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Supplemental information

N^6-methyladenosine in 7SK small nuclear RNA underlies RNA polymerase II transcription regulation

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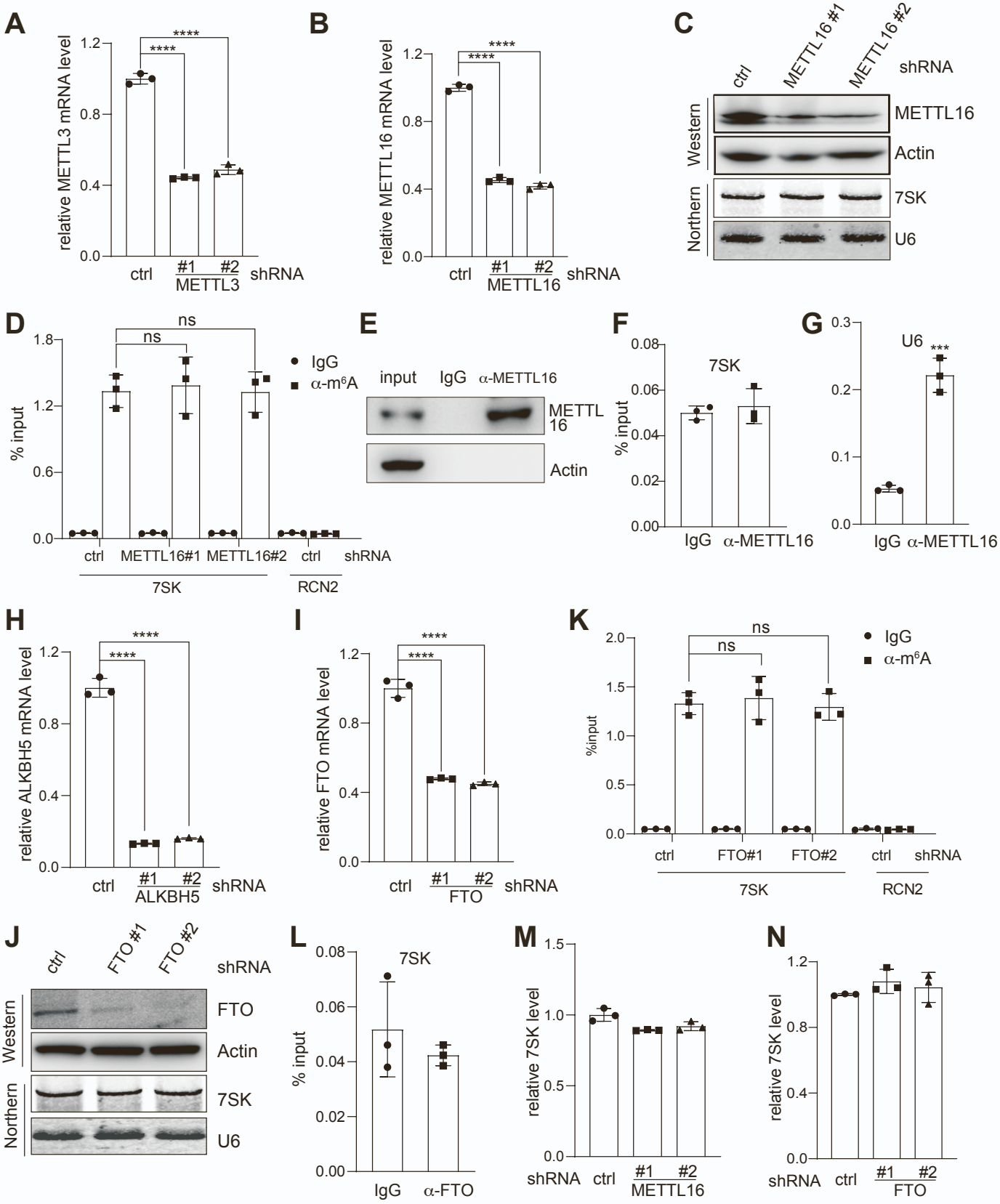
Figure S1


A
relative METTL3 mRNA level
1.2
0.8
0.4
0.0
****
****
ctrl
#1
#2
METTL3
shRNA
B
relative METTL16 mRNA level
1.2
0.8
0.4
0.0
****
****
ctrl
#1
#2
METTL16
shRNA
C
ctrl
METTL16 #1
METTL16 #2
shRNA
Western
METTL16
Actin
Northern
7SK
U6
D
% input
1.8
1.2
0.6
0.0
ns
ns
IgG
α-m⁶A
ctrl
METTL16#1
METTL16#2
ctrl
shRNA
7SK
RCN2
E
input
IgG
α-METTL16
METTL 16
Actin
F
7SK
% input
0.08
0.06
0.04
0.02
0.00
IgG
α-METTL16
G
U6
0.3
0.2
0.1
0.0
***
IgG
α-METTL16
H
relative ALKBH5 mRNA level
1.2
0.8
0.4
0.0
****
****
ctrl
#1
#2
ALKBH5
shRNA
I
relative FTO mRNA level
1.2
0.8
0.4
0.0
****
****
ctrl
#1
#2
FTO
shRNA
K
%input
2.0
1.5
1.0
0.5
0.0
ns
ns
IgG
α-m⁶A
ctrl
FTO#1
FTO#2
ctrl
shRNA
7SK
RCN2
J
ctrl
FTO #1
FTO #2
shRNA
Western
FTO
Actin
Northern
7SK
U6
L
7SK
% input
0.08
0.06
0.04
0.02
0.00
IgG
α-FTO
M
relative 7SK level
1.5
1.0
0.5
0.0
shRNA
ctrl
#1
#2
METTL16
N
relative 7SK level
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0.8
0.4
0.0
shRNA
ctrl
#1
#2
FTO

Figure S2

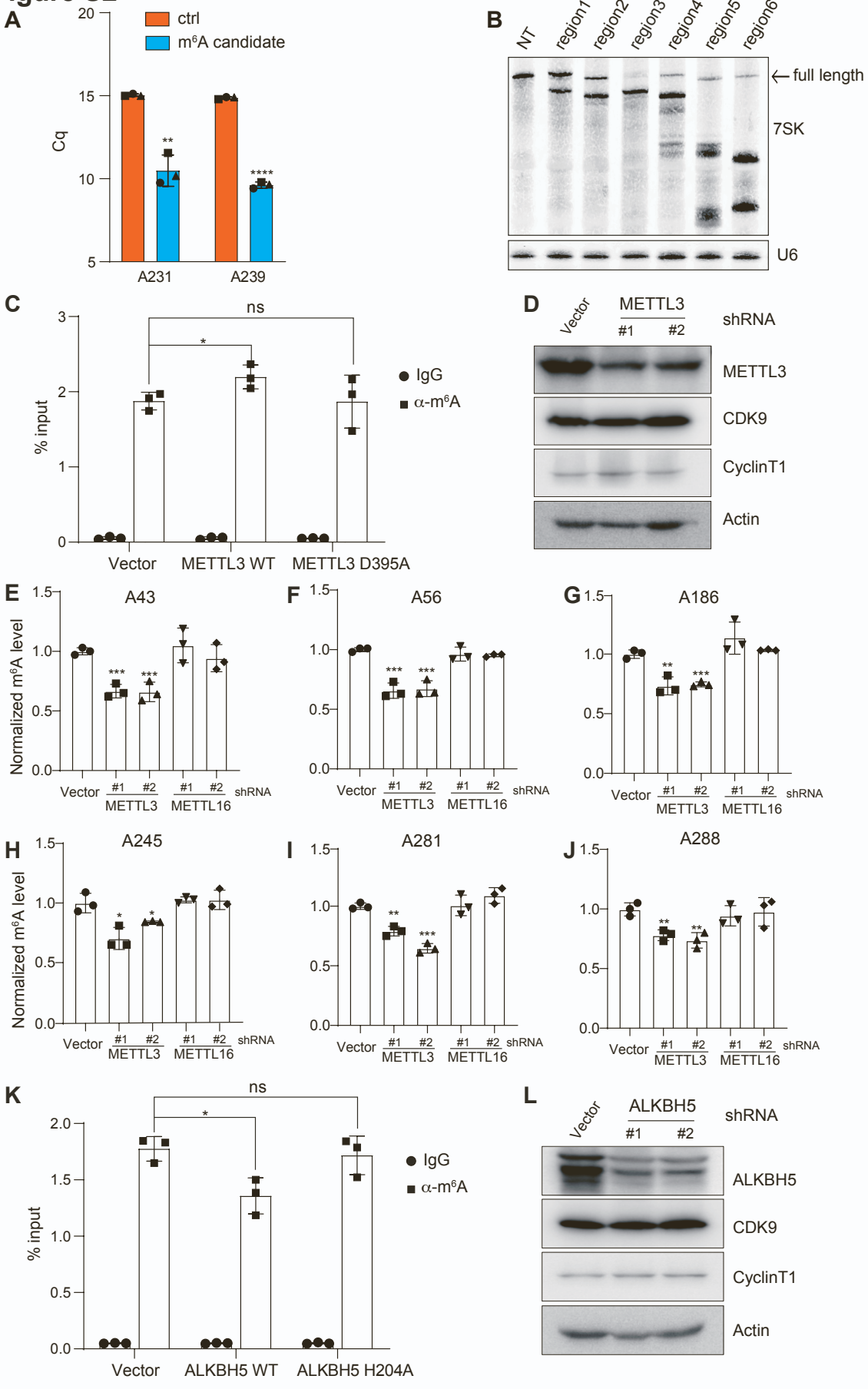
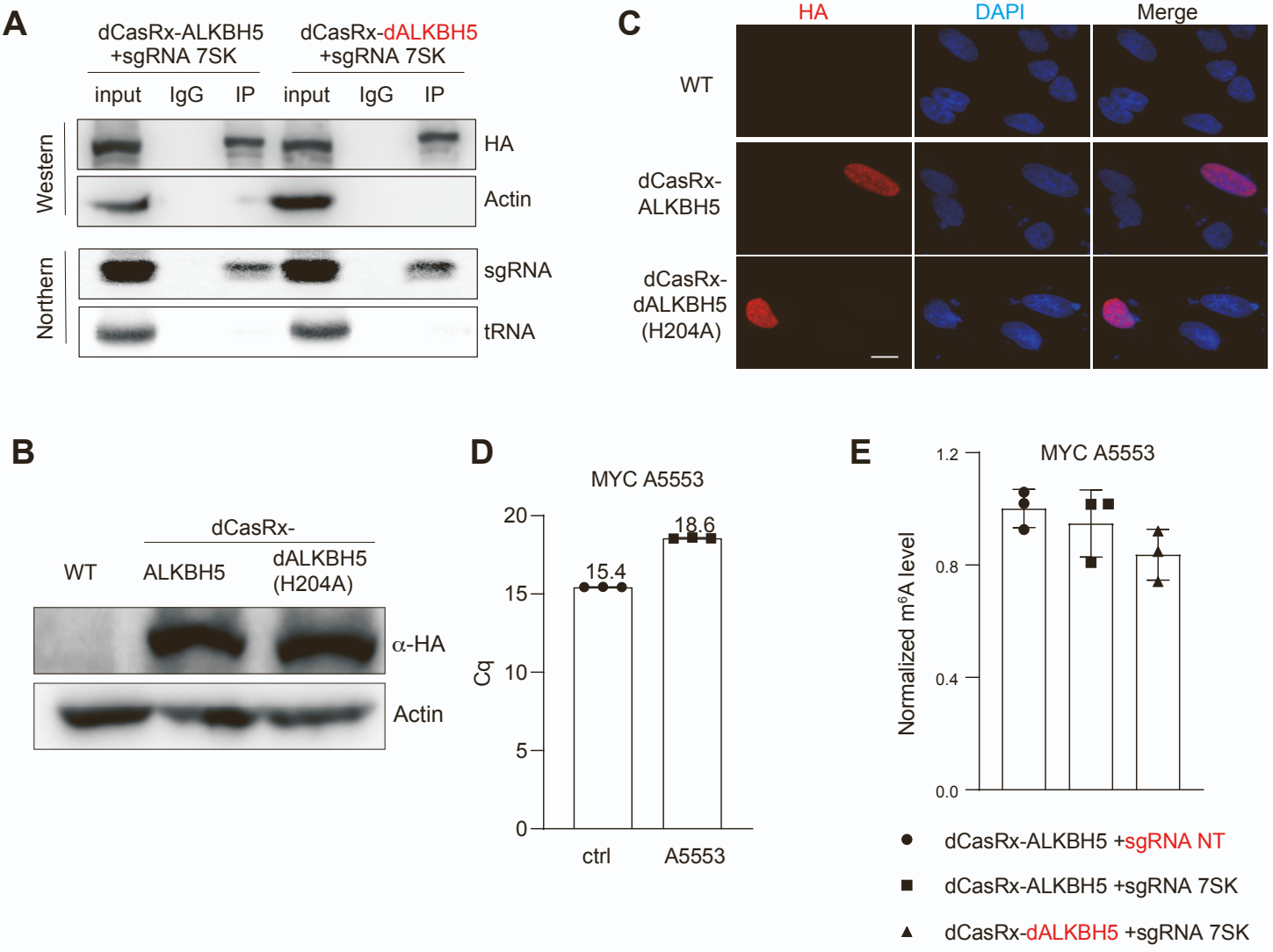

A
ctrl
m6A candidate
Cq
A231
A239
B
NT
region1
region2
region3
region4
region5
region6
full length
7SK
U6
C
ns
% input
IgG
α-m6A
Vector
METTL3 WT
METTL3 D395A
D
Vector
METTL3
shRNA
METTL3
CDK9
CyclinT1
Actin
E
A43
F
A56
G
A186
Normalized m6A level
Vector
METTL3
METTL16
shRNA
H
A245
I
A281
J
A288
K
ns
% input
IgG
α-m6A
Vector
ALKBH5 WT
ALKBH5 H204A
L
Vector
ALKBH5
shRNA
ALKBH5
CDK9
CyclinT1
Actin

Figure S3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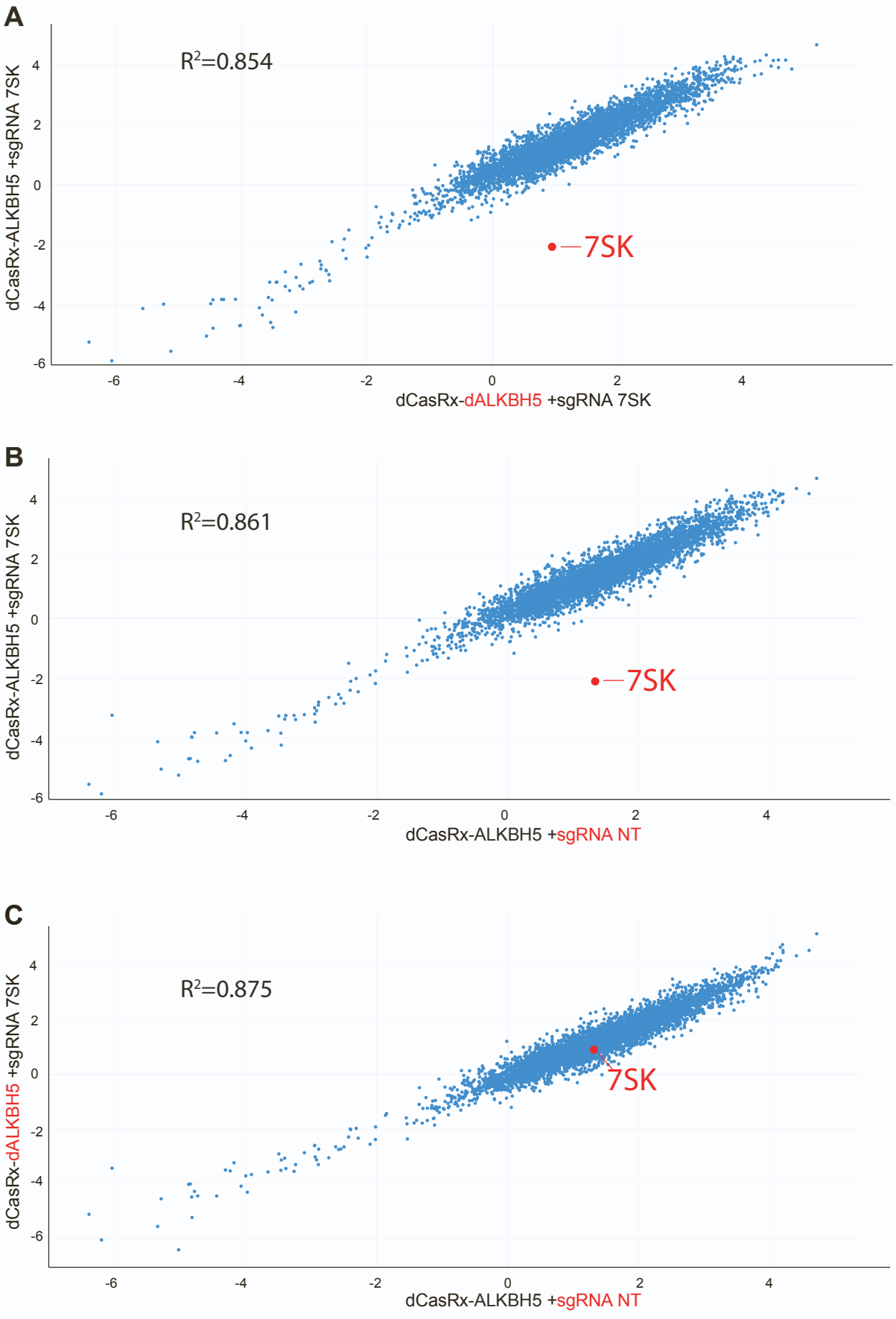
dCasRx-ALKBH5 +sgRNA 7SK
dCasRx-dALKBH5 +sgRNA 7SK
input IgG IP input IgG IP
Western
HA
Actin
Northern
sgRNA
tRNA
C
HA
DAPI
Merge
WT
dCasRx-ALKBH5
dCasRx-dALKBH5 (H204A)
B
dCasRx-
WT
ALKBH5
dALKBH5 (H204A)
α-HA
Actin
D
MYC A5553
Cq
20
15
10
5
0
15.4
18.6
ctrl
A5553
E
MYC A5553
Normalized m6A level
1.2
0.8
0.4
0.0
dCasRx-ALKBH5 +sgRNA NT
dCasRx-ALKBH5 +sgRNA 7SK
dCasRx-dALKBH5 +sgRNA 7SK

Figure S4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7SK
dCasRx-ALKBH5 +sgRNA 7SK
dCasRx-dALKBH5 +sgRNA 7SK
B
R²=0.861
7SK
dCasRx-ALKBH5 +sgRNA 7SK
dCasRx-ALKBH5 +sgRNA NT
C
R²=0.875
7SK
dCasRx-dALKBH5 +sgRNA 7SK
dCasRx-ALKBH5 +sgRNA NT

Figure S5

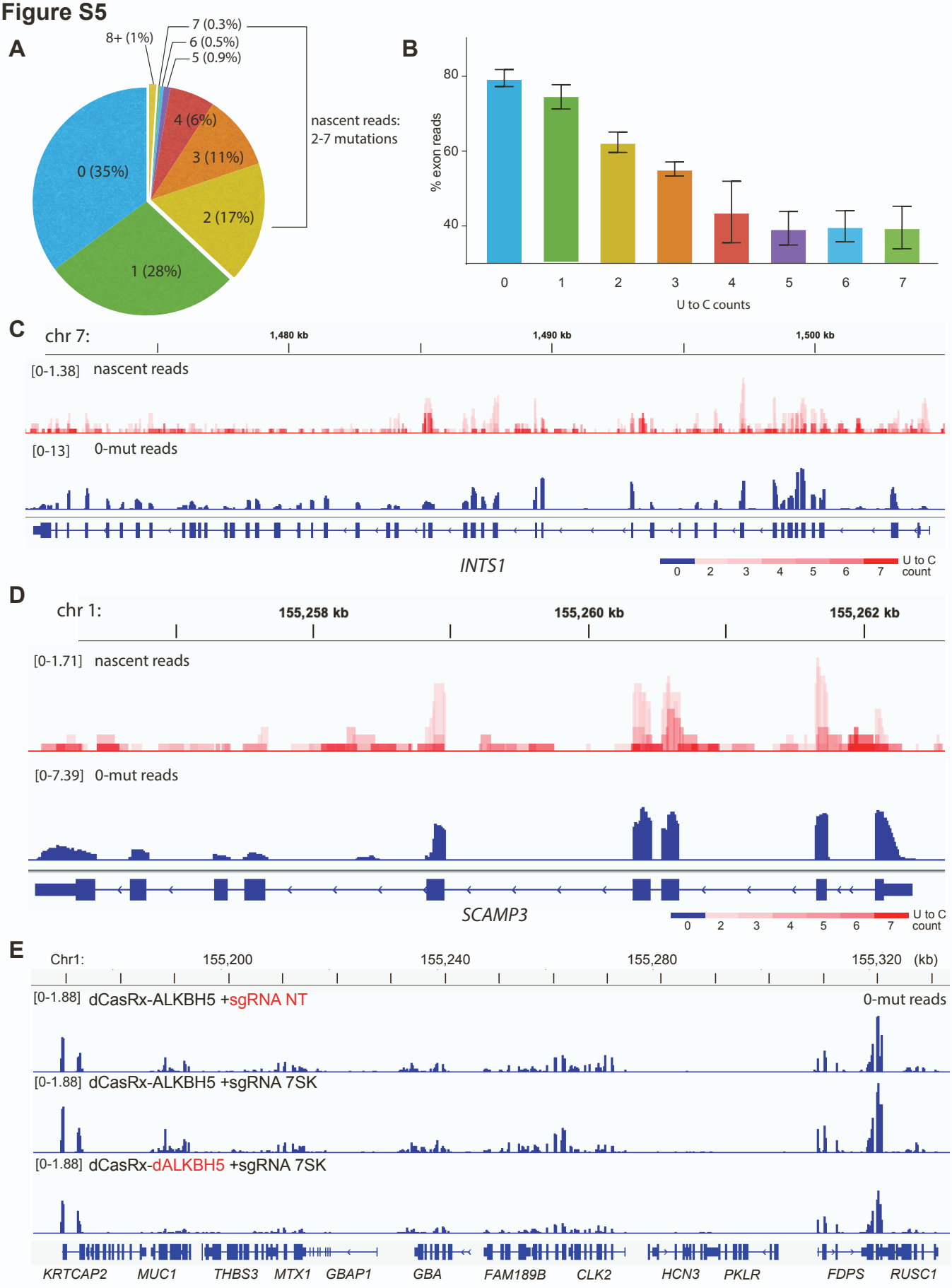
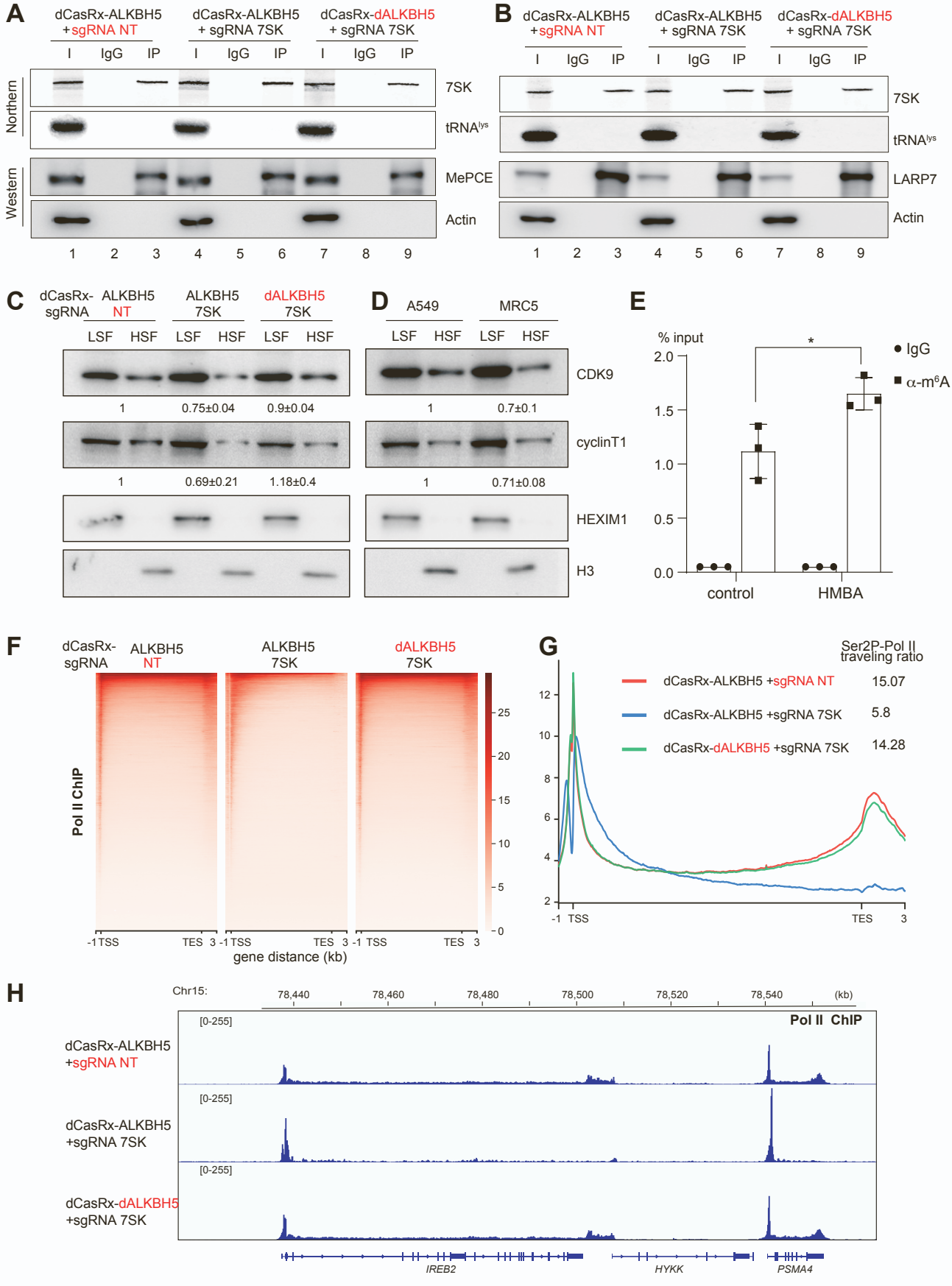

A
8+ (1%)
7 (0.3%)
6 (0.5%)
5 (0.9%)
4 (6%)
3 (11%)
2 (17%)
1 (28%)
0 (35%)
nascent reads:
2-7 mutations
B
% exon reads
80
60
40
0
1
2
3
4
5
6
7
U to C counts
C
chr 7:
1,480 kb
1,490 kb
1,500 kb
[0-1.38] nascent reads
[0-13] 0-mut reads
INTS1
0 2 3 4 5 6 7
U to C count
D
chr 1:
155,258 kb
155,260 kb
155,262 kb
[0-1.71] nascent reads
[0-7.39] 0-mut reads
SCAMP3
0 2 3 4 5 6 7
U to C count
E
Chr1:
155,200
155,240
155,280
155,320 (kb)
[0-1.88] dCasRx-ALKBH5 +sgRNA NT
0-mut reads
[0-1.88] dCasRx-ALKBH5 +sgRNA 7SK
[0-1.88] dCasRx-dALKBH5 +sgRNA 7SK
KRTCAP2
MUC1
THBS3
MTX1
GBAP1
GBA
FAM189B
CLK2
HCN3
PKLR
FDPS
RUSC1

Figure S6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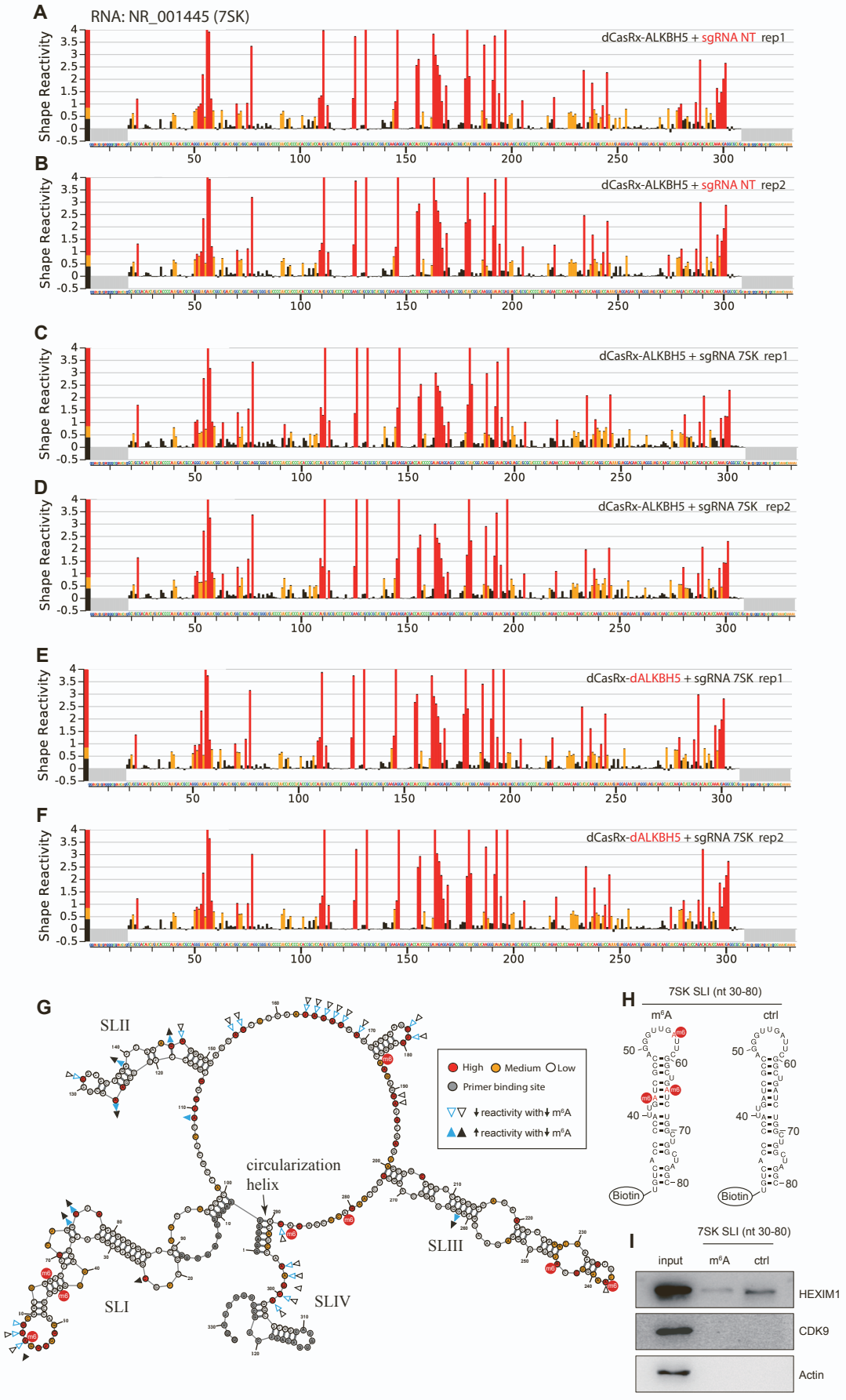
dCasRx-ALKBH5 +sgRNA NT
dCasRx-ALKBH5 + sgRNA 7SK
dCasRx-dALKBH5 + sgRNA 7SK
I IgG IP
Northern
Western
7SK
tRNAlys
MePCE
Actin
1 2 3 4 5 6 7 8 9
B
LARP7
C
dCasRx-sgRNA
ALKBH5 NT
ALKBH5 7SK
dALKBH5 7SK
LSF HSF
CDK9
1 0.75±0.04 0.9±0.04
cyclinT1
1 0.69±0.21 1.18±0.4
HEXIM1
H3
D
A549
MRC5
1 0.7±0.1
1 0.71±0.08
E
% input
IgG
α-m6A
control
HMBA
F
Pol II ChIP
-1 TSS TES 3
gene distance (kb)
G
Ser2P-Pol II traveling ratio
dCasRx-ALKBH5 +sgRNA NT 15.07
dCasRx-ALKBH5 +sgRNA 7SK 5.8
dCasRx-dALKBH5 +sgRNA 7SK 14.28
H
Chr15:
78,440 78,460 78,480 78,500 78,520 78,540 (kb)
[0-255]
Pol II ChIP
IREB2
HYKK
PSMA4

Figure S7


A
RNA: NR_001445 (7SK)
dCasRx-ALKBH5 + sgRNA NT rep1
B
dCasRx-ALKBH5 + sgRNA NT rep2
C
dCasRx-ALKBH5 + sgRNA 7SK rep1
D
dCasRx-ALKBH5 + sgRNA 7SK rep2
E
dCasRx-dALKBH5 + sgRNA 7SK rep1
F
dCasRx-dALKBH5 + sgRNA 7SK rep2
Shape Reactivity
G
SLII
SLI
SLIII
SLIV
circularization helix
High
Medium
Low
Primer binding site
reactivity with m6A
reactivity with m6A
H
7SK SLI (nt 30-80)
m6A
ctrl
Biotin
I
7SK SLI (nt 30-80)
input
m6A
ctrl
HEXIM1
CDK9
Actin

Supplementary Figure Legends

Supplementary Figure S1. m^{6}A modification of 7SK was not affected by METTL16 or FTO, related to Figure 1. RT-qPCR analysis showing the mRNA knockdown of METTL3 (**A**), METTL16 (**B**), ALKBH5 (**H**) and FTO (**I**) using two different shRNAs stably expressed in A549 cells. Western and northern blots show the abundance of 7SK in A549 cells with METTL16 (**C**) or FTO (**J**) knockdown, with Actin and U6 as loading controls. MeRIP experiments were performed in METTL16 (**D**) or FTO (**K**) knockdown A549 cells, followed by RT-qPCR detection of 7SK and RCN2. RIP analyses were performed in A549 cell lysates using METTL16 (**E-G**) or FTO (**L**) specific antibodies, followed by RT-qPCR detection of 7SK and U6. RT-qPCR assays detect the abundance of 7SK relative to U6 in A549 cells with METTL16 (**M**) or FTO (**N**) depletion. All the error bars represent SD from three experiments. *t*-test statistics are used, ***P<0.001, ****P<0.0001; ns, not significant.

Supplementary Figure S2. Validation of m^{6}A candidates, accessible regions on 7SK and m^{6}A-7SK writer/eraser, related to Figures 2-4. (**A**) SELECT analyses of two candidate 7SK m^{6}A sites identified by the nanopore sequencing (error bars represent SD; paired *t* test statistics are used, **P<0.01, ****P<0.0001). (**B**) Endogenous RNase H cleavage assays were performed with A549 cell lysate with the addition of DNA oligonucleotides that match the putative accessible regions 1-6 as shown in Figure 2A. Total RNAs were extracted after RNase H assay and northern blot was performed to detect 7SK and U6 snRNAs. Full length 7SK is indicated with an arrow. MeRIP experiments were performed in METTL3 (**C**) or ALKBH5 (**K**) overexpression cells by plasmid transfection, followed by RT-qPCR detection of 7SK. Western blots of METTL3 (**D**) or ALKBH5 (**L**) in A549 cells stably expressing shRNA against these proteins. CDK9 and CyclinT1 levels are also detected, with Actin as a loading control. (**E-J**) SELECT analyses of six 7SK m^{6}A sites were compared in METTL3 and METTL16 knockdown cells. Error bars represent SD; *t*-test statistics are used, * P<0.05; **P<0.01 and ***P<0.001, ns, not significant.

Supplementary Figure S3. dCasRx-ALKBH5 fusion proteins expression levels, subcellular localization and detection of m^{6}A at *MYC* A5553, related to Figure 4. (**A**) Western and northern blots detect the dCasRx-ALKBH5 fusion protein level and the interaction of sgRNA with dCasRx-ALKBH5/dCasRx-dALKBH5. (**B**) Western blots demonstrate overexpression of HA-tagged dCasRx-ALKBH5 (WT and H204A) by plasmid transfection with an antibody detecting HA.

Actin is probed as a loading control. (**C**) Representative immunofluorescence images of A549 cells transfected with dCasRx-ALKBH5 (WT and H204A) plasmids. Scale bars, 10 μm. (**D**) SELECT analysis of *MYC* A^{5553} in A549 cells. (**E**) SELECT analyses of *MYC* A^{5553} in A549 cells stably expressing dCasRx-ALKBH5 with sgRNAs targeting 7SK and the control cells.

Supplementary Figure S4. 7SK-targeting dCasRx-ALKBH5 specifically modulates m^{6}A-7SK, related to Figure 4. Scatter plots of transcriptome-wide relative m^{6}A modifications [$\log_2$(m^{6}A-IP/input)] in three engineered A549 cells expressing dCasRx-ALKBH5, detected by MeRIP-seq. Comparison was made between every two cell lines. Red dots indicate 7SK.

Supplementary Figure S5. Breakdown of U-C mutations in nascent RNA-seq, related to Figure 5. (**A**) Pie chart illustrating the average percentage of mutations among all the nascent RNA-seq reads ranging from 0 to 8+ U to C mutations. (**B**) Histogram profile showing normalized exon reads distribution for 0-7 U to C mutations. Track example of 0 mutation reads (blue) or 2-7 mutations reads (red) mapped *INTS1* (**C**) and *SCAMP3* (**D**) in the control A549 cell line (dCasRx-ALKBH5+sgRNA NT). (**E**) Comparison of steady state RNA levels (0 mutation reads) across a region on chromosome 1 as shown in Figure 5F.

Supplementary Figure S6. m^{6}A affects 7SK snRNP composition and genome-wide Pol II distribution, related to Figure 6

Northern blot analyses of 7SK and $tRNA^{lys}$ from RIP experiments performed using a MePCE (**A**) or LARP7 (**B**) antibody compared with control IgG. Western blots show the IP efficiency of targeted proteins. Inputs (I) are 5% of the pellet (P). The low-salt fraction (LSF) and high-salt fraction (HSF) prepared from engineered A549 cell lines (**C**) and MRC5 cell (**D**) were subjected to western blot analysis and probed with the indicated antibodies. **(E)** MeRIP and RT-qPCR measurement of 7SK in A549 cells with or without HMBA treatment. (**F**) Heatmaps of Pol II (ChIP relative to input) occupancy in A549 cells upon dCasRx-ALKBH5 and sgRNA expression, centered at the TSS (-1 kb to TES+3 kb). (**G**) Metagene profiles of Pol II ChIP performed in the three A549 cell lines as in F, showing the distribution of Pol II and the traveling ratio. (**H**) Track examples of Pol II ChIP-seq in the region spanning genes *IREB2*, *HYKK* and *PSMA4* in three A549 cell lines as in F. Error bars represent SD; *t*-test statistic is used, * $P<0.05$.

Supplementary Figure S7. SHAPE-MaP analysis of 7SK in A549 cells with or without perturbation of m^{6}A status, related to Figure 7. (**A-F**) Each condition is shown in duplicate, as

labeled. The nucleotides and numbering across the x axis correspond to the reference sequence NR_001445. Highly reactive nucleotides are colored in red, medium in orange and low in black. Regions that do not contain structural information due to primer binding sites (nt 1-18, 319-332) are shaded in grey. Error bars indicate standard deviation. (**G**) 7SK consensus structure annotated with SHAPE-MaP reactivity. High reactivity positions are shown in red, medium in orange and low are white. Terminal regions without information due to primer binding sites are depicted in grey. The arrowheads show changes in reactivity upon reduction of m^6A levels in 7SK, comparing cells expressing dCasRx-ALKBH5 + sgRNA 7SK to cells expressing dCasRx-ALKBH5 + sgRNA NT, with the colors (cyan and black) representing two replicate experiments. Filled arrowheads indicate positions that become more reactive when m^6A is decreased while open arrowheads mark positions that are less reactive. The m^6A sites confirmed by the SELECT assay are marked with a red circle (m^6). (**H**) Schematic of biotinylated 7SK SLI (30-80nt) with and without m^6A modification. (**I**) Western blot analyses of proteins enriched by 7SK SLI with or without m^6A modification from A549 cell lysate.

Supplementary Tables

Table S1. RT-qPCR sequences used in this study, related to STAR Methods.

gene	Sequence 5' - 3'
ALKBH5 forward	CGGCGAAGGCTACACTTACG
ALKBH5 reverse	CCACCAGCTTTTGGATCACCA
METTL3 forward	TTGTCTCCAACCTTCCGTAGT
METTL3 reverse	CCAGATCAGAGAGGTGGTGTAG
METTL16 forward	CTCTGACGTGTACTCTCCTAAGG
METTL16 reverse	TACCAGCCATTCAAGGTTGCT
FTO forward	ACTTGGCTCCCTTATCTGACC
FTO reverse	TGTGCAGTGTGAGAAAGGCTT
RCN2 forward	TGGACTCAGATGGCTTTCTCA
RCN2 reverse	GACCTGAATCCTGGTTAGCTTTT
7SK forward	GCGATCTGGCTGCGACAT
7SK reverse	TCCTCTTCGACCGAGCGC
Actin forward	CCAACCGCGAGAAGATGA
Actin reverse	CCAGAGGCGTACAGGGATAG
U6 forward	GTGCTCGCTTCGGCAGC
U6 reverse	AAAATATGGAACGCTTCACGAAT

Table S2. Northern blot probe sequences used in this study, related to STAR Methods.

Name	Sequence 5' - 3'
7SK	AAAAGAAAGGCAGACUGCCACAUGCAGCGCCUCAUUUGGAUGUGUCUGGAG UCUUGGAAGCUUGACUACCCUACGUUCUCCUACAAAUGGACCUUGAGAGCUU GUUUGGAGGUUCUAGCAGGGGAGCGCAGCUACUCGUAUACCCUUGACCGAA GACCGGUCCUCCUCUAUCGGGGAUGGUCGUCCUCUUCGACCGAGCGCGCAG CUUCGGGAGGGACGCACAUGGAGCGGUGAGGGAGGAAGGGGACACCCGCCU AGCCAGCCAGAUCAGCCGAAUCAACCCUGGCGAUCAAUGGGGUGACAGAUG UCGCAGCCAGAUCGCCCUCACAUCC (*in vitro* transcribed antisense 7SK, with Us possibly containing azide modification [92])
U6	GCAGGGGCCATGCTAATCTTCTCTGTATCGT/3AzideN/
sgRNA	/5IRD800/GTTTCAAACCCCGACCAGTTGGTA
tRNA	CTCATGCTCTACCGACTGAGCTAGCCGGGCA/3AzideN/

Table S3. siRNA sequences produced from the shRNAs, related to STAR Methods.

Name	Sequence 5' - 3'
ALKBH5 shRNA#1	CCACCCAGCUAUGCUUCAGAU
ALKBH5 shRNA#2	CCUCAGGAAGACAAGAUUAGA
METTL3 shRNA#1	GCAAGUAUGUUCACUAUGAAA
METTL3 shRNA#2	GCCAAGGAACAAUCCAUUGUU
METTL16 shRNA#1	GUUUGUUAUGAGGUGGAGUUU
METTL16 shRNA#2	CAUCUGAAGUAGAGCUUGUUU
FTO shRNA#1	UCACCAAGGAGACUGCUAUUU
FTO shRNA#2	CGGUUCACAACCUCGGUUUAG

Table S4. SELECT primer sequences, related to STAR Methods.

name	Sequence 5' - 3'
qPCR-F for SELECT	ATGCAGCGACTCAGCCTCTG
qPCR-R for SELECT	TAGCCAGTACCGTAGTGCGTG
MYC A5553 target UP	tagccagtaccgtagtgcgtgGATTGCTCAGGACATTTCTG
MYC A5553 target DOWN	/5phos/TAGAAGGAATCGTTTTCCTTcagaggctgagtcgctgcat
MYC control UP	tagccagtaccgtagtgcgtgATAGGTGATTGCTCAGGACA
MYC control DOWN	5phos/TTCTGTTAGAAGGAATCGTTcagaggctgagtcgctgcat
7SK A43 target UP	tagccagtaccgtagtgcgtgAGCCGAATCAACCCTGGCGA
7SK A43 target DOWN	/5Phos/CAATGGGGTGACAGATGTCGcagaggctgagtcgctgcat
C45 ctrl UP	tagccagtaccgtagtgcgtgTCAGCCGAATCAACCCTGGC
C45 ctrl DOWN	/5Phos/ATCAATGGGGTGACAGATGTcagaggctgagtcgctgcat
A56 target UP	tagccagtaccgtagtgcgtgAGCCAGCCAGATCAGCCGAA
A56 target DOWN	/5phos/CAACCCTGGCGATCAATGGGcagaggctgagtcgctgcat
U58 ctrl UP	tagccagtaccgtagtgcgtgCTAGCCAGCCAGATCAGCCG
U58 ctrl DOWN	/5phos/ATCAACCCTGGCGATCAATGcagaggctgagtcgctgcat
A65 target UP	tagccagtaccgtagtgcgtgCACCCGCCTAGCCAGCCAGA
A65 target DOWN	/5phos/CAGCCGAATCAACCCTGGCGcagaggctgagtcgctgcat
C67 ctrl UP	tagccagtaccgtagtgcgtgGACACCCGCCTAGCCAGCCA
C67 ctrl DOWN	/5phos/ATCAGCCGAATCAACCCTGGcagaggctgagtcgctgcat
A172 target UP	tagccagtaccgtagtgcgtgTACCCTTGACCGAAGACCGG
A172 target DOWN	/5Phos/CCTCCTCTATCGGGGATGGTcagaggctgagtcgctgcat
C174 ctrl UP	tagccagtaccgtagtgcgtgTATACCCTTGACCGAAGACC
C174 ctrl DOWN	/5Phos/GTCCTCCTCTATCGGGGATGcagaggctgagtcgct

	gcat
A186 target UP	tagccagtaccgtagtgcgtgCGCAGCTACTCGTATACCCT
A186 target DOWN	/5phos/GACCGAAGACCGGTCCTCCTcagaggctgagtcgc tgcat
G188 ctrl UP	tagccagtaccgtagtgcgtgAGCGCAGCTACTCGTATACC
G188 ctrl DOWN	/5phos/TTGACCGAAGACCGGTCCTCcagaggctgagtcgc tgcat
A220 target UP	tagccagtaccgtagtgcgtgCTTGAGAGCTTGTTTGGAGG
A220 target DOWN	/5Phos/TCTAGCAGGGGAGCGCAGCTcagaggctgagtcg ctgcat
A228 target UP	tagccagtaccgtagtgcgtgAAATGGACCTTGAGAGCTTG
A228 target DOWN	/5Phos/TTGGAGGTTCTAGCAGGGGAcagaggctgagtcg ctgcat
C222 ctrl UP	tagccagtaccgtagtgcgtgACCTTGAGAGCTTGTTTGGA
C222 ctrl DOWN	/5Phos/GTTCTAGCAGGGGAGCGCAGcagaggctgagtcg ctgcat
A230 target UP	tagccagtaccgtagtgcgtgACAAATGGACCTTGAGAGCT
A230 target DOWN	/5phos/GTTTGGAGGTTCTAGCAGGGcagaggctgagtcgc tgcat
A231 target UP	tagccagtaccgtagtgcgtgTACAAATGGACCTTGAGAGC
A231 target DOWN	/5phos/TGTTTGGAGGTTCTAGCAGGcagaggctgagtcgct gcat
G232 ctrl UP	tagccagtaccgtagtgcgtgCTACAAATGGACCTTGAGAG
G232 ctrl DOWN	/5phos/TTGTTTGGAGGTTCTAGCAGcagaggctgagtcgct gcat
A238 target UP	tagccagtaccgtagtgcgtgGTTCTCCTACAAATGGACCT
A238 target DOWN	/5phos/GAGAGCTTGTTTGGAGGTTCcagaggctgagtcgct gcat
A239 target UP	tagccagtaccgtagtgcgtgCGTTCTCCTACAAATGGACC
A239 target DOWN	/5phos/TGAGAGCTTGTTTGGAGGTTcagaggctgagtcgct gcat
A245 target UP	tagccagtaccgtagtgcgtgACCCTACGTTCTCCTACAAA
A245 target DOWN	/5phos/GGACCTTGAGAGCTTGTTTGcagaggctgagtcgct

	gcat
G240 ctrl UP	tagccagtaccgtagtgcgtgACGTTCTCCTACAAATGGAC
G240 ctrl DOWN	5phos/TTGAGAGCTTGTTTGGAGGTcagaggctgagtcgct gcat
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A281 target UP	tagccagtaccgtagtgcgtgTCATTTGGATGTGTCTGGAG
A281 target DOWN	/5Phos/CTTGGAAGCTTGACTACCCTcagaggctgagtcgct gcat
A288 target UP	tagccagtaccgtagtgcgtgCAGCGCCTCATTTGGATGTG
A288 target DOWN	/5Phos/CTGGAGTCTTGGAAGCTTGAcagaggctgagtcgc tgcat
T283 ctrl UP	tagccagtaccgtagtgcgtgCCTCATTTGGATGTGTCTGG
T283 ctrl DOWN	/5Phos/GTCTTGGAAGCTTGACTACCcagaggctgagtcgct gcat

Table S5. DNA oligonucleotides for RNase H assay, related to STAR Methods.

Name	sequence 5' - 3'
NT	GATGAAAGCTGGCTACAGGAAGGCCAGACG
region 1	TGGCGATCAATGGGGTGACAGATGTCGCAG
region 2	CCGAATCAACCCTGGCGATCAATGGGGTGA
region 3	CATTTGGATGTGTCTGGAGTCTTGGAAGCT
region 4	TGTGTCTGGAGTCTTGGAAGCTTGACTACC
region 5	CGCAGCTACTCGTATACCCTTGACCGAAGA
region 6	CGTATACCCTTGACCGAAGACCGGTCCTCC

Table S6. 7SK SLI sequences for *in vitro* binding assay, related to STAR Methods.

Oligos name	SLI (30-80nt) 5' - 3'
7SK with m^6A	Biotin-UGUCACCCCAUUGm^6AUCGCCAGGGUUGm^6AUUCGGCUGm^6AUCUGGCUGGCUAGGC
7SK without m^6A	Biotin-UGUCACCCCAUUGAUCGCCAGGGUUGAUUCGGCUGAUCUGGCUGGCUAGGC

Table S7. Primer sequences for SHAPE-Map library construction, related to STAR Methods.

Primer name	Sequence 5' - 3'
7SK-RT	AAAAGAAAGGCAGACTGCCAC
Step1-F1	GTGACTGGAGTTCCTTGGCACCCGAGAATTCCANNNNNGGATGTGAGGGCGATCTG
Step1-R1	CCCTACACGACGCTCTTCCGATCTNNNNNAAAAGAAAGGCAGACTGCCACATG
Step2-F	CAAGCAGAAGACGGCATACGAGAT[barcode]GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Step2-R	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG