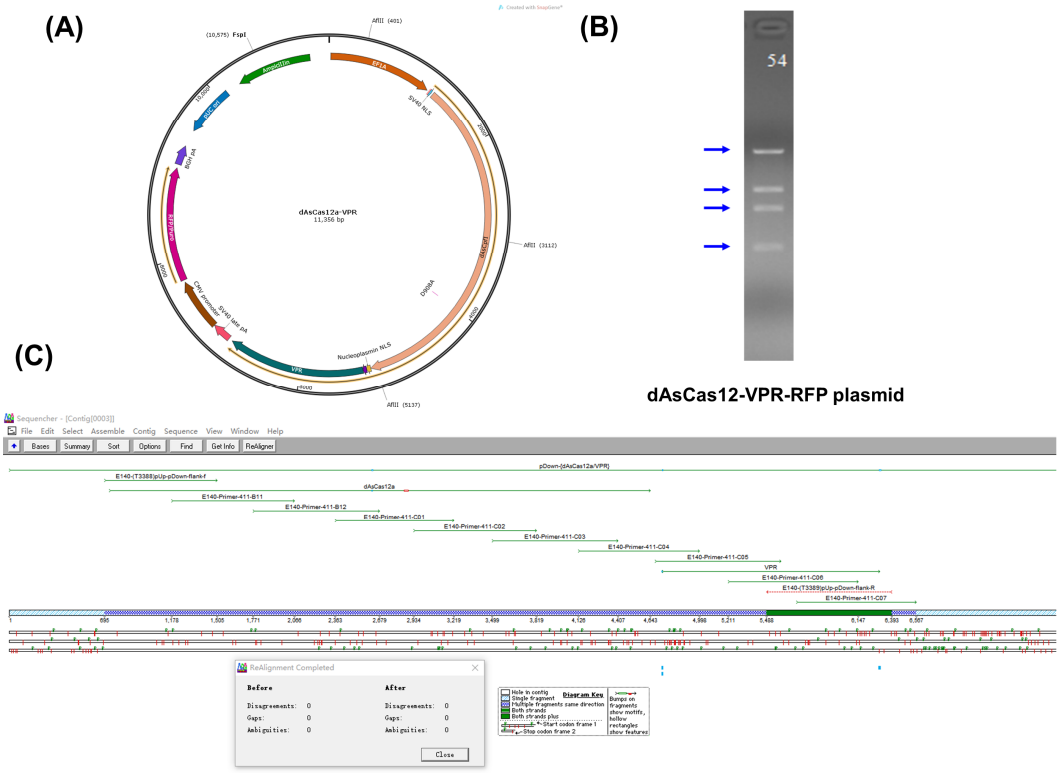

Figure S13 Characterization of dAsCas12a-VPR-RFP plasmid. (A) The plasmid map. The dAsCas12a-VPR protein was initiated by EF1A promoter and the RFP (Red Fluorescence Protein) was started by CMV promoter. (B) The result of restriction endonuclease treatment (FspI + AflII: 5438 bp, 2711 bp, 2025 bp, and 1182bp). The sites of endonuclease cleavage were labeled on plasmid map. (C) The consistency of sequencing results was evaluated by *Sequencer* software.

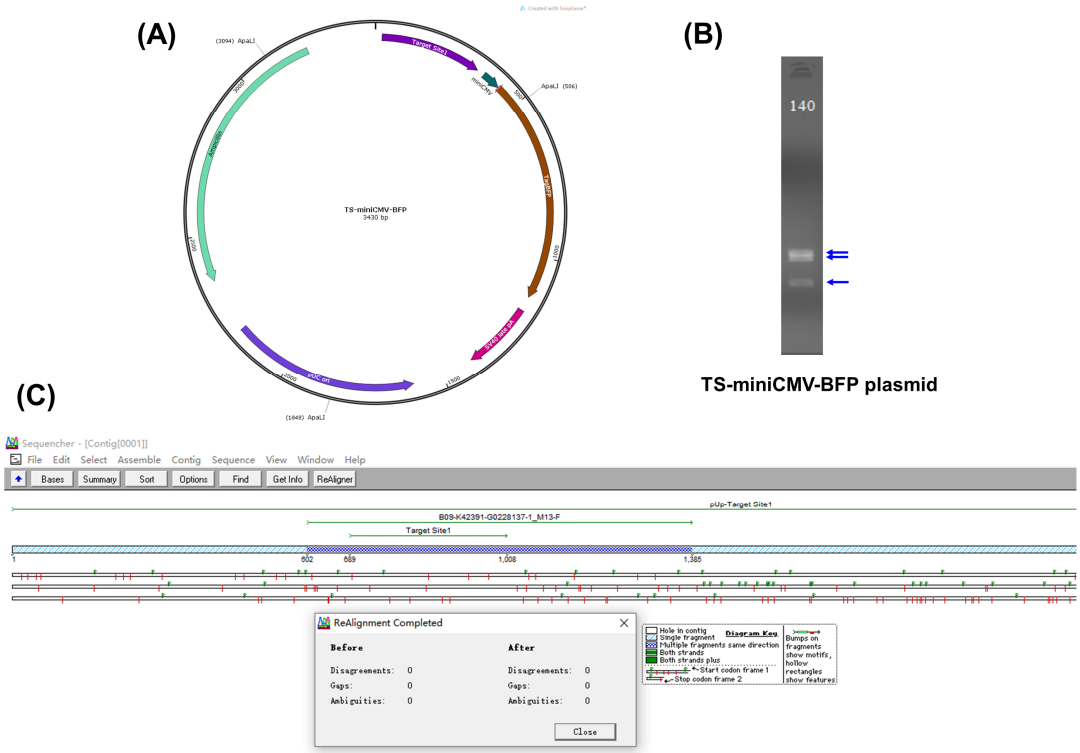

Figure S14 Characterization of TS-miniCM-BFP plasmid. (A) The plasmid map. (B) The result of restriction endonuclease treatment (ApaLI: 1342 bp, 1246 bp, 842 bp). The sites of endonuclease cleavage was labeled on plasmid map. (C) The consistency of sequencing results were evaluated by *Sequencer* software.

Confocal laser scanning microscopy (CLSM) real-time imaging demethylases in HEK293 cell lines

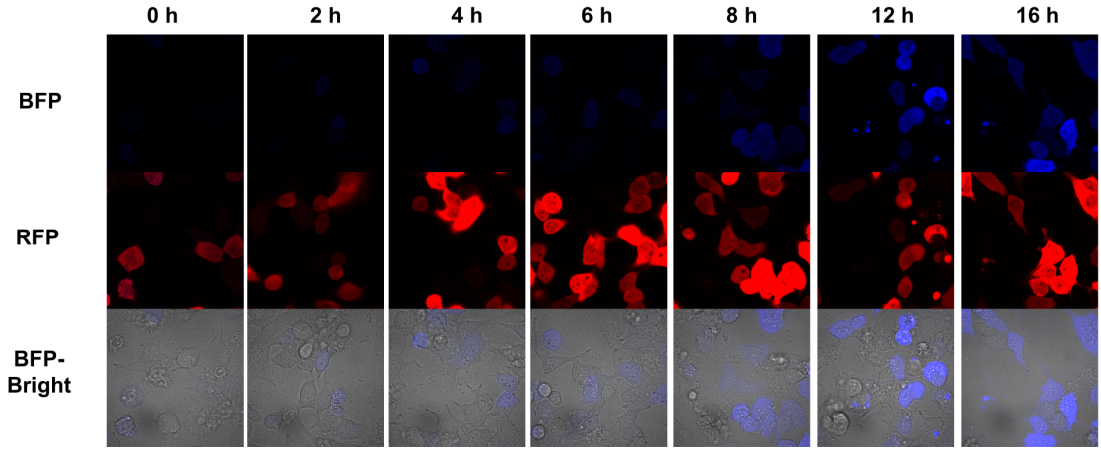

Figure S15 Images of demethylase activating m6A-deactivated CRISPR-dCas12a transcriptional circuits at different times. BFP and RFP represent the fluorescence of blue fluorescence protein and red fluorescence protein respectively. **Scale Bar: 20 μm.**

Western Blot analysis

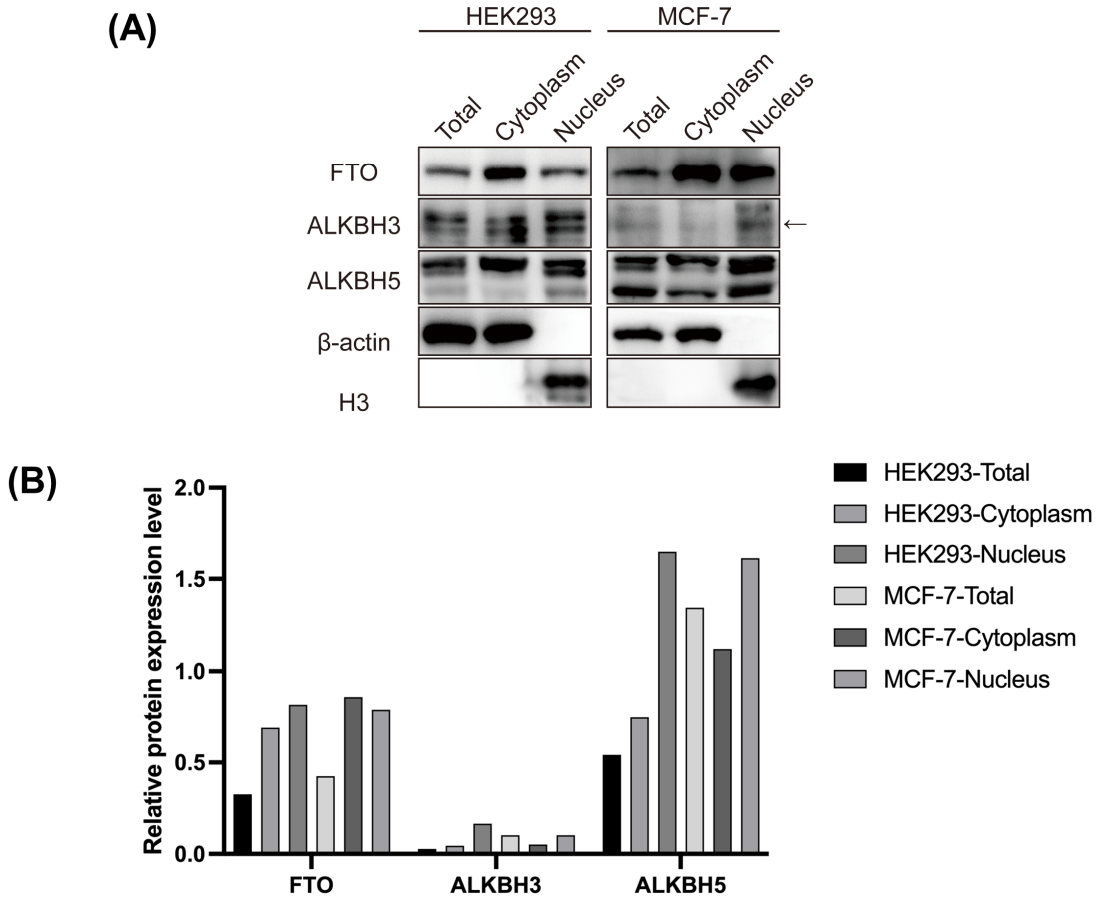

Figure S16 Western blot results of the cellular distribution of FTO, ALKBH5 and ALKBH3. (A) the images of different demethylases in HEK293 and MCF-7 cell lines. (B) the analysis of expression level. The results were analyzed by *ImageJ* software and the relative protein expression level was calculated by intensity of demethylase/ β -actin or demethylase/H3 in picture (A).

The analysis of qRT-PCR

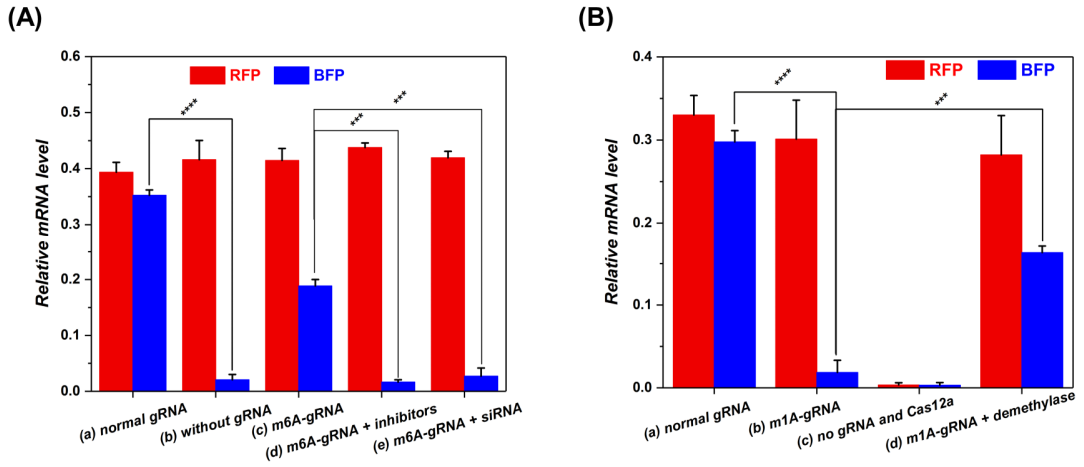

Figure S17 The analysis of mRNA levels of BFP and RFP by qRT-PCR. (A) The results of m6A -demethylases inducible CRISPR-dCas12a transcriptional circuits. (a) BFP-gRNA + dAsCas12a-VPR plasmid + miniCMV-BFP plasmid; (b) negative group: without gRNA; (c) BFP-m6A-gRNA + dAsCas12a-VPR plasmid + miniCMV-BFP plasmid; (d) pretreated by m6A demethylase's inhibitors: entacapone (80 μM) and IOX-1 (60 μM); (e) pretreated by siRNA before incubating with BFP-m6A-gRNA and two plasmids. (B) The results of m1A -demethylases inducible CRISPR-dCas12a transcriptional circuits. (a) BFP-gRNA + dAsCas12a-VPR plasmid + miniCMV-BFP plasmid; (b) BFP-m1A-gRNA + dAsCas12a-VPR plasmid + miniCMV-BFP plasmid; (c) only human ALKBH3 plasmid with GFP; (d) BFP-m1A-gRNA + dAsCas12a-VPR plasmid + miniCMV-BFP plasmid + human ALKBH3 plasmid with GFP. The error bars are the standard deviation of three parallel experiments. **** $P < 0.0001$ and *** $P < 0.001$ were obtained by Student's t-test.

As shown in Figure S17, the mRNA level changes of BFP are consistent with the fluorescence results shown in Figure 9. For m6A modified gRNA, the BFP mRNA level is lower than control group with normal gRNA, but higher than groups in the presence of inhibitors or siRNA of m6A demethylases. For m1A modifed gRNA, there is almost no BFP mRNA in cells compared with normal gRNA group. The m1A modification caused a dramatic transcriptional inhibition. To induce the de-repression of m1A modified gRNA, we transfected the m1A demethylases overexpressed plasmid into HEK293T cells. In Figure S17 B (d), the m1A demethylase could greatly activate the BFP target expression. In other words, the m1A modified gRNA has restored its activity of gene activation.

The dynamic fluorescence results of trans cleavage with m1A modification

(A)

gRNA	5'- UAAUUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU
BC-gRNA	5'- UA(m1A)$_2$UUUCU(m1A)$_8$CUCUUGU(m6A)$_{16}$G(m6A)$_{18}$UGCCAGG GACGAAGCGCAAGU
BR-gRNA-m1A	5'-UAAUUUCUACUCUUGUAGAUGCC(m6A)$_{22}$ GGG(m6A)$_{27}$CGA (m6A)$_{31}$ GCGCA(m6A)$_{37}$GU
8(m6A)-gRNA	5'- UA(m1A)$_2$UUUCU(m1A)$_8$CUCUUGU(m6A)$_{16}$G(m6A)$_{18}$UGCC(m6 A)$_{22}$ GGG(m6A)$_{27}$CGA (m6A)$_{31}$ GCGCA(m6A)$_{37}$GU

(B)

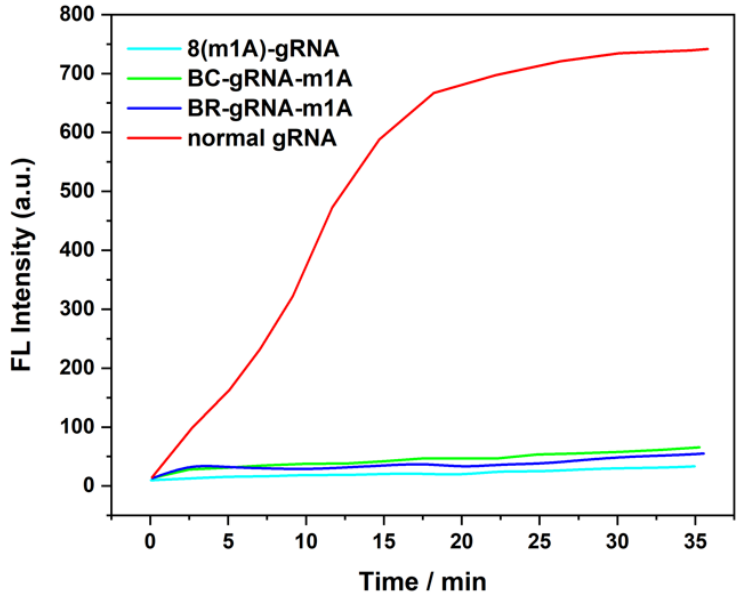

Figure S18 The effect of m1A modification on cleavage avtivity of CRIPR-Cas12a. (A) The sequence of m1A modified gRNA. (B) The kinetic curve of Cas12a with different gRNA trans cleaving FQ probe. BC-gRNA-m1A containing m1A at sites A_2, A_8, A_{16}, and A_{18}. BR-gRNA-m1A with m1A at sites A_{22}, A_{27}, A_{31}, and A_{37}. 8(m1A)-gRNA containing m1A at sites all above 8 positions.

As shown in ***Figure S18***, the BC-gRNA-m1A, BR-gRNA-m1A and 8(m1A)-gRNA exhibit significantly lower fluorescence intensity over time than normal gRNA. This phenomenon suggests that the three types of m1A-modified gRNA are capable of inhibiting the cleavage activity of CRISPR-Cas12a. Furthermore, modifying four m1A methylation sites in the recognition or conserved regions of gRNA resulted in an inhibition efficiency exceeding 90%.

From ***Figure S18 and Figure 1E***, there are significant differences in m1A and m6A effects on the cleavage activity of CRISPR-Cas12a.

(1) The m1A modifications in the recognition region of the gRNA (BR-gRNA-m1A) can completely inhibit the cleavage activity of CRISPR-Cas12a, whereas m6A only exhibits an inhibition efficiency of 40%.

(2) The m1A modifications in the conserved region of the gRNA (BC-gRNA-m1A) shows an inhibition efficiency of over 90%, while m6A can reach an inhibition efficiency of 70%. Although there are differences in their inhibition efficiencies, both modifications can effectively hinder the cleavage activity of CRISPR-Cas12a.

We speculate that the different effect of m6A and m1A modifications on cleavage activity of CRISPR-Cas12a from their distinct chemical structures and their influence on Watson-Crick pairing.

Table S1. The sequences of m6A modified gRNA.

Name	Sequence	5'-Modified	3'-Modified
gRNA	UAAUUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		
BR-gRNA	UAAUUUCUACUCUUGUAGAUGCC(m6A) GGG(m6A)CGA (m6A) GCGCA(m6A)GU		
BC-gRNA	UA(m6A)UUUCU(m6A)CUCUUGU(m6A)G(m6A)UGCCAGGGACGAAGCGCAAGU		
8(m6A)-gRNA	UA(m6A)UUUCU(m6A)CUCUUGU(m6A)G(m6A)UGCC(m6A)GGG(m6A)CGA(m6A)GCGCA(m6A)GU		
$m6A_2$	UA(m6A)UUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		
$m6A_3$	UAAUUUCU(m6A)CUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		
$m6A_4$	UAAUUUCUACUCUUGU(m6A)GAUGCCAGGGACGAAGCGCAAGU		
$m6A_5$	UAAUUUCUACUCUUGUAG(m6A)UGCCAGGGACGAAGCGCAAGU		
$m6A_{34}$	UAAUUUCU(m6A)CUCUUGU(m6A)GAUGCCAGGGACGAAGCGCAAGU		
$m6A_{345}$	UAAUUUCU(m6A)CUCUUGU(m6A)G(m6A)UGCCAGGGACGAAGCGCAAGU		
$m6A_{all}$	UA(m6A)UUUCU(m6A)CUCUUGU(m6A)G(m6A)UGCCAGGGACGAAGCGCAAGU		
FAM-$m6A_{345}$	UAAUUUCU(m6A)CUCUUGU(m6A)G(m6A)UGCCAGGGACGAAGCGCAAGU		3'-FAM
gRNA-FAM	UAAUUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		3'-FAM
BFP-gRNA	UAAUUUCUACUCUUGUAGAUACGACUCAGACCACCGCAUCA		
BFP-m6A-gRNA	UAAUUUCU(m6A)CUCUUGU(m6A)G(m6A)UACGACUCAGACCACCGCAUCA		

Note: m6A is N6-Me-rA.

Table S2. The sequences of m1A modified gRNA

Name	Sequence	5'-Modified	3'-Modified
gRNA	UAAUUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		
Con-m1A$_2$	UA**(m1A)**UUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		
Con-m1A$_3$	UAAUUUCU**(m1A)**CUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		
Con-m1A$_4$	UAAUUUCUACUCUUGU**(m1A)**GAUGCCAGGGACGAAGCGCAAGU		
Con-m1A$_5$	UAAUUUCUACUCUUGUAG**(m1A)**UGCCAGGGACGAAGCGCAAGU		
Con-m1A$_{34}$	UAAUUUCU**(m1A)**CUCUUGU**(m1A)**GAUGCCAGGGACGAAGCGCAAGU		
Con-m1A$_{345}$	UAAUUUCU**(m1A)**CUCUUGU**(m1A)**G**(m1A)**UGCCAGGGACGAAGCGCAAGU		
FAM-m1A$_{345}$	UAAUUUCU**(m1A)**CUCUUGU**(m1A)**G**(m1A)**UGCCAGGGACGAAGCGCAAGU		FAM
gRNA-FAM	UAAUUUCUACUCUUGUAGAUGCCAGGGACGAAGCGCAAGU		FAM
BFP-m1A-gRNA	UAAUUUCU**(m1A)**CUCUUGU**(m1A)**G**(m1A)**UACGACUCAGACCACCGCAUCA		

Table S3. The DNAs of *in vitro* experiments

Name	Sequence	5'-Modified	3'-Modified
FQ Probe	TTTTTT	FAM	BHQ-1
ssDNA Target	AGGTACCGTCACTTGCGCTTCGTCCCTGGCGAAA GAGGTTTACAACC		
dsDNA Target	TGG GTA ATC TGC CCT GCA CTC TGG GAT AAG CCT TGG AAA CGG GGT CTA ATA CCG GAT ATC ACA ATC TCT CGC ATG GGG GGT TGT TGA AAG TTC TGG CGG TGC AGG ATG AAC CCG CGG CCT ATC AGC TTG TTG GTG GGG TAG TGG CCT ACC AAG GCG ACG ACG GGT AGC CGG CCT GAG AGG GTG ACC GGC CAC ACT GGG ACT GAG ACA CGG CCC AGA CTC CTA CGG GAG GCA GCA GTG GGG AAT ATT GCA CAA TGG GCG CAA GCC TGA TGC AGC GAC GCC GCG TGA GGG ATG ACG GCC TTC GGG TTG TAA ACC TC**T TTC GCC AGG GAC GAA GCG CAA GT**G ACG GTA CCT GGA TAA GAA GCA CCG GCT AAC TAC GTG CCA GCA GCC GCG GTA ATA CGT AGG GTG CGA GCG TTG TCC GGA TTT ATT GGG CGT AAA GAG CTC GTA GGC GGT TTG TCG CGT CGG CCG TGA AAT CTC CAT GCT TAA CGT GGA GCG TGC GGT CGA TAC GGG CAG ACT TGA GTT CGG T		

Table S4. The primers of pRP[Exp]-Target Site-miniCMV>TagBFP Sanger Sequences

Name	Sequence	5'-Modified	3'-Modified
1427mcs-F1	CAA GTT TGT ACA AAA AAG CAG GC		
1427mcs-R1	CTG CGT TAT CCC CTG ATT CTG		
Seq-220331-1427mcs-PF1(809)	CACATGAAGCTGTACATGGAGGGCACCGTG		
Target Site 1-F1	CGT ATC ACG AGG CCC TTT CG		

Table S5. The primers of pRP[Exp]-RFP/Puro-EF1A>(dAsCas12a/VPR) Sanger Sequences

Name	Sequence	5'-Modified	3'-Modified
1172esn-F1	TTC TCA AGC CTC AGA CAG TGG		
Seq-220331-1172esn-PF1	CTG TTC AAT GGC AAA GTG CTG AAG CAA CTG		
Seq-220331-1172esn-PF2	AGT ACA AGA CCC TCC TCC GCA ACG AGA ATG		
Seq-220331-1172esn-PF3	ACC AAG AAG CCG TAT TCC GTG GAG AAG TTC		
Seq-220331-1172esn-PF4	ACC CAC TCC TGT ATC ACA TCT CGT TTC AGC		
Seq-220331-1172esn-PF5	GAG CGG AAC CTC ATC TAC ATC ACC GTC ATC		
Seq-220331-1172esn-PF6	AAA ACC GGA GAC TTC ATC CTG CAT TTC AAG		
Seq-220331-1172esn-PF7(1802)	CGC AAT AAG CGT CCT GCT GCC ACC AAA AAG		
Seq-220331-1172esn-PF8	AAC TAC GAC GAG TTC CCT ACC ATG GTG TTC		
Seq-220331-1172esn-PF9(782)	CTG TCT GGC GAC GAG GAC TTC AGC TCT ATC		
1172esn-R1	ATA ATC AAT GTC AAC GCG TAC C		
1172esn-F1	TTC TCA AGC CTC AGA CAG TGG		

Table S6. The sequences of ROIs in plasmid

Name	Sequence	5'-Modified	3'-Modified
VPR effector	gacgcattggacgattttgatctggatatgctgggaagtgacgccctcgatgattttgaccttgacatgcttggttcggatgcccttgatgactttgacc tcgacatgctcggcagtgacgcccttgatgatttcgacctggacatgctgattaactctagaagttccggatctccgaaaaagaaacgcaaagttgg tagccagtacctgcccgacaccgacgaccggcaccggatcgaggaaaagcggaagcggacctacgagacattcaagagcatcatgaagaagt ccccttcagcggccccaccgaccctagacctccacctagaagaatcgccgtgcccagcagatccagcgccagcgtgccaaaacctgcccccc agccttacccttcaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccccagcggccagatctctcaggcctctgctct ggctccagcccctcctcaggtgctgcctcaggctcctgctcctgcaccagctccagccatggtgtctgcactggctcaggcaccagcacccgtgc ctgtgctggctcctggacctccacaggctgtggctccaccagcccctaaacctacacaggccggcgagggcacactgtctgaagctctgctgca gctgcagttcgacgacgaggatctgggagccctgctgggaaacagcaccgatcctgccgtgttcaccgacctggccagcgtggacaacagcga gttccagcagctgctgaaccagggcatccctgtggcccctcacaccaccgagcccatgctgatggaataccccgaggccatcacccggctcgtg acaggcgctcagaggcctcctgatccagctcctgcccctctgggagcaccaggcctgcctaatggactgctgtctggcgacgaggacttcagctc tatcgccgatatggatttctcagccttgctgggctctggcagcggcagccgggattccagggaagggatgtttttgccgaagcctgaggccggctc cgctattagtgacgtgtttgagggccgcgaggtgtgccagccaaaacgaatccggccatttcatcctccaggaagtccatgggccaaccgcccac tccccgccagcctcgcaccaacaccaaccggtccagtacatgagccagtcgggtcactgaccccggcaccagtccctcagccactggatccag cgcccgcagtgactcccgaggccagtcacctgttggaggatcccgatgaagagacgagccaggctgtcaaagcccttcgggagatggccgata ctgtgattccccagaaggaagaggctgcaatctgtggccaaatggaccttcccatccgcccccaaggggccatctggatgagctgacaaccaca cttgagtccatgaccgaggatctgaacctggactcacccctgaccccggaattgaacgagattctggatacctttcctgaacgacgagtgcctcttgc atgccatgcatatcagcacaggactgtccatcttcgacacatctctgttt		
Target Site	tttgacgactcagaccaccgcatcaggcaaagctctatttgacgactcagaccaccgcatcaggccgtatgtcgaggtttgacgactcagaccacc gcatcaggaaaggaagcagccactttgacgactcagaccaccgcatcaggattacgttagtttgacgactcagaccaccgcatcagggaagtagt cttgcggtatttgacgactcagaccaccgcatcaggcaaagctctatttgacgactcagaccaccgcatcaggaaaggaagcagccactttgacg actcagaccaccgcatcaggaccatttgtcgagg		

ROI: **R**egion **O**f **I**nterest

Table S7. The sequence of dAsCas12a in plasmid

Name	Sequence	5'-Modified	3'-Modified
dCas12a	acgcagttcgaggggttcaccaacctctaccaggtcagcaagacgctgcggttcgagctgattccgcagggcaagaccctcaagcacatccagg agcagggctttatcgaggaggacaaagcgcggaacgaccactacaaggagctcaagccgatcatcgaccggatctacaagacctacgcggac cagtgcctgcagctcgtgcagctcgactgggagaacctctcggccgccatcgactcctaccgcaaggagaagacggaggagacacgcaacgc gctcatcgaagaacaggctacgtatcgcaacgctatccacgactacttcatcgggcgcacagataatctcaccgacgccatcaacaagcgccacg ccgaaatctataaggggctgtttaaggccgagctgttcaatggcaaagtgctgaagcaactgggcaccgtcacgaccacagagcatgagaacgc cctcctccggtcgttcgacaagtttacgacatactttagcggcttttacgagaaccgcaaaaacgtgttcagcgccgaggacattagcaccgccatc ccgcataggatcgtgcaagacaacttcccgaagttcaaggagaactgccacatcttcacccgcctcatcacggccgtgccgtccctccgcgagca cttcgagaacgtgaagaaggccatcgggatcttcgtgtccacctcgatcgaagaggtgttctccttccccttctacaatcagctgctgacgcagacc cagatcgacctctacaaccagctcctcggcggcatctcccgcgaggccggcaccgagaagatcaagggcctgaacgaggtgctcaatctcgcc atccagaagaacgacgaaaccgcgcacatcattgcctcactcccacataggtttatccccctgtttaagcagatcctctccgaccgcaacacgttgt ccttcatcctcgaggagttcaagagcgacgaggaggtcatccagtccttctgcaagtacaagaccctcctccgcaacgagaatgtgctcgaaacc gccgaggcgctgttcaatgagctcaatagcatcgatctcacccacatcttcatctcccataagaagctggaaaccatctccagcgccctgtgcgacc actgggacacccctccgcaacgccctctacgagcggcgcatcagcgagctcaccggcaagatcacgaagtcggcgaaagagaaagtgcaaag gagcctcaagcacgaggacattaacctgcaggagatcatctccgccgcgggcaaggagctgtccgaggctttcaagcagaagacctcggagat cctctctcacgcccacgcggccctggaccagccgctgccgacgaccctgaaaaagcaagaggaaaaggaaatcctcaagtcccagctggaca gcctcctggggctgtaccaccttctcgactggttcgccgtggacgagtccaacgaggtcgatccggagtttagcgcccgcctcaccgggatcaag cttgagatggaacctagcttgagcttctataataaggcgcgcaattacgcgaccaagaagccgtattccgtggagaagttcaagctgaacttccaaa tgcccaccctggccagcgggtgggacgttaacaaagagaagaacaacggagccattcttttcgtgaaaaatgggttgtattatttgggaattatgcc gaaacaaaaaggcaggtacaaggcgctcagcttcgagccaactgagaagacctccgagggcttcgacaagatgtactacgattattttcctgacg ctgcaaagatgataccgaagtgcagcactcagcttaaggcggtgacggcgcactttcagacccatacccaccccatcctcctctccaacaacttca tcgagccgctcgagatcaccaaggagatatacgatctgaataatccagaaaaggaacccaagaagttccagaccgcctacgccaagaagacgg gcgatcaaaaggggtatagagaggcgctctgcaagtggatcgacttcacgcgcgatttcctcagcaagtacaccaagacaacctccatcgatctct cttccctccgcccctcttcccagtacaaggacctcggggagtactacgccgaactcaacccactcctgtatcacatctcgtttcagcgtatcgcgga aaaggagatcatggacgccgtcgaaaccggcaagttgtatctttttccaaatctataacaaggacttcgcgaagggccaccacgggaagccaaac ctgcacaccctctactggacaggcctcttcagcccggaaaatctcgcgaagacgagcataaagctgaacggccaggcagaactcttctacaggc cgaagtccaggatgaagcgcatggctcatcgcctcggtgagaagatgctgaacaagaagctgaaagatcaaaagacgccaatccctgatacact gtatcaggagctgtacgattacgtgaaccaccgcctctcacacgacctcagcgacgaggcccgcgcgctcctgccaaacgtcatcacgaaggag gtcagccacgagatcataaaggatcggcggtttacctctgacaagttcttttttccatgtccccatcacgctgaactaccaggccgcgaatagcccgt ccaagttcaaccagcgggtcaacgcgtatctcaaggagcacccagagacacccataatcgggattgcccgggggggagcggaacctcatctaca tcaccgtcatcgactccaccggaaagattctcgagcaacggtcgctcaataccatccagcagttcgactaccagaagaagctcgacaaccggga gaaggaacgcgtcgccgcgaggcaggcctggtccgtagtgggcacgatcaaagacctgaagcagggctatctcagccaggtcatccatgagat agtggatctcatgatccactaccaagccgtcgtggtcctcgagaatctcaatttcggattcaaatccaagcgcacaggcatcgccgagaaggcggt gtaccaacagttcgagaaaatgcttatcgacaagctcaattgcctggtgctcaaggactatccggcggagaaggtcgggggggtcctcaatccgt atcagctgaccgaccagtttacgtcatttgcgaagatgggcacccagagcggcttcctttttctatgtcccggccccatatacctcaaagattgatccct tgaccggattcgtggacccgtttgtctggaagaccatcaagaaccatgagtcgcgtaagcatttcctggagggtttcgacttcctgcactatgatgta aaaaccggagacttcatcctgcatttcaagatgaatcggaacctctccttccagcggggactccctggcttcatgcccgcttgggatatcgtttttgag aaaaatgaaacccaattcgacgccaaaggcacgcctttcatcgcgggcaagaggattgtccctgtaattgagaaccatagattcaccgggcgttac cgtgacctgtaccccgcaaacgaactcatcgccctcctggaggagaaaggcatcgttttccgcgacgggtcaaatatcctccccaaactgctcga gaacgatgatagccacgctattgacacgatggtagcgctcatcagatccgtgctgcaaatgagaaattcaaatgctgccactggggaggattacat caactcccctgtgcgtgatctcaatggcgtgtgcttcgattctagatttcagaatcctgagtggccgatggatgccgatgctaacggcgcataccaca tagcattgaaaggacaactgttgttgaaccatctcaaggagagcaaggaccttaagctgcagaacggcatcagcaaccaggattggcttgcctata tccaagagctccgcaat		

Table S8. The sequence of ALKBH3 overexpressed plasmid

Name	Sequence	5'-Modified	3'-Modified
Human ALKBH3(NM_139178.4)	atggaggaaaaaagacggcgagcccgagttcagggagcctgggctgcccctgttaaaagccaggccattgctcagccagctaccact gctaagagccatctccaccagaagcctggccagacctggaagaacaaagagcatcatctctctgacagagagtttgtgttcaaagaacct cagcaggtagtacgtagagctcctgagccacgagtgattgacagagagggtgtgtatgaaatcagcctgtcacccacaggtgtatctag ggtctgtttgtatcctggctttgttgacgtgaaagaagctgactggatattggaacagctttgtcaagatgttccctggaaacagaggactgg catcagagaggatataacttatcagcaaccaagacttacagcatggtatggagaacttccttacacttattcaagaatcactatggaaccaa atcctcactggcaccctgtgctgcgcacactaaagaaccgcattgaagagaacactggccacaccttcaactccttactctgcaatctttat cgcaatgagaaggacagcgtggactggcacagtgatgatgaaccctcactagggaggtgccccattattgcttcactaagttttggtgcc acacgcacatttgagatgagaaagaagccaccaccagaagagaatggagactacacatatgtggaaagagtgaagataccctggatc atgggaccttgttaatcatggaaggagcgacacaagctgactggcagcatcgagtgcccaaagaatacctctagagaaccgagagt gaacctgacctttcggacagtctatccagaccctcgaggggcaccctggtga		

Table S9. The sequence of siRNA[9]

Name	Sequence	5'-Modified	3'-Modified
siRNA-FTO	UCUCACAAGCAGCGGCUAUUU		

Table S10. The qRT-PCR primers of BFP and RFP

Name	Sequence
Primer-F-BFP	GACGGCTGCCTCATCTAC
Primer-R-BFP	TCTTAGCGGGTTTCTTGG
Primer-F-RFP	GAGGCTGAAGCTGAAGGAC
Primer-R-RFP	GATGGTGTAGTCCTCGTTGTG

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