Supporting Information

N1-methyladenosine detection with CRISPR-Cas13a/C2c2

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1. Materials and methods

Materials and chemicals. All chemicals were purchased from Beijing Innochem Sci. & Tech. Co. Ltd. (Beijing, China) unless mentioned otherwise. Plasmid (p2CT-His-MBP-Lbu_C2c2_WT) of LbuCas13a is purchased from Addgene (Plasmid #83482). Plasmid of AlkB is the gift from Prof Chengqi Yi's group in Peking University. All of the DNA oligonucleotides were synthesized and purified by GeneCreate Co., Ltd. (Wuhan, China). All the RNA oligonucleotides were synthesized by Takara Biotechnology (Dalian, China). RevertAid Reverse Transcriptase, RNA Clean & Concentrator™ kits and TranscriptAid T7 High Yield Transcription Kits were acquired with Thermo Scientific™ (Thermo Scientific, USA). NTP (N = A, U, C, G) and dNTP (N = A, T, C, G) were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). DNA concentration was quantified by NanoDrop 2000c (Thermo Scientific, USA). Degradase Plus and enzyme reaction buffer were purchased from Zymo Research (Zymo Research, USA). Gel Imaging was monitored

with Pharos FX Molecular imager (Bio-Rad, USA). pH was measured with Mettler Toledo, FE20-Five Easy™ pH (Mettler Toledo, Switzerland). Fluorescent emission spectra were acquired with PerkinElmer LS 55 (PerkinElmer, USA).

LbuCas13a expression and purification. Expression vectors were transformed into Rosetta2 E. coli cells grown in 2×YT broth at 37 °C. E. coli cells were induced during log phase with 0.5 mM ITPG, and the temperature was reduced to 16 °C for overnight expression of His-MBP-Cas13a. Cells were subsequently harvested, resuspended in lysis buffer (50 mM Tris-HCl pH 7.0, 500mM NaCl, 5% glycerol, 1mM TCEP, 0.5 mM PMSF, and EDTA-free protease inhibitor (Roche)) and lysed by sonication, and the lysates were clarified by centrifugation. Soluble His-MBP-Cas13a was isolated over metal ion affinity chromatography, and protein-containing eluate was incubated with TEV protease at 4 °C overnight while dialyzing into ion exchange buffer (50mM Tris-HCl pH 7.0, 250 mM KCl, 5% glycerol, 1 mM TCEP) in order to cleave off the His6-MBP tag. Cleaved protein was loaded onto a HiTrap SP column and eluted over a linear KCl (0.25–1.5M) gradient. Cation exchange chromatography fractions were pooled and concentrated with 30kDa cutoff concentrators (Thermo Fisher). The Cas13a protein was further purified via size-exclusion chromatography on an S200 column and stored in gel filtration buffer (20mM Tris-HCl pH 7.0, 200 mM KCl, 5% glycerol, 1 mM TCEP) for subsequent enzymatic assays.

Synthesis of crRNAs. crRNA DNA was annealed to a short T7 primer (final concentrations 10 uM) and incubated with T7 polymerase overnight at 37 °C using the TranscriptAid T7 High Yield Transcription Kits. crRNAs were purified using RNA Clean & Concentrator™ kits.

A-ssRNA and m^{1}A-ssRNA fluorescence detection by Cas13a. Detection assays were performed with 39 nM purified LwCas13a (1.95 μM, 1 μL), 4 nM crRNA-18N (200 nM, 1 μl), 40 nM quenched fluorescent RNA reporter (10 μM, 2 μL), 4 nM of background random RNA, 4 nM input nucleic acid target A-ssRNA (200 nM, 1 μL) or m^{1}A-ssRNA (200 nM, 1 μL), in nuclease assay buffer (40 mM Tris-HCl, 60 mM NaCl, 6 mM $MgCl_2$, pH 7.3, 5 μL) and 40 μL water. crRNA was incubated with target A-ssRNA, m^{1}A-ssRNA and reporter RNA at room temperature for 5 min, respectively. And then Cas13a was added allowing this mixture for 10 min at 37 °C, 65 °C for 10 min.

crRNA selection of m^{1}A detection. crRNA-18, crRNA-18N, crRNA-20, crRNA-20N and reporter RNA was incubated with target A-ssRNA and m^{1}A-ssRNA at room temperature for 5 min, respectively. And then Cas13a was added allowing this mixture for 10 min at 37 °C, 65 °C for 10 min.

Temperature selection for Cas13a cleavage. crRNA-18 and reporter RNA was incubated with target A-ssRNA and m^{1}A-ssRNA at room temperature for 5 min, respectively. And then Cas13a was added allowing this mixture for 10 min at 25 °C, 30 °C, 37 °C and 40 °C, respectively. The mixture then was keeping 65 °C for 10 min.

Time selection for Cas13a cleavage. crRNA-18 and reporter RNA was incubated with target A-ssRNA and m^{1}A-ssRNA at room temperature for 5 min, respectively. And then Cas13a was added

allowing this mixture for at 37 °C for 0 min, 1 min, 3 min, 8 min, 15 min and 20 min, respectively. The mixture then was keeping 65 °C for 10 min.

Sensitivity of m^{1}A-ssRNA detection by Cas13a. crRNA-18 and reporter RNA was incubated with target A-ssRNA (40 pM, 4 pM, 40 fM, 4 fM) and m^{1}A-ssRNA (40 pM, 4 pM, 40 fM, 4 fM) at room temperature for 5 min, respectively. And then Cas13a was added allowing this mixture for at 37 °C for 10 min, 65 °C for 10 min.

Quantitating m^{1}A-ssRNA by Cas13a. m^{1}A-ssRNA and A-ssRNA were mixed with different molar ratio (0:10, 1:9, 3:7, 5:5, 7:3, 9:1, 10:0). The mixture RNA was incubated with crRNA-18 and reporter RNA at room temperature for 5 min, respectively. And then Cas13a was added allowing this mixture for at 37 °C for 10 min, 65 °C for 10 min.

Expression and purification of Recombinant AlkB. A truncated AlkB with deletion of the N-terminal 11 amino acids was cloned into pET30a (Novagen), transformed to E. coli BL21(DE3), A 2 μL starter culture was grown overnight in Terrific Broth growth media (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) (LB), which was used to inoculate 5 mL of LB for growth at 37 °C and 200 RPM until the OD600 reached 0.6–0.8. At this time, protein expression was induced by supplementation with IPTG (Sigma) to a final concentration of 1 mM, and cells were cooled to 25 °C for 12 h for protein expression. Cells were then centrifuged at 8000 g for 5 min at 4 °C. Cell pellet was crushed and resuspended in lysis buffer A (500 mM NaCl, 1 mM DTT , 5 % Glycerol , 10 mM Tris-HCl , pH 7.2) supplemented with 1 mM protease inhibitors, followed by sonication with the following conditions: amplitude of 35 for 5 second on and 5 seconds off with a total sonication time of 30 min. Lysate was cleared by centrifugation for 30 min at 4 °C at 10,000 g. Supernatant was applied to 200 μL Ni-NTA chromatography at 4 °C for 1h with 70RPM, then 3000 g for 5 min. The pecitate was followed by gel-filtration chromatography with 200 mM imidazole wash buffer (500 mM NaCl, 1 mM DTT, 5 % Glycerol, 10 mM Tris-HCl, pH 7.2) and Mono-Q anion exchange chromatography (GE Healthcare). Digestion was confirmed by SDS-PAGE and Coomassie Blue staining. Such purification procedure effectively avoided RNA contamination from E. coli (expression host).

In vitro demethylation treatment. In vitro demethylation mediated by the purified AlkB protein: Total RNA was isolated by Trizol reagent following the manufacturer's guidelines, 10 μg full-length total RNA was fragmented at 75 °C for 15 min using magnesium RNA fragmentation buffer (NEB) and fragmented total RNA was desalted and concentrated by ethanol precipitation.10 μg fragmented total RNA was denatured at 65 °C for 5 min, and then subjected to demethylation reaction in a 500 mL demethylation mixture containing 0.4 nmol purified AlkB, 50 mM MES, pH 6.5, 283 mM of $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$, 300 mM 2-ketoglutarate, 2 mM L-ascorbic acid, 1 U/mL SUPERaseIn RNase Inhibitor. The demethylation reaction was incubated at 37 °C for 2 h and quenched by adding 5 mM EDTA. The demethylated RNA was then purified by phenol chloroform extraction.

m^{1}A and m^{6}A analysis by HPLC-MS. 200 ng (+) and (-) AlkB fragmented total RNA were digested into nucleosides by 0.5 U nuclease P1 in 20 mL buffer containing 10 mM ammonium

acetate, pH 5.3 at 42 °C for 6 h, followed by the addition of 2.5 mL 0.5 M MES buffer, pH 6.5 and 0.5 U alkaline phosphatase. The mixture was incubated at 37 °C for another 6 h and diluted to 50 mL. 5 mL of the solution was injected into HPLC-MS. The nucleosides were separated by ultra-performance liquid chromatography with a C18 column, and then detected by triple-quadrupole mass spectrometer (AB SCIEX QTRAP 5500) in the positive ion multiple reaction-monitoring (MRM) mode. The mass transitions of m/z 282.0 to 150.1 (m^1A), m/z 282.0 to 150.1 (m^6A), m/z 268.0 to 136.0 (A) were monitored and recorded.

Reverse transcription analysis. 1 μg (+) and (-) AlkB fragmented total RNA were incubated with FAM labeled primer 28s-P (1 μM, 1 μL) at 65 °C for 5 min, then drop one degree per 30 s. Then, 2 μL of reverse transcription buffer, 1 μL 0.1 M DTT, 1 μL 5 mM dNTPs and 1.5 μL RevertAid Reverse Transcriptase (200 U/μL) were added to get the total volume of 10 μL. The reverse transcription was performed at 42 °C for 15 min, and then 20 μL of deionized formamide was added. The temperature was immediately heated up to 95 °C for 10 min, then turned down to 4 °C. The cDNAs were size fractionated by 8 M urea 20 % denaturing polyacrylamide gel. The gel was scanned with Pharos FX Molecular imager operated in the fluorescence mode (λex = 488 nm).

28S rRNA fluorescence analysis by Cas13a detection. 4 μg (+) and (-) AlkB fragmented total RNA were incubated with crRNA-28s (1 μM, 1 μL) at 65 °C for 5 min, then drop one degree per 30 s, respectively. then LwCas13a (1.95 μM, 1 μL) and 40 nM quenched fluorescent RNA reporter (10 μM, 2 μL) were added allowing this mixture at 37 °C for 10 min, 65 °C for 10 min.

2. Temperature selection for Cas13a cleavage.

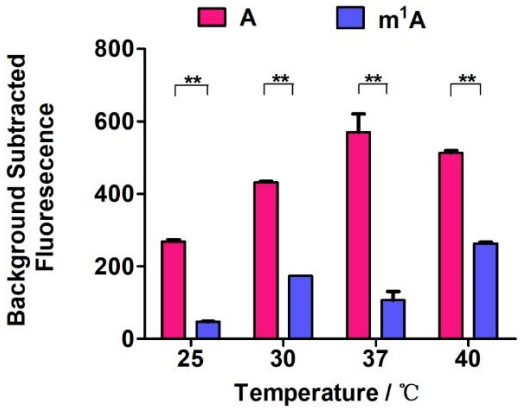

Fig. S1 Temperature-dependent emission intensity of A-ssRNA and m^1A-ssRNA using Cas13a. (n=3 technical replicates, two-tailed Student t test; **, $p < 0.01$; bars represent mean ± s.e.m.)

3. Time selection for Cas13a cleavage.

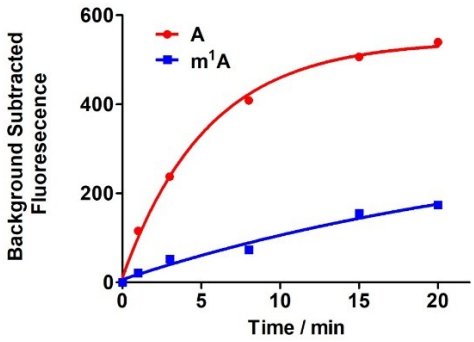

Fig. S2 Time-dependent emission intensity change at 520 nm of A-ssRNA and m^1A-ssRNA via Cas13a.

4. Expression and purification of Cas13a

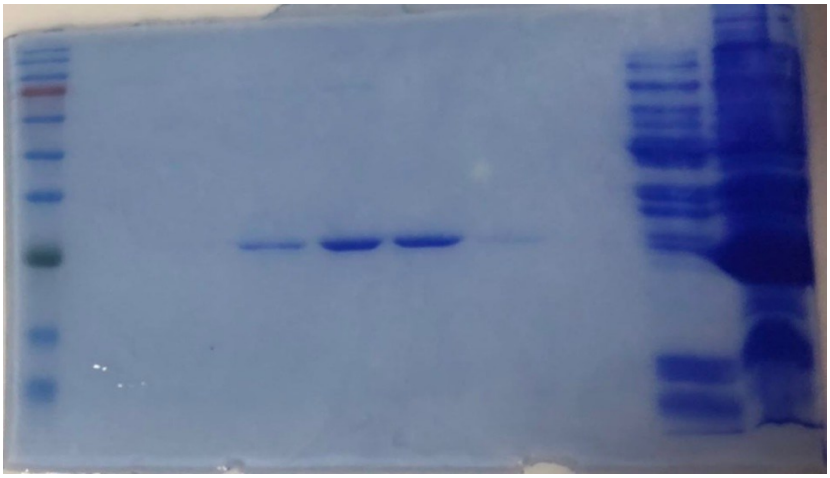

Fig. S3 SDS-PAGE and Coomassie Blue staining of final size exclusion of AlkB protein.

5. m^1A and m^6A analysis by HPLC-MS.

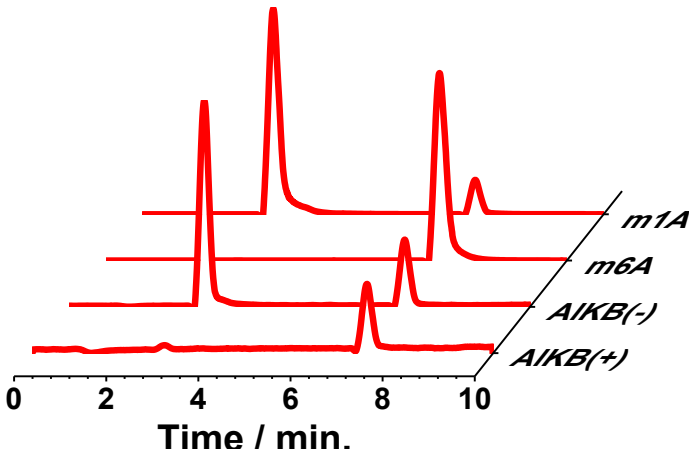

Fig. S4 HPLC-MS extracted $[M+H]^+$ ion count for standard m^1A, standard m^6A, and oxynucleosides after digestion of (+) and (-) AlkB fragment RNA.

6. Reverse transcription analysis.

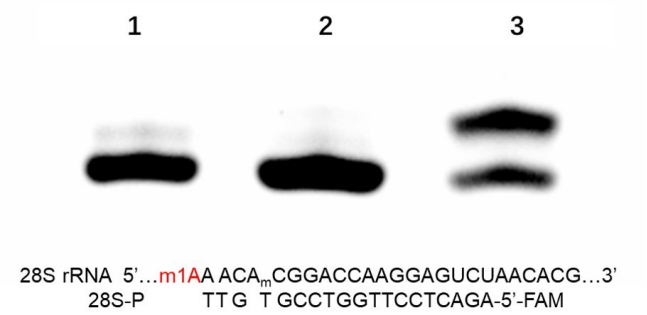

Fig. S5 Gel assay of the reverse transcription of (+) and (-) AlkB fragmented total RNA. Lane 1 shows primer 28s-P, lane 2 shows (-) AlkB fragment RNA, lane 3 shows (+) AlkB fragment RNA.

7. Fluorescence analysis of 28S rRNA

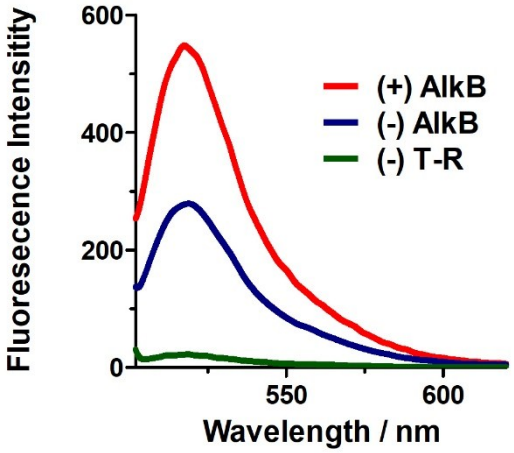

Fig. S6 Fluorescence spectra of the detection of target 28S rRNA with (+) and (-) AlkB and

no target RNA via the Cas13a collateral detection.

8. Reverse transcription analysis of dynamic m^{1}A demethylation of 28S rRNA.

Fig. S7 Gel assay of the reverse transcription of (+) AlkB fragmented total RNA. Lane 1 and 7 shows primer 28s-P, lane 2 shows fragment RNA treated with AlkB for 10 min; lane 3 shows fragment RNA treated with AlkB for 30 min; lane 4 shows fragment RNA treated with AlkB for 1 h; lane 5 shows fragment RNA treated with AlkB for 2 h; lane 6 shows fragment RNA treated with AlkB for 0 min;

9. The sequences used for this study

Table S1. DNA and RNA sequences used in this study.

Name	Sequence
crRNA-18N DNA template	5'-CTGGACTATGGAATTCTCTGTTTTAGTCCCCTTCATTTTTGGGGTGGTCCCTATAGTGAGTCGTATTA-3'
crRNA-18 DNA template	5'-CTGGACAATGGAATTCTCTGTTTTAGTCCCCTTCATTTTTGGGGTGGTCCCTATAGTGAGTCGTATTA-3'
crRNA-20N DNA template	5'-ACTGGACTATGGAATTCTCGTGTTTTAGTCCCCTTCATTTTTGGGGTGGTCCCTATAGTGAGTCGTATTA -3'
crRNA-20 DNA template	5'-ACTGGACTATGGAATACTCGTGTTTTAGTCCCCTTCATTTTTGGGGTGGTCCCTATAGTGAGTCGTATTA-3'
crRNA-28s DNA template	5'-CCCGACTTGAAACACGGTCCGTTTTAGTCCCCTTCGTTTTTGGGGTTGGTCCCTATAGTGAGTCGTATTA-3'
T7 DNA promoter	5'-TTATACGACTCACTATAG-3'
reporter RNA	5'-FAM-UUUUU-BHQ1-3'
DNA primer	5' FAM-AGACTCCTTGGTCCGTGTT- 3'
A-ssRNA	5'-GGACUGGACUAUGGAAUUCUCGGGUGCCAAGG-3'
m^{1}A-ssRNA	5'-GGACUGGm^{1}ACUAUGGAAUUCUCGGGUGCCAAGG-3'
crRNA-18N	5'-GGACCACCCCAAAAAUGAAGGGGACUAAAACAGAGAAUUCCAUAGUCCAG-3'
crRNA-18	5'-

	GGACCACCCCAAAAAUGAAGGGGACUAAAACAGAGAAUUCCAUUGUCCAG-3’
crRNA-20N	5’-GGACCACCCCAAAAAUGAAGGGGACUAAAACACGAGAAUUCCAUAGUCCAGU-3’
crRNA-20	5’-GGACCACCCCAAAAAUGAAGGGGACUAAAACACGAGUAUUCCAUAGUCCAGU-3’
crRNA-28s	5’-GGACCACCCCAAAAAUGAAGGGGACUAAAACGGACCGUGUUUCAAGUCGGG-3’

Reference

[1] X. Li, X. Xiong, M. Zhang, K. Wang, C. Ying, J. Zhou, Y. Mao, L. Jia, D. Yi and X. W. Chen, *Molecular Cell*, 2017, **68**, 993-1005.

[2] A. Eastseletsky, M. R. O’Connell, S. C. Knight, D. Burstein, J. H. D. Cate, R. Tjian and J. A. Doudna, Nature, 2016, **538**, 270-273.