Supplementary Material for

Sequence-specific m^{6}A demethylation in RNA by FTO fused to RCas9

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SUPPLEMENTARY FIGURES

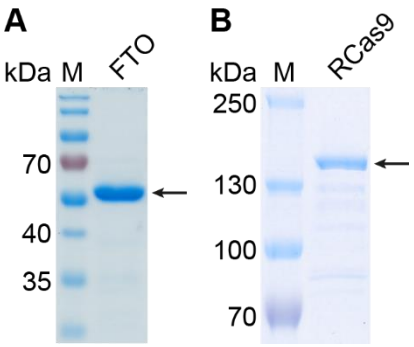

Figure S1: SDS-PAGE analysis of purified FTO (A) and RCas9 (B).

Both SDS-polyacrylamide gels (12% for A, 8% for B) were stained with Coomassie blue G-250. Expected size for FTO: 57 kDa. Expected size for RCas9: 159 kDa.

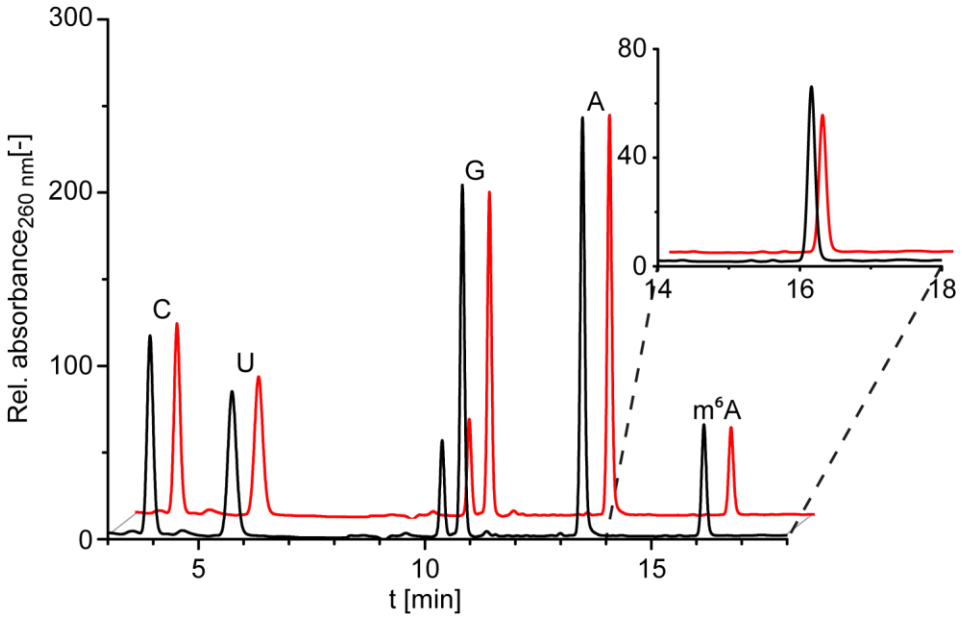

Figure S2: HPLC analysis of FTO activity under RCas9-FTO conditions.

Chromatogram of HPLC analysis of activity assays using 1 µM FTO and 10 µM 15 nt-m^{6}A RNA after digestion into nucleosides. The reaction times 0 h (black) and 1 h (red) are shown, indicating ~16% demethylation (two biological replicates). Inset shows a magnification of m^{6}A peaks between 14-18 min. This Figure is a supplement to Fig. 1C for RCas9-FTO in the main text.

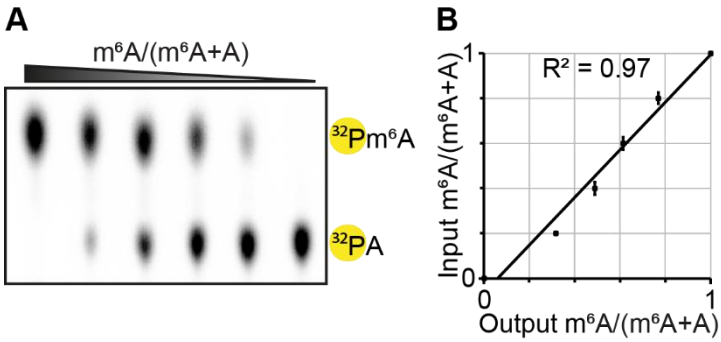

Figure S3: Analysis of the established SCARLET assay.

For SCARLET, two control RNAs were mixed in defined ratios, one containing A and one m^6A at a defined position. A) TLC showing separate spots for m^6AMP and AMP. Samples with decreasing ratios of $m^6A/(m^6A+A)$ were loaded. B) Evaluation of used (input) and measured (output) $m^6A/(m^6A+A)$ ratios from SCARLET assay. Datapoints and error bars show average and standard deviation of three independent experiments. Linear regression was used to determine the coefficient of determination (R^2= 0.97).

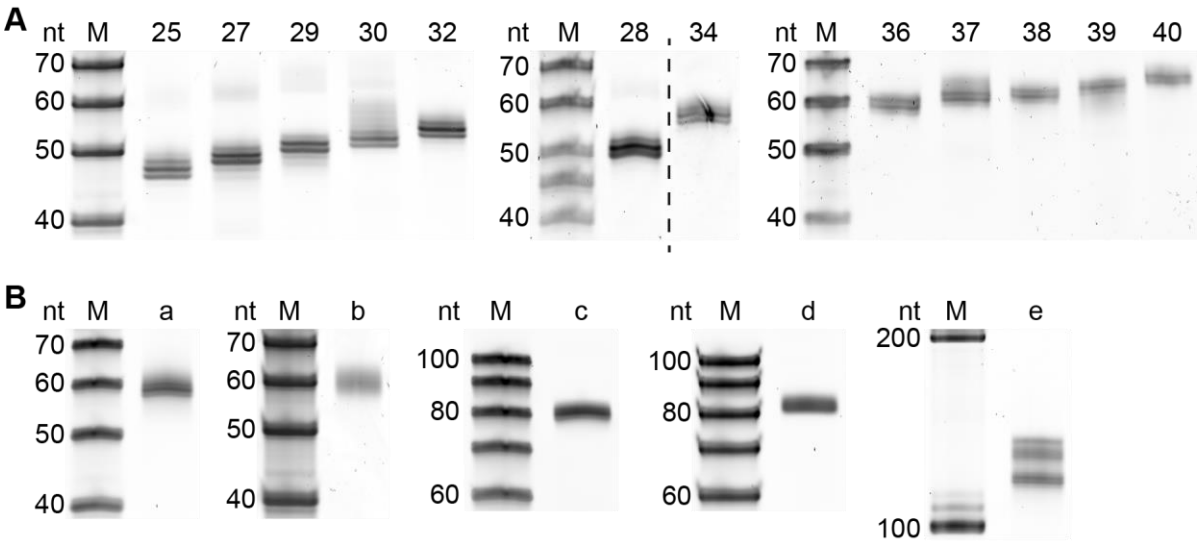

Figure S4: Urea TBE PAGE analysis of RNAs from IVT and splint ligation.

Denat. polyacrylamide gels were stained with SYBR Gold. A) The purified RNAs 25 nt to 40 nt are resolved on a 15% gel (expected sizes: 41-56 nt). B) The 15% gels show the purified *in vitro* transcripts of *M2515mut* (a) and *M2515* (b; expected size for both: 53 nt) as well as the purified splint ligation products of *M2515mut* (c) and *M2515* (d; expected size for both: 73 nt). The 10% gel on the right shows the purified sgRNA2 (e; expected size: 116 nt).

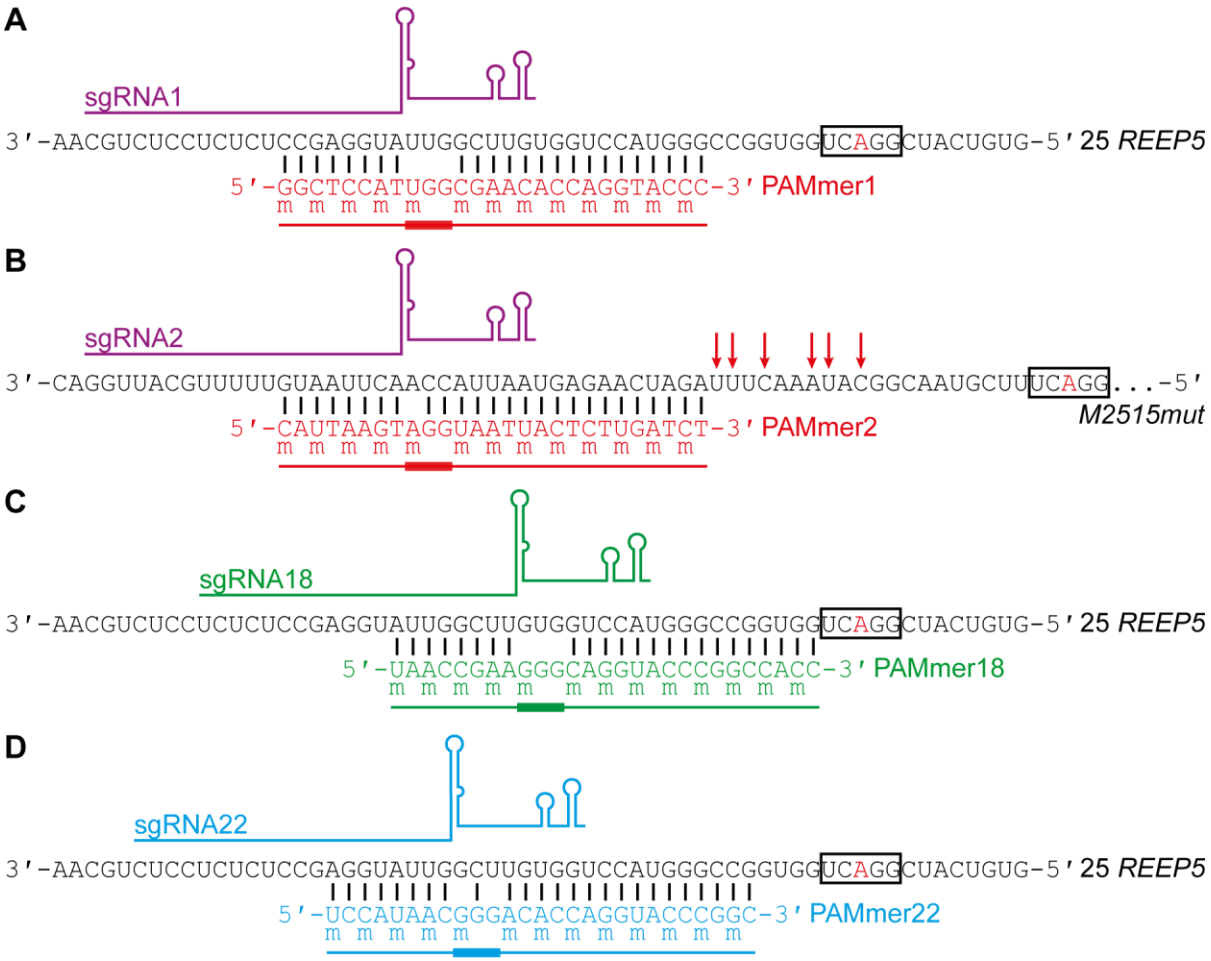

Figure S5: Scheme of PAMmer and sgRNA interactions.

A-D) Interactions of sgRNAs and PAMmers with their respective target RNAs containing m^6A (marked in red) in DRACH motif (black box). All PAMmers contain an 8 nt 5′-sequence part complementary to the protospacer, a 3 nt-long PAM (bold part of the line) and a 16 nt-long 3′-sequence part. The PAMmer is composed of deoxyribonucleotides and 2′-*O*-methylated ribonucleotides (m). The PAM is either completely or partially non-complementary to the target RNA. Red arrows indicate mutated nucleotides in *M2515mut* RNA.

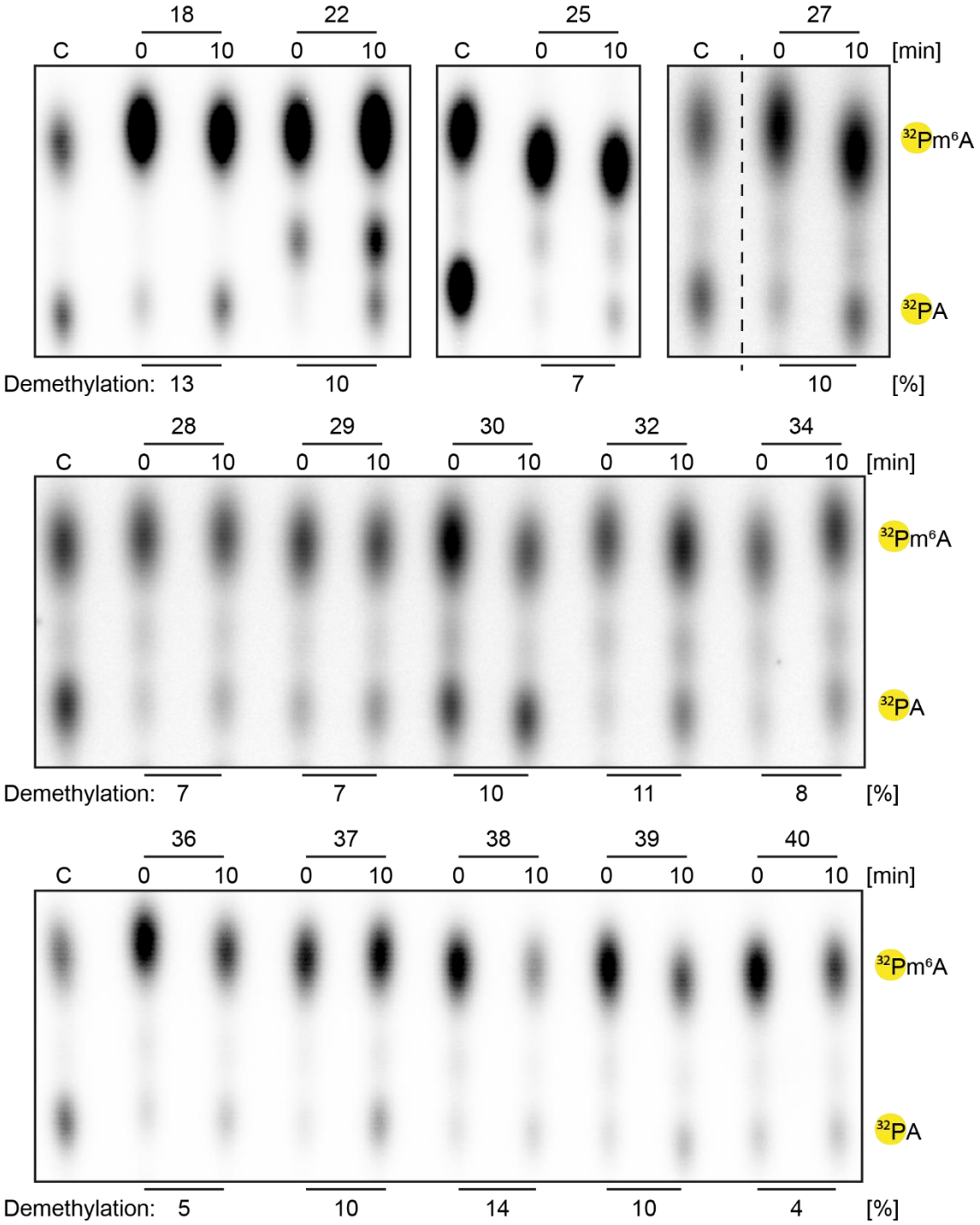

Figure S6: SCARLET analysis of PAM-to-m^{6}A distance measurements.

Representative TLCs of SCARLET analysis of RCas9-FTO activity assays with 25-40 nt *REEP5* RNAs and sgRNAs/PAMmers 1, 18 and 22. Each TLC shows a control lane (C) to indicate m^{6}AMP (upper) and AMP (lower) spots. For each distance, the m^{6}A/(m^{6}A+A) ratio is evaluated for the 0 min and 10 min assay sample. The demethylation is calculated normalized to the respective 0 min value and indicated below the TLCs.

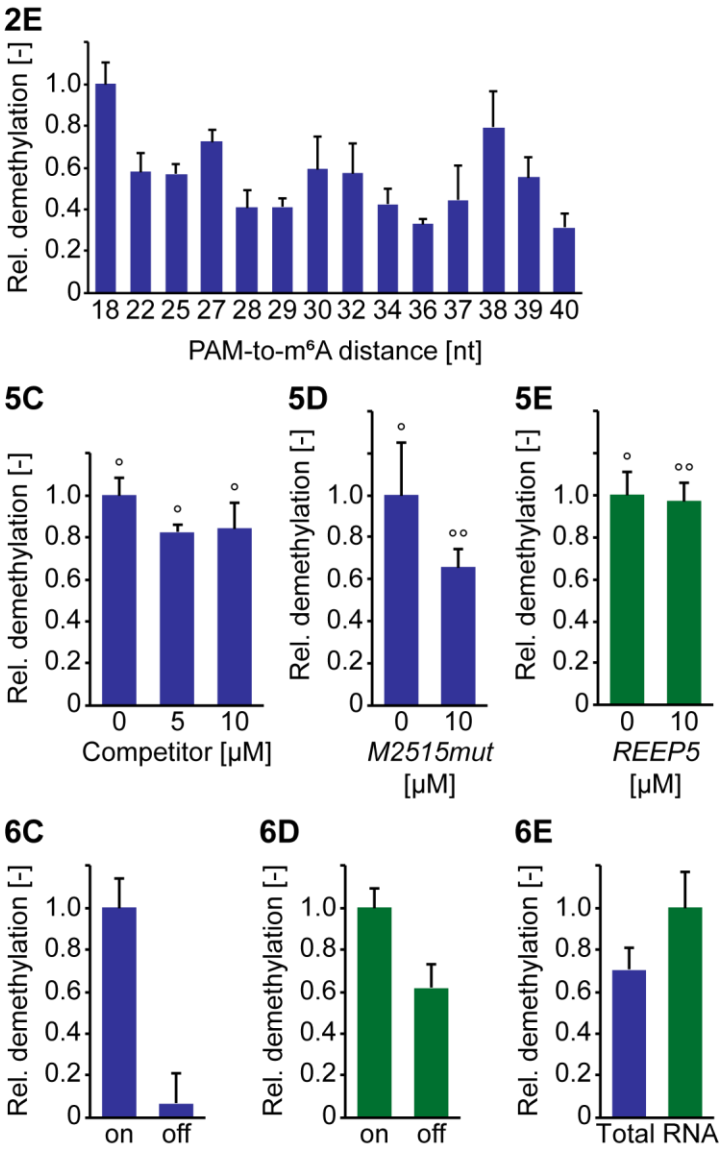

Figure S7: Diagrams of Figure 2E, 5C-E and 6C-E with normalized values.

2E) Diagram of RCas9-FTO demethylation activity on different *REEP5* RNA constructs determined by SCARLET and normalized to the 18 nt distance value. Bars show average and standard deviation of three independent biological replicates. 5C-E) Effect of addition of an unspecific competitor RNA on demethylation activity of RCas9-FTO. Demethylation shown in the diagrams was determined by SCARLET and normalized to the value without competitor. Bars show mean and standard deviation from three independent biological replicates (°-marked, 10 min incubation) or two independent biological replicates, each performed in two independent technical replicates (°°-marked, 30 min incubation). 6C-D) Analysis of on- and off-target effects of sequence-specific demethylation in a stoichiometric mixture of target and non-target RNA normalized to on-target demethylation. 6E) Effect of sequence-specific demethylation of *REEP5* and *M2515mut* in cellular total RNA normalized to *M2515mut* demethylation. 6C-E) Bars show average and standard deviation of two independent biological replicates, each performed in two independent technical replicates. In all diagrams *REEP5* is shown in blue and *M2515mut* in green.

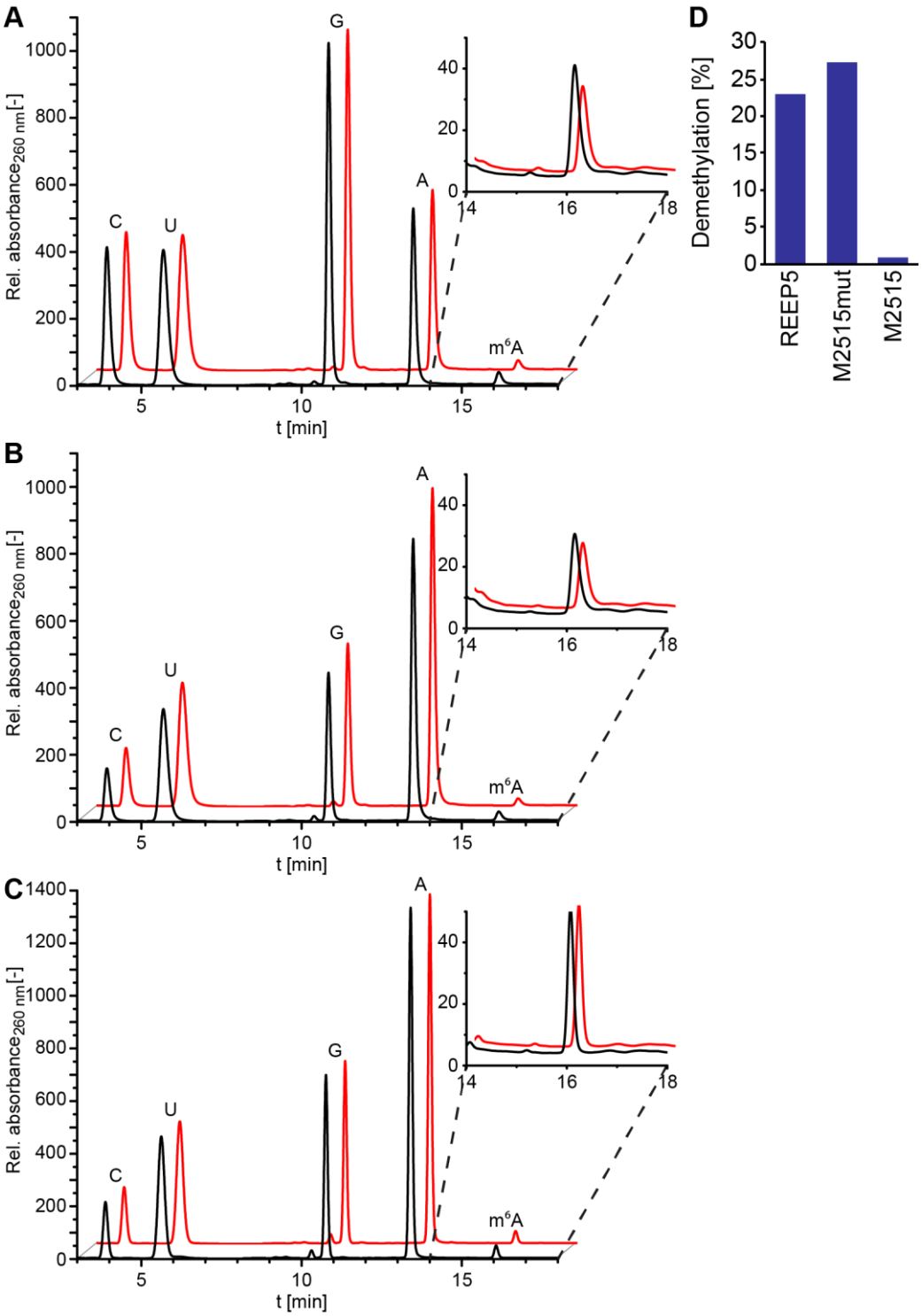

Figure S8: Analysis of FTO activity on *REEP5* and *MALAT1*.

A-C) Chromatograms of HPLC analysis of activity assays using 5 µM FTO and 10 µM RNA. The reaction times 0 h (black) and 1 h (red) are shown. A) The chromatogram indicates 23% demethylation of 38 nt *REEP5* RNA. In B), a demethylation of 27% of *M2515mut* was calculated, whereas the natural *M2515 MALAT1* RNA was 1% demethylated (C). Insets show a magnification of m^6A peaks between 14-18 min. D) Comparison of calculated demethylation values.

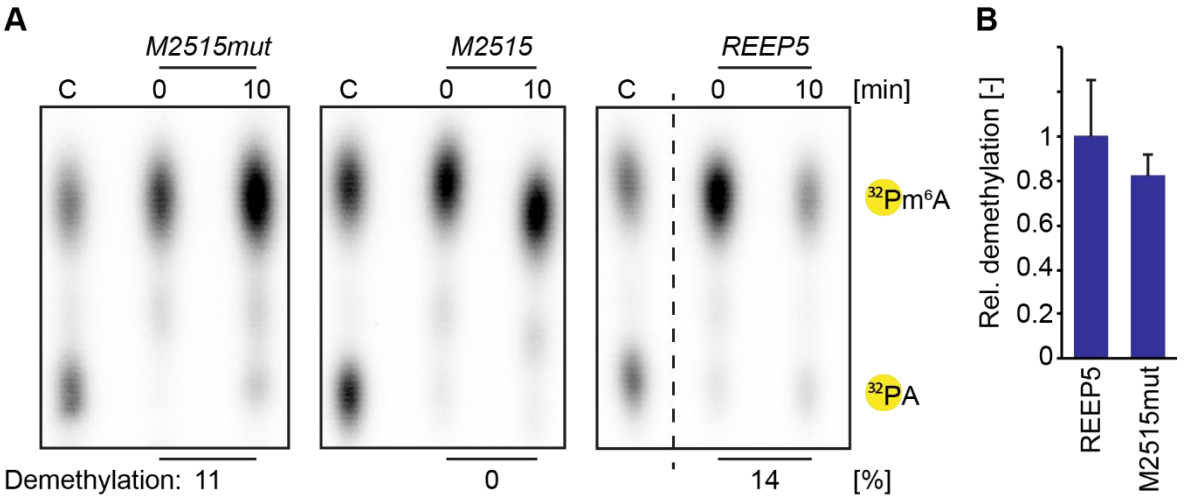

Figure S9: SCARLET analysis of RCas9-FTO activity on *MALAT1* and *REEP5* RNAs.

A) Representative TLCs of RCas9-FTO activity on *M2515mut*, *M2515* and 38 nt *REEP5* RNA. Each TLC shows a control lane (C) to indicate m^6AMP (upper) and AMP (lower) spots. B) Analysis of *M2515mut* demethylation normalized to demethylation of the 38 nt *REEP5* RNA. Data and error bars show average and standard deviation of three independent biological replicates.

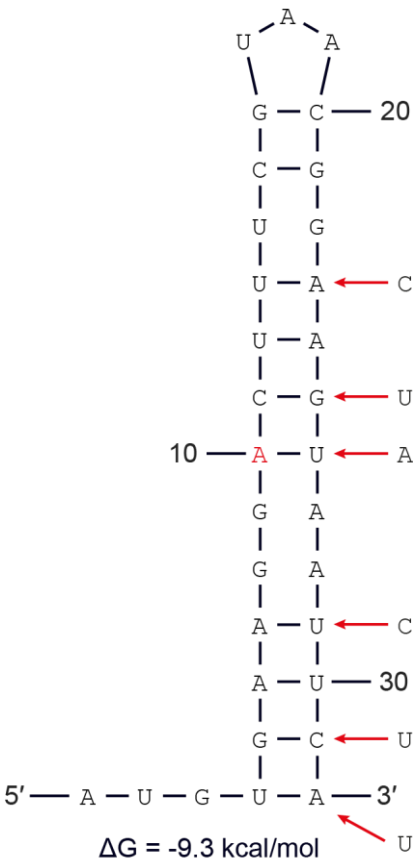

Figure S10: Secondary structure of *M2515 MALAT1* RNA.

The secondary structure of *M2515 MALAT1* forms a stem-loop structure with m^6A (marked in red) in the stem (Liu *et al.* 2013, Liu *et al.* 2015, Zhou *et al.* 2016) as calculated by mfold (Zuker 2003). To avoid stem-loop formation six mutations were introduced at the indicated positions (red arrows).

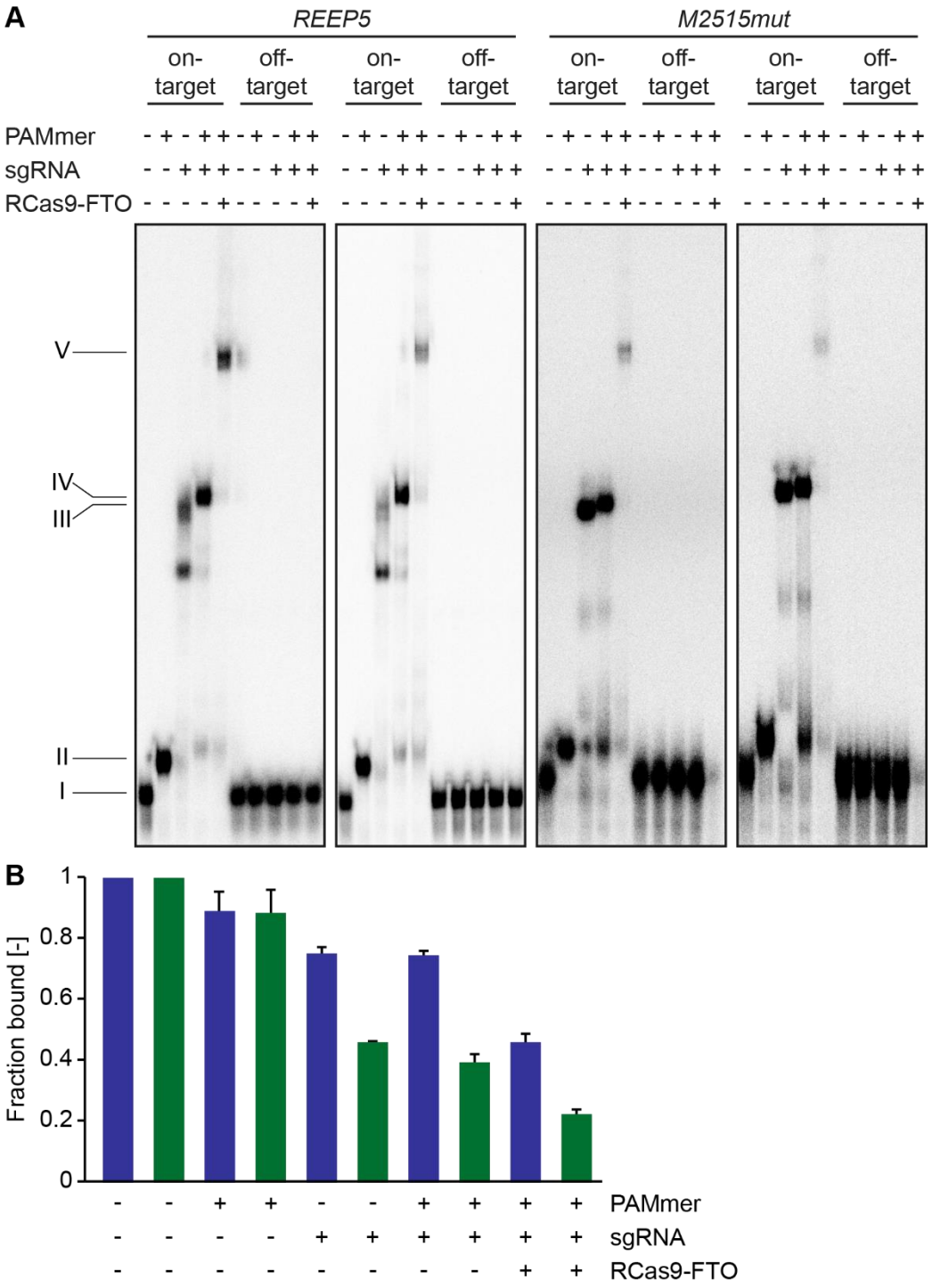

Figure S11: Binding studies of sequence-specific interaction of RCas9-FTO with *REEP5* and *M2515mut* RNAs.

A) EMSA experiments with *REEP5* (left part) and *M2515mut* (right part) target RNAs with PAM-to-m^{6}A distance of 38 nt. For binding of *REEP5*, the sgRNA1/PAMmer1 combination was used, whereas for the off-target samples the sgRNA2/PAMmer2 were used. For the *M2515mut* RNA it was done vice versa. Adding a sequence-specific motif to the target RNA results in a shift, which is numbered from II-V (complexes in Figure 3). B) Quantified complex formation of PAMmer, sgRNA, PAMmer-sgRNA together as well as the RNP complex with the target RNAs

REEP5 (blue) and *M2515mut* (green) normalized to the input target RNAs (n = 3 independent biological replicates).

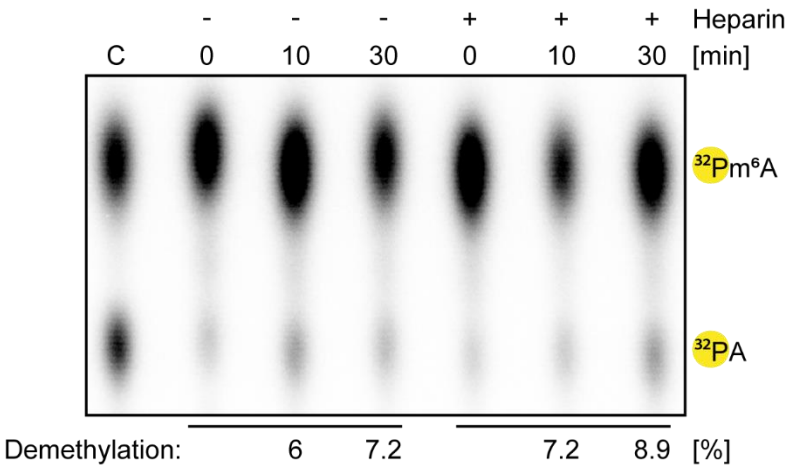

Figure S12: SCARLET analysis of heparin influence on RCas9-FTO activity on *REEP5* RNA.

TLC of RCas9-FTO activity on 38 nt *REEP5* RNA without and with addition of heparin and with incubation for 10 min and 30 min. The control lane (C) indicates m^6AMP (upper) and AMP (lower) spots.

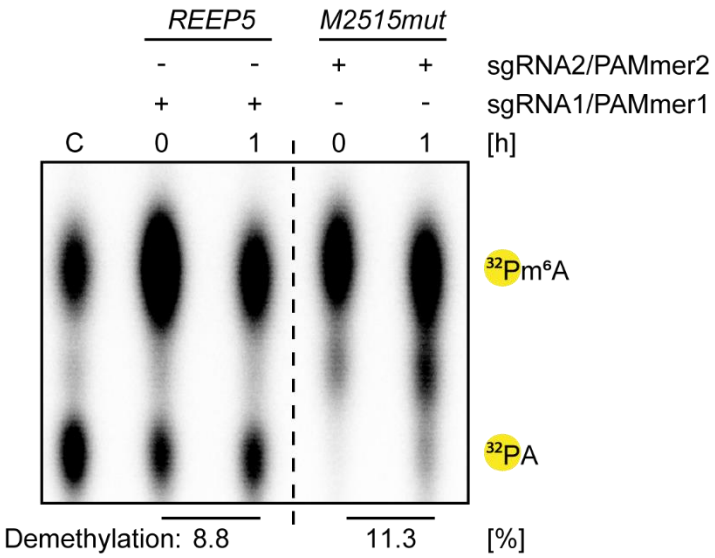

Figure S13: SCARLET analysis of RCas9-FTO activity on *REEP5* and *M2515mut* RNAs in total RNA.

A) Representative TLC of RCas9-FTO activity on 38 nt *REEP5* RNA and *M2515mut* in total RNA isolated from HeLa cells. The control lane (C) indicates m^6AMP (upper) and AMP (lower) spots.

Figure S14: Amino acid sequences.

RCas9

MHHHHHHGKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDII
FWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKT
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DLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSA
GINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPN
IPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNTSENLYFQGAASMDKKYSIGLAIGTN
SVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYL
QEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSK
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LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQS
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EDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFM
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MARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI
NRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFD
NLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDF
RKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA
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GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
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ASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA
ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

FTO

MGSSHHHHHHSSGLVPRGSHMTPKDDEFYQQWQLKYPKLILREASSVSEELHKEVQEAFLTLHKHGCL
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QIETIQALEELAAKEKANEDAVPLCMSADFPRVGMGSSYNGQDEVDIKSRAAYNVTLLNFMDPQKMPY
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FDLTDIVSELRGQLLEAKP

RCas9-FTO

MHHHHHHGKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDII
FWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKT
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LTAILASLTARQNLRREWHARCQSRIARTLPADQKPECRPYWEKDDASMPLPFDLTDIVSELRGQLLE
AKP

[His_6, MBP, TEV, FTO]

SUPPLEMENTARY TABLES

Table S1: Plasmids

Vector	Insert	Resistance	Creator
pET-28a(+)	FTO (NΔ31aa)	Kanamycin	Chengqi Yi (Peking University)
pEC-K-MBP (pET-based)	dCas9 (RCas9)	Kanamycin	Jennifer Doudna (UC Berkeley)
pEC-K-MBP (pET-based)	RCas9-FTO	Kanamycin	This study
pCAG	sgRNA1	Kanamycin	This study
pCAG	sgRNA2	Kanamycin	This study
pCAG	sgRNA18	Kanamycin	This study
pCAG	sgRNA22	Kanamycin	This study

Table S2: DNA oligonucleotides

Name	Sequence	Creator
Primer for cloning of RCas9-FTO		
RCas9_TAA_SDM_fwd	GTCAGCTAGGAGGTGACTCTAGAGGCCGGTGCTTTGCAG	This study
RCas9_TAA_SDM_rev	CTGCAAAGCACCGGCCTCTAGAGTCACCTCCTAGCTGAC	This study
RCas9_Xba1_SDM_fwd	TGTGAGCGGATAACAATTCCCCTCAAGGAATAATTTTGTTTAACTTTAAGAAG	This study
RCas9_Xba1_SDM_rev	CTTCTTAAAGTTAAACAAAATTATTCCTTGAGGGGAATTGTTATCCGCTCACA	This study
pEC_GGGGS-FTO_fwd	TATTCTAGAGGCGGCGGTGGTTCTGTCGACATGACGCCGAAAGATGACG	This study
FTO_rev	TTATTCTCGAGTCATGGTTTGGCTTCCAG	This study
Primer for cloning of sgRNAs		
BbsI_gBlock	TAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGGTCTTCGAGAAGACCTGTTTTAGAGCTATGCTGGAAACAGCATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT	This study
Spacer1_BbsI_fwd	CACCGCAGAGGAGAGAGGCTCCAT	This study
Spacer1_BbsI_rev	AAACATGGAGCCTCTCTCCTCTGC	This study
Spacer18_BbsI_fwd	CACCGAGAGAGGCTCCATAACCGAA	This study

Spacer18_BbsI_rev	AAACTTCGGTTATGGAGCCTCTCTC	This study
Spacer22_BbsI_fwd	CACCGAGGAGAGAGGCTCCATAAC	This study
Spacer22_BbsI_rev	AAACGTTATGGAGCCTCTCTCCTC	This study
Spacer2_BbsI_fwd	CACCGCCAATGCAAAAACATTAAGT	This study
Spacer2_BbsI_rev	AAACACTTAATGTTTTTGCATTGGC	This study
Primer for PCR of T7 template		
sgRNA1_fwd	TAATACGACTCACTATAGGGCAGAGGAGAGAGGC TCCAT	This study
sgRNA18_fwd	TAATACGACTCACTATAGGGAGAGAGGCTCCATAA CCGAAG	This study
sgRNA22_fwd	TAATACGACTCACTATAGGGAGGAGAGAGGCTCC ATAACG	This study
sgRNA2_fwd	TAATACGACTCACTATAGGGCCAATGCAAAAACAT TAAGTG	This study
sgRNA_rev	AAAAAAAGCACCGACTCGG	This study
Oligonucleotides as T7 templates		
25_fwd	TAATACGACTCACTATAGGGTACCTGGTGTTCGGT TATGGAGCCTCTCTCCTCTGCAA	This study
25_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCA GGTACCCTATAGTGAGTCGTATTA	This study
27_fwd	TAATACGACTCACTATAGGGGGTACCTGGTGTTCG GTTATGGAGCCTCTCTCCTCTGCAA	This study
27_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCA GGTACCCCCTATAGTGAGTCGTATTA	This study
28_fwd	TAATACGACTCACTATAGGAGGGTACCTGGTGTTC GGTTATGGAGCCTCTCTCCTCTGCAA	This study
28_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCA GGTACCCTCCTATAGTGAGTCGTATTA	This study
29_fwd	TAATACGACTCACTATAGGACGGGTACCTGGTGTT CGGTTATGGAGCCTCTCTCCTCTGCAA	This study
29_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCA GGTACCCGTCCTATAGTGAGTCGTATTA	This study
30_fwd	TAATACGACTCACTATAGGACTGGGTACCTGGTGT TCGGTTATGGAGCCTCTCTCCTCTGCAA	This study
30_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCA GGTACCCAGTCCTATAGTGAGTCGTATTA	This study

32_fwd	TAATACGACTCACTATAGGACTTCGGGTACCTGGTGTTCGGTTATGGAGCCTCTCTCCTCTGCAA	This study
32_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCAGGTACCCGAAGTCCTATAGTGAGTCGTATTA	This study
34_fwd	TAATACGACTCACTATAGGACTTCCTGGGTACCTGGTGTTCGGTTATGGAGCCTCTCTCCTCTGCAA	This study
34_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCAGGTACCCAGGAAGTCCTATAGTGAGTCGTATTA	This study
36_fwd	TAATACGACTCACTATAGGACTTCCTACGGGTACCTGGTGTTCGGTTATGGAGCCTCTCTCCTCTGCAA	This study
36_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCAGGTACCCGTAGGAAGTCCTATAGTGAGTCGTATTA	This study
37_fwd	TAATACGACTCACTATAGGACTTCCTACAGGGTACCTGGTGTTCGGTTATGGAGCCTCTCTCCTCTGCAA	This study
37_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCAGGTACCCTGTAGGAAGTCCTATAGTGAGTCGTATTA	This study
38_fwd	TAATACGACTCACTATAGGACTTCCTACAAGGGTACCTGGTGTTCGGTTATGGAGCCTCTCTCCTCTGCAA	This study
38_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCAGGTACCCTTGTAGGAAGTCCTATAGTGAGTCGTATTA	This study
39_fwd	TAATACGACTCACTATAGGACTTCCTACAATGGGTACCTGGTGTTCGGTTATGGAGCCTCTCTCCTCTGCAA	This study
39_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCAGGTACCCATTGTAGGAAGTCCTATAGTGAGTCGTATTA	This study

40_fwd	TAATACGACTCACTATAGGACTTCCTACAATCGGG TACCTGGTGTTCGGTTATGGAGCCTCTCTCCTCTG CAA	This study
40_rev	TTGCAGAGGAGAGAGGCTCCATAACCGAACACCA GGTACCCGATTGTAGGAAGTCCTATAGTGAGTCGT ATTA	This study
M2515mut_fwd	TAATACGACTCACTATAGGCATAAACTTTAGATCAA GAGTAATTACCAACTTAATGTTTTTGCATTGGAC	This study
M2515mut_rev	GTCCAATGCAAAAACATTAAGTTGGTAATTACTCTT GATCTAAAGTTTATGCCTATAGTGAGTCGTATTA	This study
M2515_fwd	TAATACGACTCACTATAGGAAGTAATTCAAGATCAA GAGTAATTACCAACTTAATGTTTTTGCATTGGAC	This study
M2515_rev	GTCCAATGCAAAAACATTAAGTTGGTAATTACTCTT GATCTTGAATTACTTCCTATAGTGAGTCGTATTA	This study
PAMmers for RCas9 targeting (m = 2′-*O*-Me RNA, DNA)		
PAMmer1	mGGmCTmCCmATmUGGmCGmAAmCAmCCmAGm GTmACmCC	This study
PAMmer1 (EMSA)	GGCTCCATTGGCGAACACCAGGTACCC	This study
PAMmer18	mUAmACmCGmAAmGGGmCAmGGmUAmCCmCGm GCmCAmCC	This study
PAMmer22	mUCmCAmUAmACmGGGmACmACmCAmGGmUAm CCmCGmGC	This study
PAMmer2	mCAmUTmAAmGTmAGGmUAmATmUAmCTmCTmU GmATmCT	This study
PAMmer2 (EMSA)	CATTAAGTAGGTAATTACTCTTGATCT	This study
Splints for production of longer m^6A RNAs		
Splint 1 (25)	CTCCATAACCGAACACCAGGTACCCGGCCACCAG TCCGATGACAC	This study
Splint 3 (27)	CCATAACCGAACACCAGGTACCCCCGGCCACCAG TCCGATGACAC	This study
Splint 4 (28)	CATAACCGAACACCAGGTACCCTCCGGCCACCAG TCCGATGACAC	This study
Splint 5 (29)	ATAACCGAACACCAGGTACCCGTCCGGCCACCAG TCCGATGACAC	This study
Splint 6 (30)	TAACCGAACACCAGGTACCCAGTCCGGCCACCAG TCCGATGACAC	This study
Splint 8 (32)	ACCGAACACCAGGTACCCGAAGTCCGGCCACCAG TCCGATGACAC	This study
Splint 10 (34)	CGAACACCAGGTACCCAGGAAGTCCGGCCACCAG TCCGATGACAC	This study

Splint 12 (36)	AACACCAGGTACCCGTAGGAAGTCCGGCCACCAG TCCGATGACAC	This study
Splint 13 (37)	ACACCAGGTACCCTGTAGGAAGTCCGGCCACCAG TCCGATGACAC	This study
Splint 14 (38)	CACCAGGTACCCTTGTAGGAAGTCCGGCCACCAG TCCGATGACAC	This study
Splint 15 (39)	ACCAGGTACCCATTGTAGGAAGTCCGGCCACCAG TCCGATGACAC	This study
Splint 16 (40)	CCAGGTACCCGATTGTAGGAAGTCCGGCCACCAG TCCGATGACAC	This study
Splint M2515mut	TTACTCTTGATCTAAAGTTTATGCCGTTACGAAAGT CCTTCACAT	This study
Splint M2515	TTACTCTTGATCTTGAATTACTTCCGTTACGAAAGT CCTTCACAT	This study
SCARLET chimeras (m = 2′-*O*-Me RNA, DNA), ssDNA and splints		
REEP5 chimera	mGmGmCmCmAmCmCmAmGmUCCGAmUmGmAm C	This study
M2515 chimera	mUmAmCmGmAmAmAmGmUCCTTmCmAmCmAmU	This study
ssDNA	GGAGAGACAACTTAAAGAGACTTAAAAGATTAATT TAAAATTTATCAAAAAGAGTATTGACTTAAAGTCTA ACCTATAGGATACTTACAGCCATCGCCGGTCCTGT GAGTTAATAG	(Liu *et al.* 2013)
Control Splint	AACCGAACACCAGGTACAAGGCCACCAGTCTATTA ACTCACAGGACCGGCGATGGCTG	This study
Splint I (25)	AACCGAACACCAGGTACCCGGCCACCAGTCTATT AACTCACAGGACCGGCGATGGCTG	This study
Splint III (27)	CCGAACACCAGGTACCCCCGGCCACCAGTCTATT AACTCACAGGACCGGCGATGGCTG	This study
Splint IV (28)	CGAACACCAGGTACCCTCCGGCCACCAGTCTATT AACTCACAGGACCGGCGATGGCTG	This study
Splint V (29)	GAACACCAGGTACCCGTCCGGCCACCAGTCTATT AACTCACAGGACCGGCGATGGCTG	This study
Splint VI (30)	AACACCAGGTACCCAGTCCGGCCACCAGTCTATTA ACTCACAGGACCGGCGATGGCTG	This study
Splint VIII (32)	CACCAGGTACCCGAAGTCCGGCCACCAGTCTATT AACTCACAGGACCGGCGATGGCTG	This study

Splint X (34)	CCAGGTACCCAGGAAGTCCGGCCACCAGTCTATT AACTCACAGGACCGGCGATGGCTG	This study
Splint XII (36)	AGGTACCCGTAGGAAGTCCGGCCACCAGTCTATT AACTCACAGGACCGGCGATGGCTG	This study
Splint XIII (37)	GGTACCCTGTAGGAAGTCCGGCCACCAGTCTATT AACTCACAGGACCGGCGATGGCTG	This study
Splint XIV (38)	GTACCCTTGTAGGAAGTCCGGCCACCAGTCTATTA ACTCACAGGACCGGCGATGGCTG	This study
Splint XV (39)	TACCCATTGTAGGAAGTCCGGCCACCAGTCTATTA ACTCACAGGACCGGCGATGGCTG	This study
Splint XVI (40)	ACCCGATTGTAGGAAGTCCGGCCACCAGTCTATTA ACTCACAGGACCGGCGATGGCTG	This study
Splint M2515mut (SCARLET)	TGATCTAAAGTTTATGCCGTTACGAAAGTCTATTAA CTCACAGGACCGGCGATGGCTG	This study
Splint M2515 (SCARLET)	TGATCTTGAATTACTTCCGTTACGAAAGTCTATTAA CTCACAGGACCGGCGATGGCTG	(Liu *et al.* 2013)

Table S3: RNA oligonucleotides

Name	**Sequence**	**Creator**
m^{6}A RNA parts for production of longer m^{6}A RNAs		
REEP5 m^{6}A	GUGUCAUCGGm^{6}ACUGGUGGCC	This study
MALAT1 m^{6}A	AUGUGAAGGm^{6}ACUUUCGUAAC	This study
Splint ligated m^{6}A RNAs		
25	GUGUCAUCGGm^{6}ACUGGUGGCCGGGUACCUGGU GUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
27	GUGUCAUCGGm^{6}ACUGGUGGCCGGGGGUACCUG GUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
28	GUGUCAUCGGm^{6}ACUGGUGGCCGGAGGGUACCU GGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
29	GUGUCAUCGGm^{6}ACUGGUGGCCGGACGGGUACC UGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCA A	This study

30	GUGUCAUCGGm^{6}ACUGGUGGCCGGACUGGGUACCUGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
32	GUGUCAUCGGm^{6}ACUGGUGGCCGGACUUCGGGUACCUGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
34	GUGUCAUCGGm^{6}ACUGGUGGCCGGACUUCCUGGGUACCUGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
36	GUGUCAUCGGm^{6}ACUGGUGGCCGGACUUCCUACGGGUACCUGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
37	GUGUCAUCGGm^{6}ACUGGUGGCCGGACUUCCUACAGGGUACCUGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
38	GUGUCAUCGGm^{6}ACUGGUGGCCGGACUUCCUACAAGGGUACCUGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
39	GUGUCAUCGGm^{6}ACUGGUGGCCGGACUUCCUACAAUGGGUACCUGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
40	GUGUCAUCGGm^{6}ACUGGUGGCCGGACUUCCUACAAUCGGGUACCUGGUGUUCGGUUAUGGAGCCUCUCUCCUCUGCAA	This study
M2515mut	AUGUGAAGGm^{6}ACUUUCGUAACGGCAUAAACUUUAGAUCAAGAGUAAUUACCAACUUAAUGUUUUUGCAUUGGAC	This study
M2515	AUGUGAAGGm^{6}ACUUUCGUAACGGAAGUAAUUCAAGAUCAAGAGUAAUUACCAACUUAAUGUUUUUGCAUUGGAC	This study
sgRNAs		
sgRNA1	GGGCAGAGGAGAGAGGCUCCAUGUUUUAGAGCUAUGCUGGAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU	This study
sgRNA18	GGGAGAGAGGCUCCAUAACCGAAGUUUUAGAGCUAUGCUGGAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU	This study
sgRNA22	GGGAGGAGAGAGGCUCCAUAACGUUUUAGAGCUAUGCUGGAAACAGCAUAGCAAGUUAAAAUAAGGC	This study

	UAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAG UCGGUGCUUUUUUU	
sgRNA2	GGGCCAAUGCAAAAACAUUAAGUGUUUUAGAGCU AUGCUGGAAACAGCAUAGCAAGUUAAAAUAAGGC UAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAG UCGGUGCUUUUUUU	This study
Control *REEP5* RNAs for SCARLET		
A RNA	GUCAUCGGACUGGUGGCCUUGUACCUGGUGUUC GGUUAUG	This study
m^{6}A RNA	GUCAUCGGm^{6}ACUGGUGGCCUUGUACCUGGUGU UCGGUUAUG	This study
m^{6}A RNAs for HPLC analysis		
15 nt-m^{6}A RNA	AUUGUCAm^{6}ACAGCAGC	(Jia *et al.* 2011)
Random RNA as competitor in activity assays		
Competitor	GGGCCAGAAUUGUAUGGUUUAAUCCCAUAUAUU CG	This study

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