Supplementary data for

Epitranscriptomic editing of the RNA N6-methyladenosine modification by dCasRx conjugated methyltransferase and demethylase

Xia et al.

Supplementary Figure S1

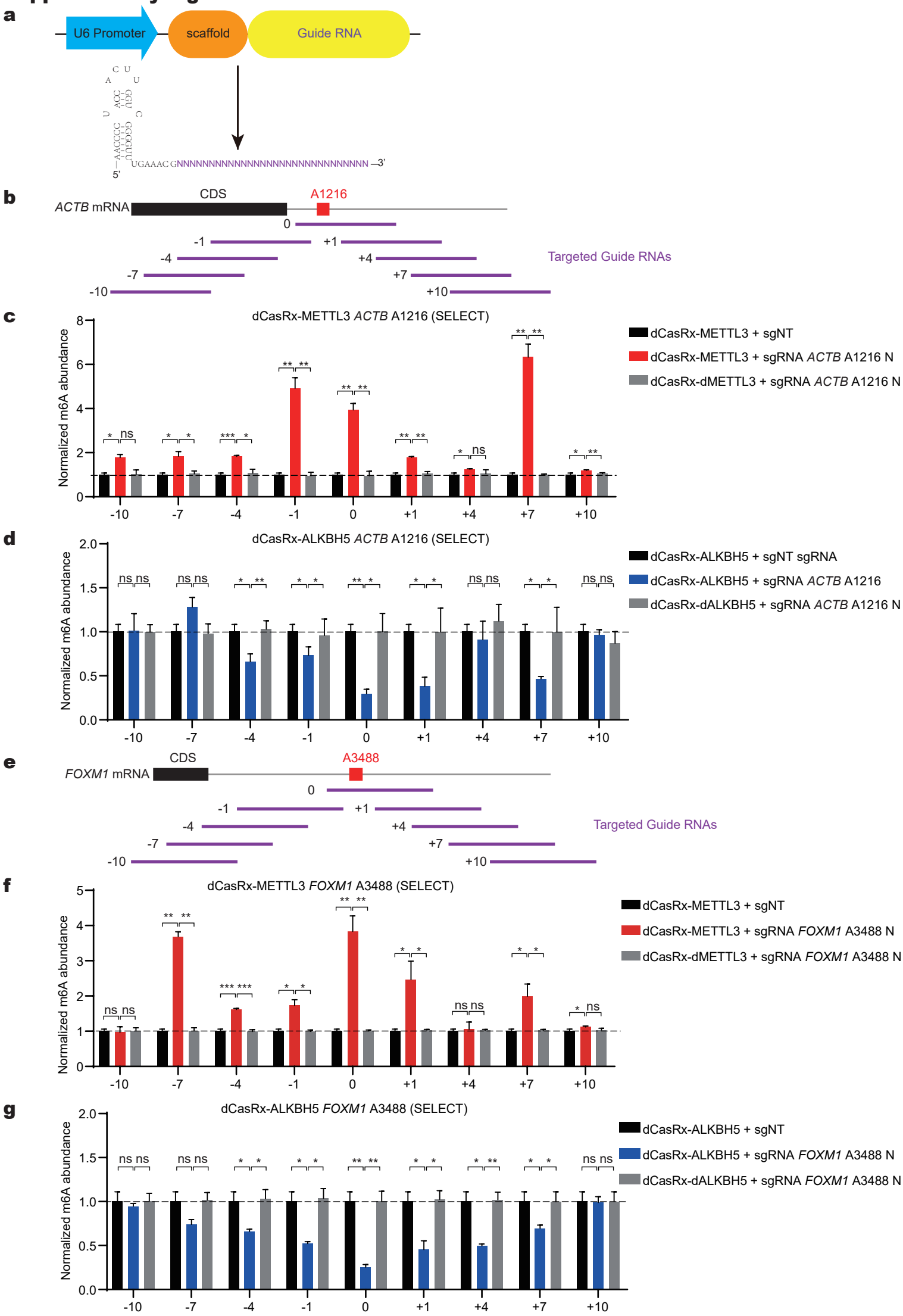

Figure S1. dCasRx epitranscriptomic editors editing window on *ACTB* and *FOXM1* mRNA.

a, Schematic diagram of sgRNA designed for dCasRx epitranscriptomic editors. **b**, **e**, Illustration of the sgRNAs designed for targeting *ACTB* A1216 (**b**), *FOXM1* A3488 (**e**). Each 30-nt sgRNAs (purple) ending (-1, -4, -7, -10) or starting (+1, +4, +7, +10) at the indicated bp from the targeted site, and 0-nt sgRNA represented the sgRNA covered on targeted site. **c**, **f**, Normalized abundance of m6A altered by dCasRx-METTL3 with sgRNAs targeting *ACTB* A1216 (**c**), *FOXM1* A3488 (**f**). **d**, **g**, Normalized abundance of m6A altered by dCasRx-ALKBH5 with sgRNAs targeting *ACTB* A1216 (**d**), *FOXM1* A3488 (**g**). Data is represented as mean ± SEM. (ANOVA; *, P value < 0.05; **, P value < 0.01; ***, P value < 0.001;n = 3).

Supplementary Figure S2

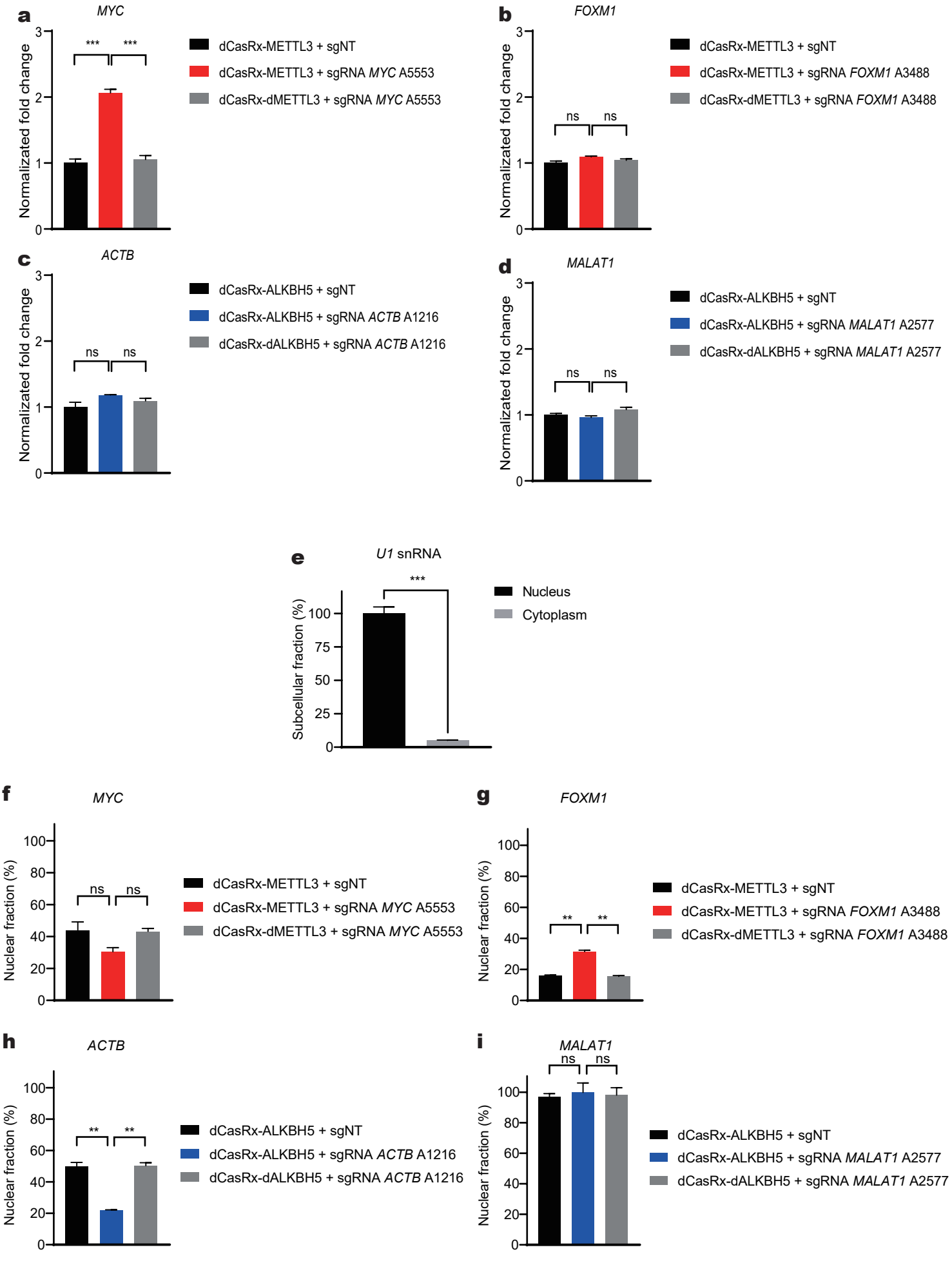

Figure S2. The effect of changes in m6A level on transcripts altered splicing and nuclear export efficiency mediated by dCasRx epitranscriptome editors.

a-**d**, qRT–PCR results of specific transcripts, *MYC* (**a**), *FOXM1* (**b**), *ACTB* (**c**) and *MALAT1* (**d**), were shown as fold change in the ratio of matured RNA to precursor RNA after manipulating m6A by dCasRx epitranscriptome editors. Data were calculated using a modified version of the $2^{-\Delta\Delta CT}$ method to show changes in matured RNA, where CT was the threshold cycle. First, the CT values for the common amplicons were normalized to the levels of its corresponding precursor RNA, where $\Delta CT = CT^{\text{matured RNA}} - CT^{\text{precursor RNA}}$. Then, the fold changes were normalized to the sgNT group following with the equation $\Delta\Delta CT = \Delta CT^{\text{target sgRNA or defunction enzyme}} - \Delta CT^{\text{sgNT}}$. Normalized fold change = $2^{-\Delta\Delta CT}$. **e**, Distribution of *U1* snRNA transcripts in subcellular fractions assessed by qPCR. **f**-**i**, qRT–PCR results of specific transcripts, *MYC* (**f**), *FOXM1* (**g**), *ACTB* (**h**) and *MALAT1* (**i**), were shown as the percentage of target transcripts in the nucleus to it in whole cell after manipulating m6A by dCasRx epitranscriptome editors. Data are displayed as mean ± SEM (ANOVA; ns: not significant; **,$P < 0.01$, ***,$P < 0.001$; n=3).

Supplementary Figure S3

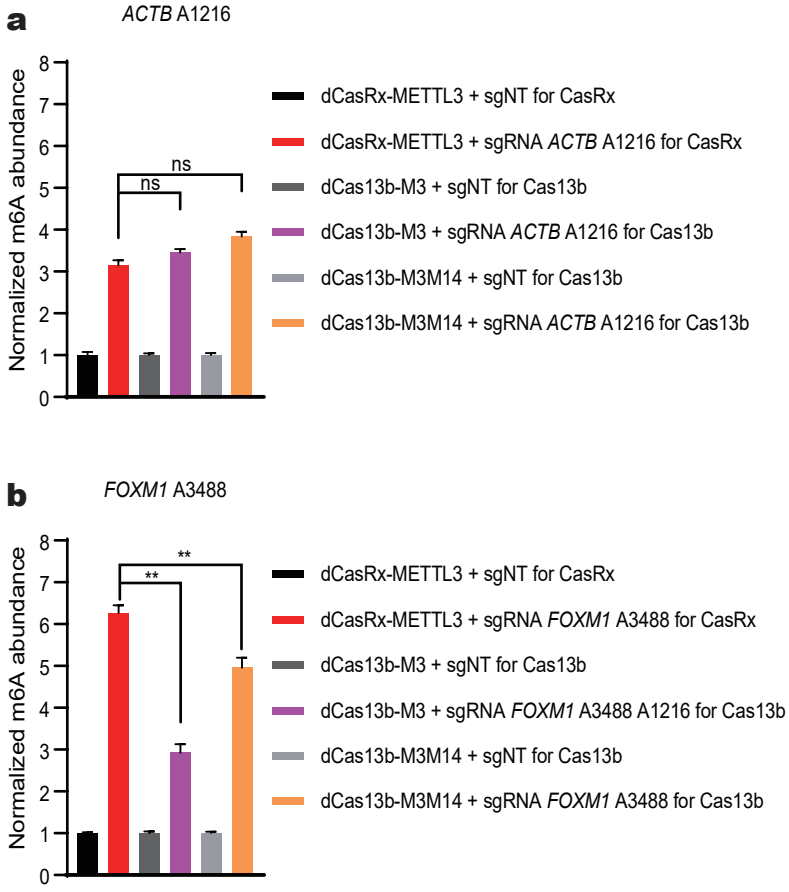

Figure S3. Comparison of m6A editing efficiency between dCasRx-based system and dCas13b-based system. a-**b**, Normalized abundance of m6A at *ACTB* A1216 (**a**) and *FOXM1* A3488 (**b**) detected by SELECT. It used same sgRNA sequences but different sgRNA scaffold to fit different system. Data are displayed as mean ± SEM (ANOVA; ns: not significant; **, $P < 0.01$; n=3).

Supplementary Figure S4

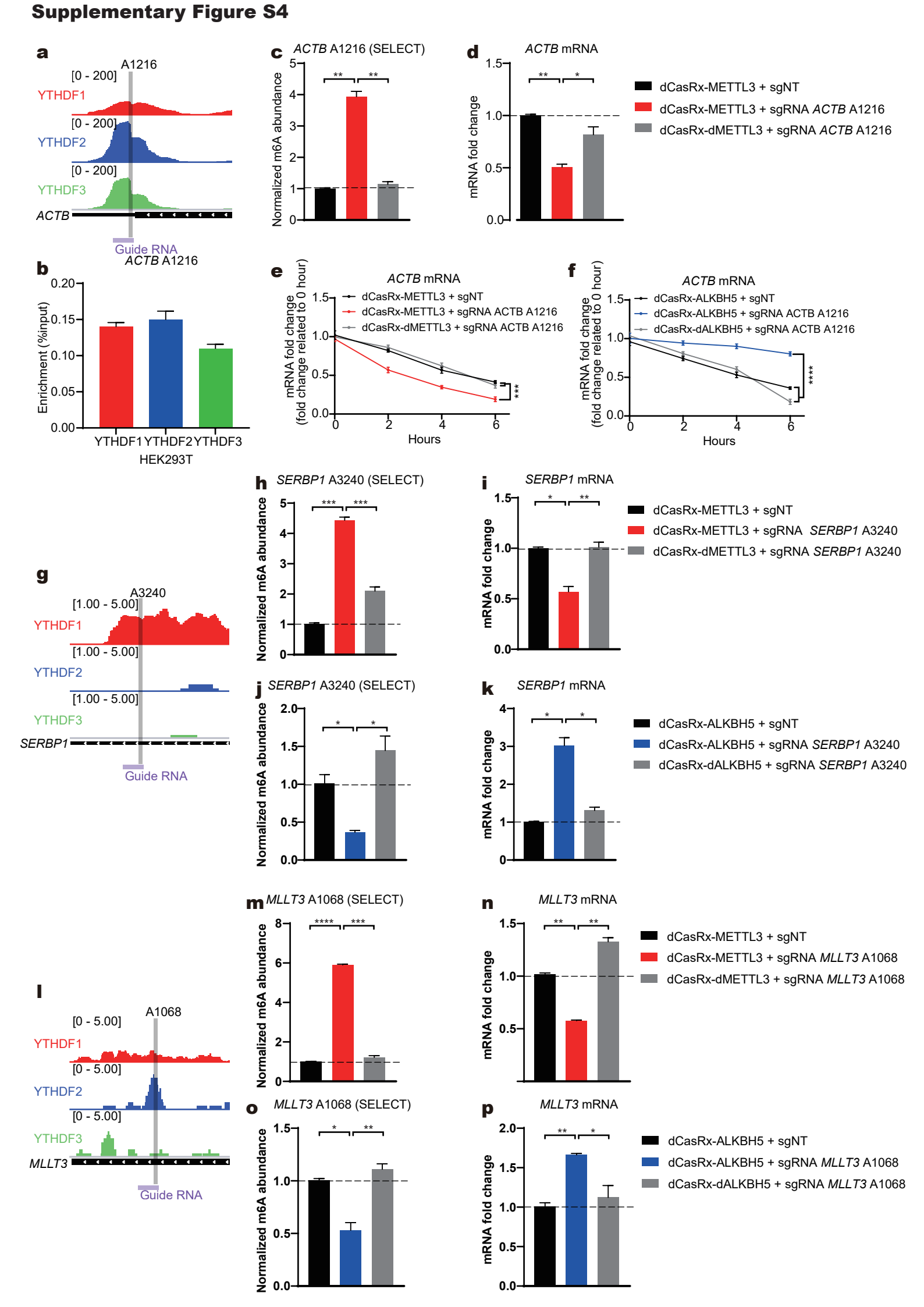

Figure S4. m6A sites binding with DF paralogs control the degradation of endogenous transcripts in HEK293T cells.

a, Schematic diagrams of distribution of DF paralogs, DF1 (red), DF2 (blue), and DF3 (green), in endogenous *ACTB* mRNA. Grey bars represent dCasRx-METTL3 or dCasRx-ALKBH5 targeted sites. Purple bars represent sgRNA binding location. Distributions of DF paralogs was based on a database GSE78030 (11). **b**, The combination of YTHDF paralogs at *ACTB* A1216 in HEK293T cells, quantified by YTHDF paralog RIP coupled with RT-qPCR. **g**, **i**, Schematic diagrams of distribution of DF paralogs in endogenous *SERBP1* (**g**) and *MLLT3* (**i**) mRNA. **c**, **h**, **m**, Normalized abundance of altered m6A at *ACTB* A1216 (**c**), *SERBP1* A3240 (**h**), *MLLT3* A1068 (**m**) edited by dCasRx-METTL3. **d**, **i**, **n**, Abundance of *ACTB* mRNA (**d**), *SERBP1* mRNA (**i**), *MLLT3* mRNA (**n**) decreased after dCasRx-METTL3 editing. **e**, mRNA degradation measurement of *ACTB* in HEK293T cells edited with dCasRx-METTL3. **f**, mRNA degradation measurement of *ACTB* in HEK293T cells edited with dCasRx-ALKBH5. **j**, **o**, Normalized abundance of altered m6A at *SERBP1* A3240 (**j**), *MLLT3* A1068 (**o**) edited by dCasRx-ALKBH5. **k**, **p**, Abundance of *SERBP1* mRNA (**k**), *MLLT3* mRNA (**p**) increased after dCasRx-ALKBH5 editing. Data is represented as mean ± SEM. (ANOVA; *, P value < 0.05; **, P value < 0.01; ***, P value < 0.001; ****, P value < 0.0001;n = 3).

Supplementary Figure S5

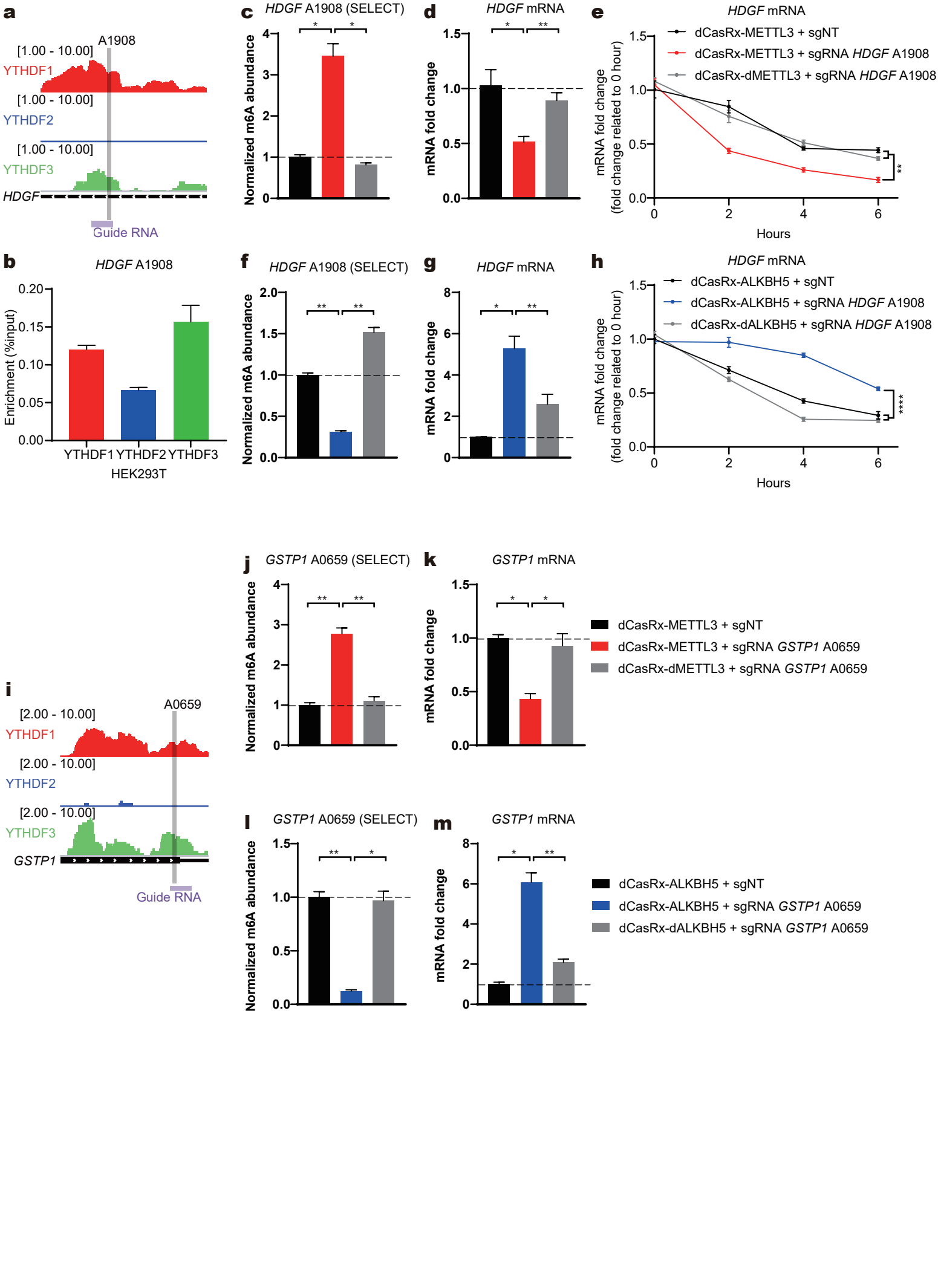

Figure S5. m6A sites binding both DF1 and DF3 control the degradation of endogenous transcripts in HEK293T cell.

a,**i**, Schematic diagrams of distribution of DF paralogs, DF1 (red), DF2 (blue), and DF3 (green), in endogenous *HDGF* (**a**) and GSTP1 (**i**) mRNA. Grey bars represent dCasRx-METTL3 or dCasRx-ALKBH5 targeted sites. Purple bars represent sgRNA binding location. Distributions of DF paralogs was based on a database GSE78030 (11). **b**, The combination of YTHDF paralogs at HDGF A1908 in HEK293T cells, quantified by YTHDF paralog RIP coupled with RT-qPCR. **c**, **j**, Normalized abundance of altered m6A at *HDGF* A1908 (**c**), *GSTP1* A0859 (**j**) edited by dCasRx-METTL3. **d**, **k**, Abundance of *HDGF* mRNA (**d**, *GSTP1* mRNA (**k**) decreased after dCasRx-METTL3 editing. **e**, mRNA degradation measurement of *HDGF* in HEK293T cells edited with dCasRx-METTL3. **h**, mRNA degradation measurement of *HDGF* in HEK293T cells edited with dCasRx-ALKBH5. **f**, **l**, Normalized abundance of altered m6A at *HDGF* A1908 (**f**), *GSTP1* A0859 (**l**) edited by dCasRx-ALKBH5. **g**, **m**, Abundance of *HDGF* mRNA (**g**), *GSTP1* mRNA (**m**) increased after dCasRx-ALKBH5 editing. Data is represented as mean ± SEM. (ANOVA, *: P value < 0.05; **: P value < 0.01; ****: P value < 0.0001. n = 3).

Supplementary Figure S6

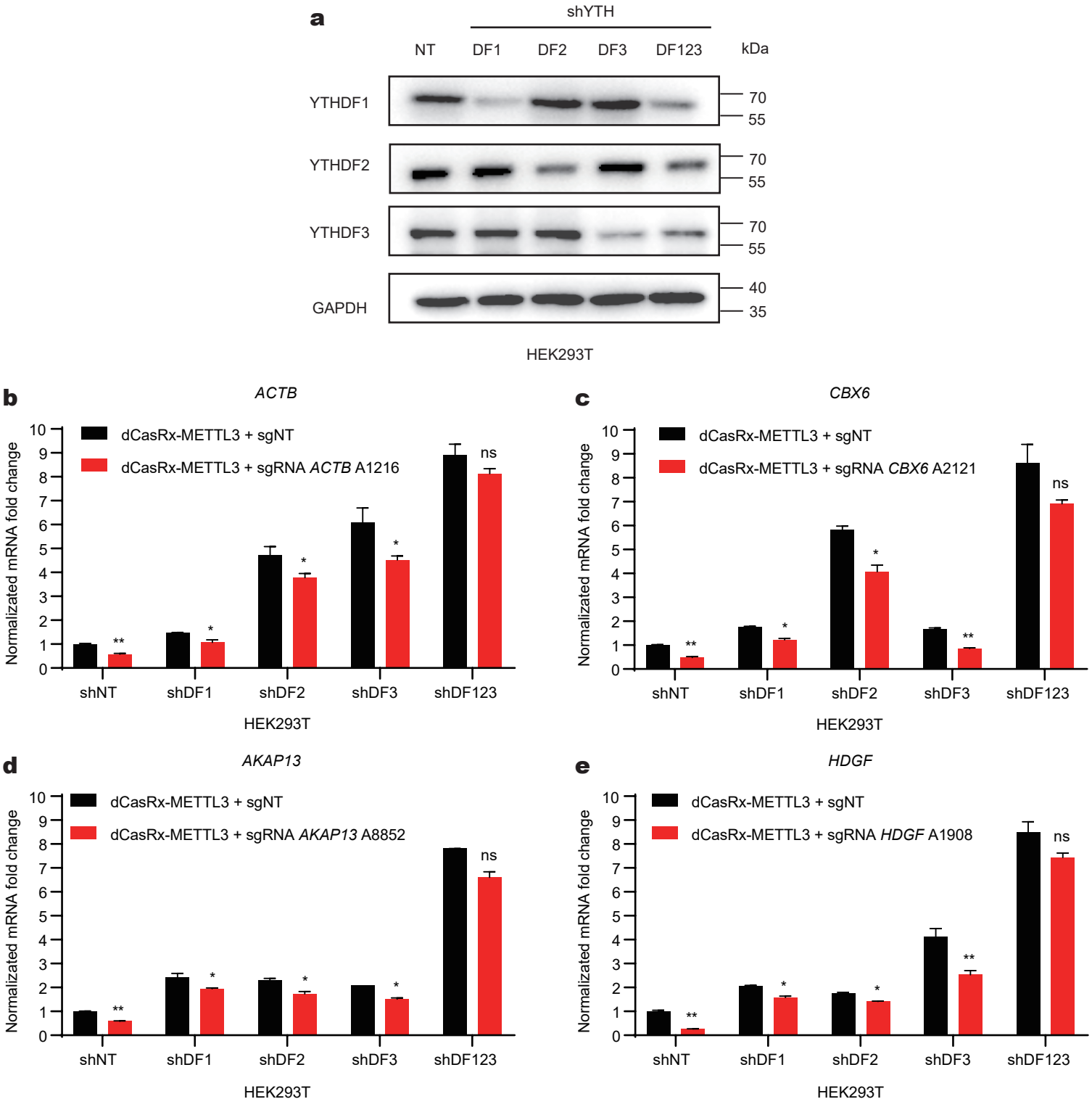

Figure S6: Depletion of YTHDF Paralogs rescued the decrease of transcripts induced by upregulated m6A levels in HEK293T cells.

a, Western blot results of knockdown YTHDF paralogs efficiency in living HEK293T cells. NT: non-target shRNA; DF1: shRNA targeted to YTHDF1; DF2: shRNA targeted to YTHDF2; DF3: shRNA targeted to YTHDF3; DF123: shRNAs targeted to YTHDF1/2/3. **b**-**e**, qRT–PCR results of select genes, *MYC* (**b**), *FOXM1* (**c**), *ACTB* (**d**) and *MALAT1* (**e**), shown as normalized mRNA fold change of sgNT and targeted group in HEK293T cells with depletion of different YTHDF paralogs. Data are displayed as mean ± SEM (ANOVA; ns: not significant; *,$P < 0.05$, **,$P < 0.01$;n=3).

Supplementary Table S1. dCasRx sgRNA sequences used in this study.

Name	Guide RNA spacer sequence
Non-targeting guide	gCGTCTGGCCTTCCTGTAGCCAGCTTTCATC
MYC A5553 guide	gTTCATAGGTGATTGCTCAGGACATTTCTGT
FOXM1 A3488 guide	gGTATGATTGGGGACATTATCAGAGAAACAT
MALAT1 A2577 guide	gAAAATAATCTTAACTCAAAGTCCAATGCAA
ACTB A1216 guide	gGTAACGCAACTAAGTCATAGTCCGCCTAGA
SQLE A0724 guide	gGATCAGACCAGTTTTTAAAAATCATATAAA
CBX6 A2121 guide	gCCTACTGAGGCGAGAGGCAGTCCAGGCCTT
SERBP1 A3240 guide	gACTGAGAAGTTGTGTTTTGAGTAGCAGGTG
AKAP13 A8852 guide	gGGGCAGTCACAAATCATGGCCGAAGCAGAG
MLLT3 A1068 guide	gCGGCCTTTTACTAGGAGCCTTCTTATCTTG
HDGF A1908 guide	gGAAGGAGCAGAATGGAGAGCACACAAAGGG
GSTP1 A0659 guide	gTCCCGCTCAGAGTCCCCCCAACCCTCACTG
ACTB A1216 guide -10	gGCATTTGCGGTGGACGATGGAGGGGCCGGA
ACTB A1216 guide -7	gGAAGCATTTGCGGTGGACGATGGAGGGGCC
ACTB A1216 guide -4	gCTAGAAGCATTTGCGGTGGACGATGGAGGG
ACTB A1216 guide -1	gCGCCTAGAAGCATTTGCGGTGGACGATGGA
ACTB A1216 guide 0	gGTAACGCAACTAAGTCATAGTCCGCCTAGA
ACTB A1216 guide +1	gAAGAAAGGGTGTAACGCAACTAAGTCATAG
ACTB A1216 guide +4	gGTCAAGAAAGGGTGTAACGCAACTAAGTCA
ACTB A1216 guide +7	gTTTGTCAAGAAAGGGTGTAACGCAACTAAG
ACTB A1216 guide +10	gGGTTTTGTCAAGAAAGGGTGTAACGCAACT
FOXM1 A3488 guide -10	gATGATTGGGGACATTATCAGAGAAACATCT
FOXM1 A3488 guide -7	gGGTATGATTGGGGACATTATCAGAGAAACA
FOXM1 A3488 guide -4	gCCTGGTATGATTGGGGACATTATCAGAGAA
FOXM1 A3488 guide -1	gCTCCCTGGTATGATTGGGGACATTATCAGA
FOXM1 A3488 guide 0	gCCTGAGTTCTCGTCAATGCCAGTCTCCCTG
FOXM1 A3488 guide +1	gAGCCTCCACCTGAGTTCTCGTCAATGCCAG
FOXM1 A3488 guide +4	gTCAAGCCTCCACCTGAGTTCTCGTCAATGC
FOXM1 A3488 guide +7	gTTCTCAAGCCTCCACCTGAGTTCTCGTCAA
FOXM1 A3488 guide +10	gGCCTTCTCAAGCCTCCACCTGAGTTCTCGT

Supplementary Table S2. dCas13b sgRNA sequences used in this study.

Name	**Guide RNA spacer sequence**
Non-targeting guide	gGTAATGCCTGGCTTGTCGACGCATAGTCTG
ACTB A1216 guide	gGAAGCATTTGCGGTGGACGATGGAGGGGCC
FOXM1 A3488 guide	gGTATGATTGGGGACATTATCAGAGAAACAT

Supplementary Table S3. RT-qPCR sequences used in this study.

Name	Sequence
18S forward	GGCCCTGTAATTGGAATGAGTC
18S reverse	CCAAGATCCAACTACGAGCTT
MYC forward	GGCTCCTGGCAAAAGGTCA
MYC reverse	CTGCGTAGTTGTGCTGATGT
FOXM1 forward	CGTCGGCCACTGATTCTCAAA
FOXM1 reverse	GGCAGGGGATCTCTTAGGTTC
MALAT1 forward	GACGGAGGTTGAGATGAAGCT
MALAT1 reverse	ATTCGGGGCTCTGTAGTCCT
ACTB forward	CATGTACGTTGCTATCCAGGC
ACTB reverse	CTCCTTAATGTCACGCACGAT
SQLE forward	GGCATTGCCACTTTCACCTAT
SQLE reverse	GGCCTGAGAGAATATCCGAGAAG
CBX6 forward	ACCCAAACCCAAAACTTTCCT
CBX6 reverse	GTCTCCGAGAAGGGCGAAAT
SERBP1 forward	CCTGGGCACTTACAGGAAGG
SERBP1 reverse	GGTCCGATTCGTCGTCAAATAAC
AKAP13 forward	GTCAACGGGCACACTTTCAG
AKAP13 reverse	GGAGGCTAGACTTTCTCGGC
MLLT3 forward	TTTGTGGAGAAAGTCGTCTTCC
MLLT3 reverse	GAGGTGATTCACTGGTGGATG
HDGF forward	CTCTTCCCTTACGAGGAATCCA
HDGF reverse	CCTTGACAGTAGGGTTGTTCTC
GSTP1 forward	CCCTACACCGTGGTCTATTTCC
GSTP1 reverse	CAGGAGGCTTTGAGTGAGC
*U1*snRNA forward	CCATGATCACGAAGGTGGTTT
*U1*snRNA reverse	ATGCAGTCGAGTTTCCCACAT
pre-*MYC* forward	GCTTCTCAGAGGCTTGGCGG
pre-*MYC* reverse	CTGGAATTACTACAGCGAGTT
pre-*FOXM1* forward	CGTTGGTTCACCTTATCTCT
pre-*FOXM1* reverse	AACCCTTCTCCAAACAGGAG
pre-*ACTB* forward	CAGGTCGGCTGTGGGGTCCT
pre-*ACTB* reverse	CGCTCAGGAGGAGCAATGAT
pre-*MALAT1* forward	GCATTCAAGTTCCATAAGCTG
pre-*MALAT1* reverse	ATTCGATCACCTTCCGCCGC

Supplementary Table S4. SELECT primer sequences used in this study.

Name	Sequence
qPCR-F for SELECT	ATGCAGCGACTCAGCCTCTG
qPCR-R for SELECT	TAGCCAGTACCGTAGTGCGTG
Control A UP	**tagccagtaccgtagtgcgtg**ATAGGTGATTGCTCAGGACA
Control A UP	**5phos/**TTCTGTTAGAAGGAATCGTT**cagaggctgagtcgctgcat**
MYC A5553 target UP	**tagccagtaccgtagtgcgtg**GATTGCTCAGGACATTTCTG
MYC A5553 target DOWN	**5phos/**TAGAAGGAATCGTTTTCCTT**cagaggctgagtcgctgcat**
FOXM1 A3488 target UP	**tagccagtaccgtagtgcgtg**TGAGTTCTCGTCAATGCCAG
FOXM1 A3488 target DOWN	**5phos/**CTCCCTGGTATGATTGGGGA**cagaggctgagtcgctgcat**
MALAT1 A2577 target UP	**tagccagtaccgtagtgcgtg**GGATTTAAAAAATAATCTTAACTCAAAG
MALAT1 A2577 target DOWN	**5phos/**CCAATGCAAAAACATTAAGT**cagaggctgagtcgctgcat**
ACTB A1216 target UP	**tagccagtaccgtagtgcgtg**GTAACGCAACTAAGTCATAG
ACTB A1216 target DOWN	**5phos/**CCGCCTAGAAGCATTTGCGG**cagaggctgagtcgctgcat**
SQLE A0724 target UP	**tagccagtaccgtagtgcgtg**CAGACCAGTTTTTAAAAATCATATAAAG
SQLE A0724 target DOWN	**5phos/**TAGTGTAAAAGTATGTGAAGCC**cagaggctgagtcgctgcat**
CBX6 A2121 target UP	**tagccagtaccgtagtgcgtg**CCTACTGAGGCGAGAGGCAG
CBX6 A2121 target DOWN	**5phos/**CCAGGCCTTCAATGCCCCTG**cagaggctgagtcgctgcat**
SERBP1 A3240 target UP	**tagccagtaccgtagtgcgtg**TGTGTTTTGAGTAGCAGGTG
SERBP1 A3240 target DOWN	**5phos/**TTTCTATAGTATGTTGCTGG**cagaggctgagtcgctgcat**
AKAP13 A8852 target UP	**tagccagtaccgtagtgcgtg**AAATCATGGCCGAAGCAGAG
AKAP13 A8852 target DOWN	**5phos/**CTGGGCCTCCTTCCCCACCC**cagaggctgagtcgctgcat**
MLLT3 A1068 target UP	**tagccagtaccgtagtgcgtg**CTAGGAGCCTTCTTATCTTG
MLLT3 A1068 target DOWN	**5phos/**CCACTGGTGATGGTGAGTAA**cagaggctgagtcgctgcat**
HDGF A1908 target UP	**tagccagtaccgtagtgcgtg**AATGGAGAGCACACAAAGGG
HDGF A1908 target DOWN	**5phos/**TAGGGGTCTTTAAAATTTTT**cagaggctgagtcgctgcat**
GSTP1 A0659 target UP	**tagccagtaccgtagtgcgtg**AGTCCCCCCAACCCTCACTG
GSTP1 A0659 target DOWN	**5phos/**TTCCCGTTGCCATTGATGGG**cagaggctgagtcgctgcat**

Supplementary Table S5. Primers used for m6A-RIP assay in this study.

Name	**Sequence**
MYC A5553 target forward	AGGAAAAGTAAGGAAAACGATTCC
MYC A5553 target reverse	TGATCATGCATTTGAAACAAGTTC
FOXM1 A3488 target forward	TGCCCAGATGTGCGCTATTA
FOXM1 A3488 target reverse	CTTCTCAAGCCTCCACCTGA
MALAT1 A2577 target forward	CGTAACGGAAGTAATTCAAG
MALAT1 A2577 target reverse	GTCAATTAATGCTAGTCCTC
ACTB A1216 target forward	ATCGTCCACCGCAAATGCTT
ACTB A1216 target reverse	TCATCTTGTTTTCTGCGCAAGT

Supplementary Table S6. Primers used for YTHDF-RIP assay in this study.

Name	Sequence
ACTB A1216 target forward	ATCGTCCACCGCAAATGCTT
ACTB A1216 target reverse	TCATCTTGTTTTCTGCGCAAGT
SQLE A0724 target forward	TACAACTTGGCTTCACATAC
SQLE A0724 target reverse	GTAAACAGTGTCCCAGGACG
CBX6 A2121 target forward	CAGTTCCTTTGAACAGGGGC
CBX6 A2121 target reverse	CACAGCAAACCTCCAGACCC
AKAP13 A8852 target forward	ATATTGAGTGTCGGGTGGGG
AKAP13 A8852 target reverse	TGAGTAGGGCCAGCCCAACC
HDGF A1908 target forward	AAAAAAAAATTTTAAAGACC
HDGF A1908 target reverse	CCCAGTGCACCTCAGAAATG

Supplementary Table S7. shRNA sequences used in this study.

Name	Sequence
Non-targeting shRNA	CAACAAGATGAAGAGCACCAA
shRNA YTHDF1	ACAGACAGTGTGATGGATGAT
shRNA YTHDF2	TACTGATTAAGTCAGGATTAA
shRNA YTHDF3	TAAGTCAAAGAAGACGTATTA