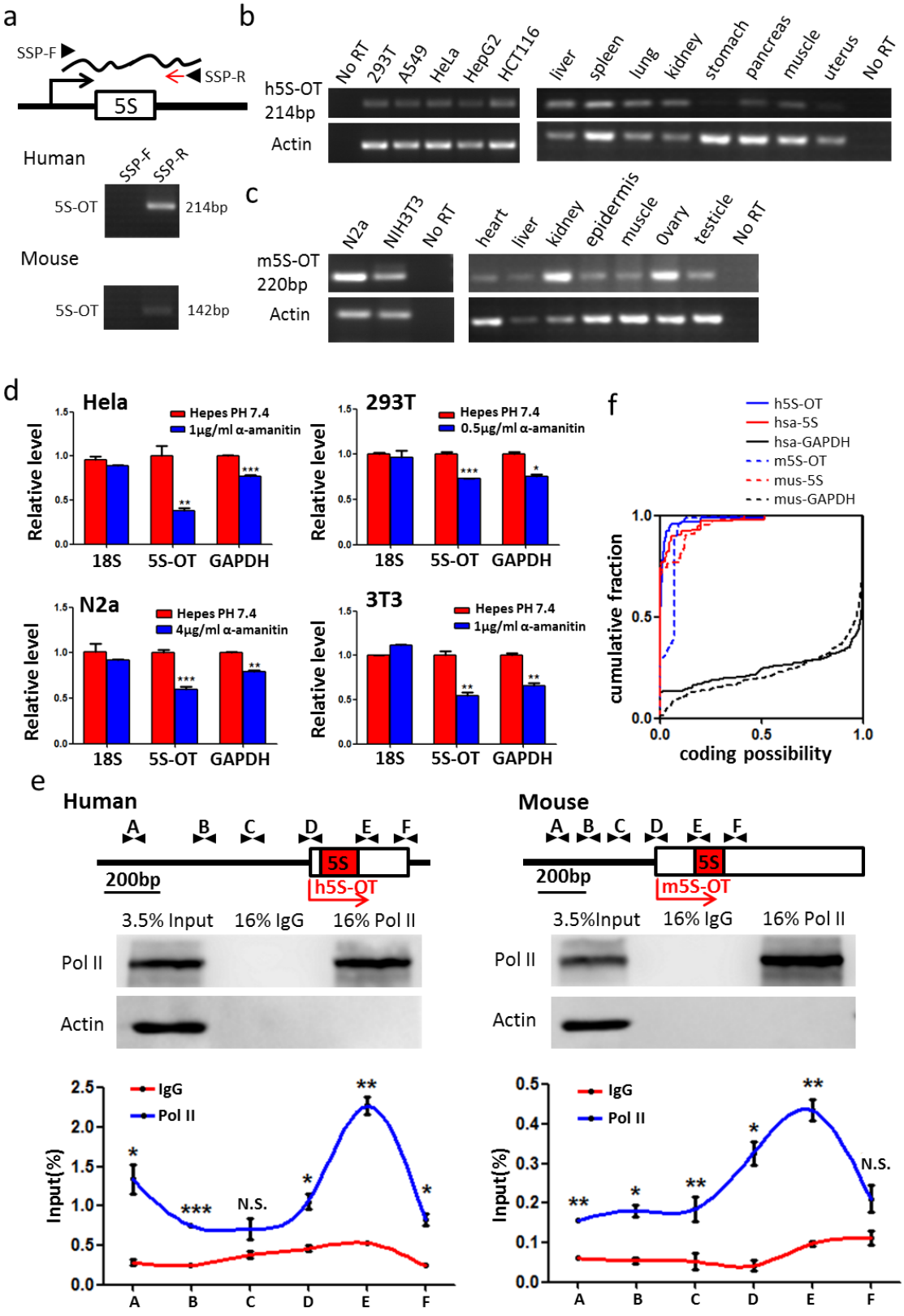
Supplementary Figure1



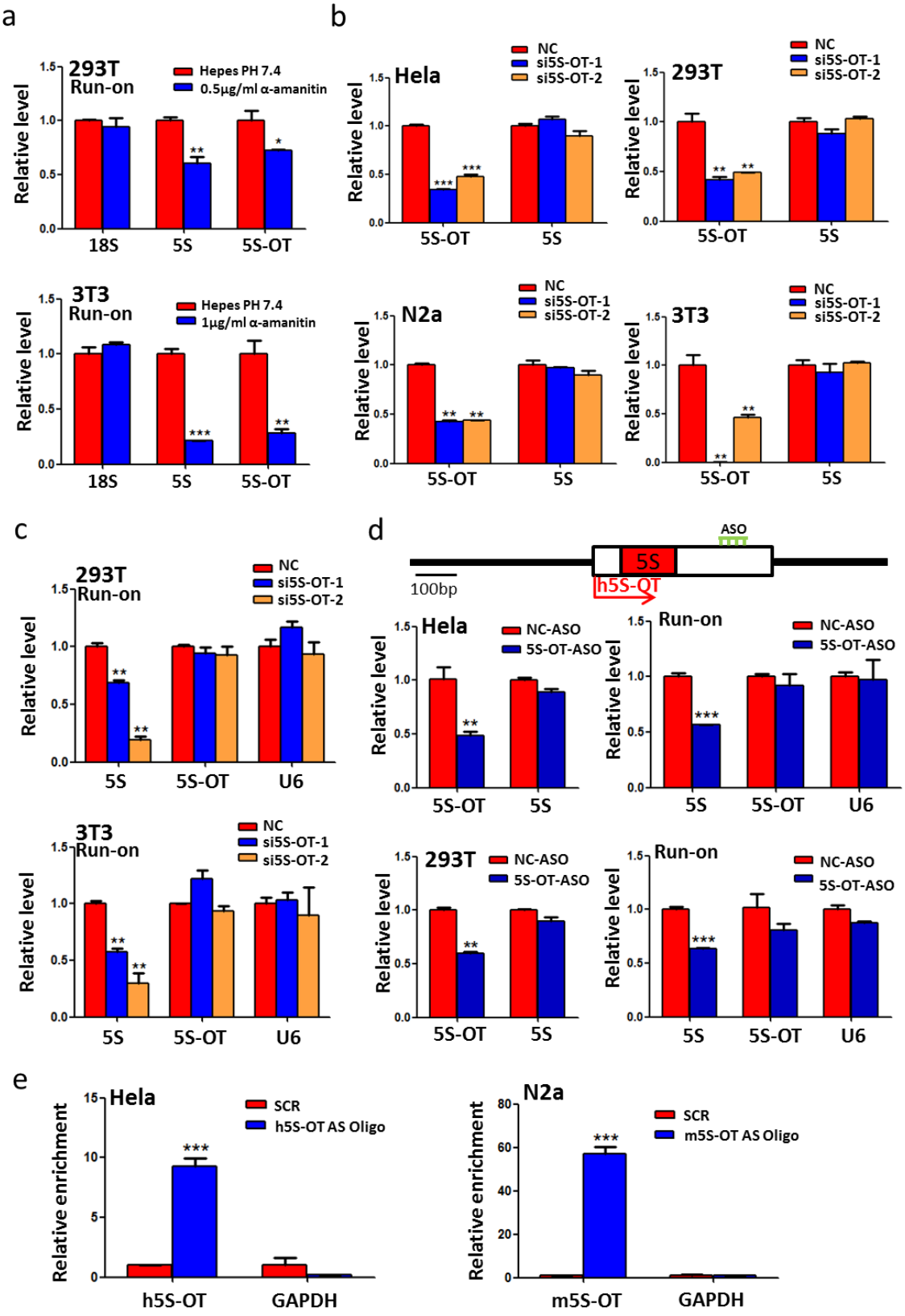
Nature Structural & Molecular Biology: doi:10.1038/nsmb.3302

Supplementary Figure 1

Characterization of mammalian 5S-OT as a pol II transcript.
a, Strand specific RT-PCR of 5S-OT in human and mice. SSP-F, RT primer with sense direction to 5S rRNA. SSP-R, RT primer with antisense direction to 5S rRNA.
b, RT-PCR of h5S-OT in different tissues and cell lines. Actin was shown as a positive control.
c, RT-PCR of m5S-OT in different tissues and cell lines. Actin was shown as a positive control.
d, Real-time PCR showing the decrease of 5S-OT level after 24h α-amanitin treatment in cells of human (Hela and 293T) and mice (N2a and 3T3). GAPDH mRNA, positive control; 18S rRNA (a pol I transcript), negative control.
e, ChIP showing binding of pol II to the promoter and gene body of h5S-OT (Hela cells) and m5S-OT (N2a cells). Actin as negative controls in western blots; 18S promoter as negative controls in Real-time PCR. The pattern of pol II binding for h5S-OT and m5S-OT has a peak at the first nucleosome from the TSS (~ 200 bp downstream), a feature of some "paused, expressed" genes in metazoans (Adelman K & Lis JT, 2012).
f, Cumulative probability distribution of coding potential as measured by CPAT for both noncoding transcripts and coding transcripts[12]. Hsa-5S rRNA and mus-5S rRNA are negative controls, and hsa-GAPDH and mus-GAPDH are positive controls.
Error bars, s.e.m. from triplicate experiments. N. S., not significant; *P < 0.05; **P < 0.01; ***P < 0.001 by two-tailed Student's t test.

Adelman, K. & Lis, J. T. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat Rev Genet* **13**, 720-31 (2012).

Supplementary Figure2



Nature Structural & Molecular Biology: doi:10.1038/nsmb.3302

Supplementary Figure 2

Cis effect of mammalian 5S-OT.

a, Nuclear run-on assays showing the decrease of 5S and 5S-OT in transcription after 24h α-amanitin treatment at a concentration of 0.5 μg/ml and 1.0 μg/ml in 293T and 3T3 respectively. 18S rRNA (a pol I transcript), negative control.

b, Knocking down 5S-OT with siRNAs in human (Hela and 293T) or mice (N2a and 3T3) cells did not affect the total levels of 5S rRNA.

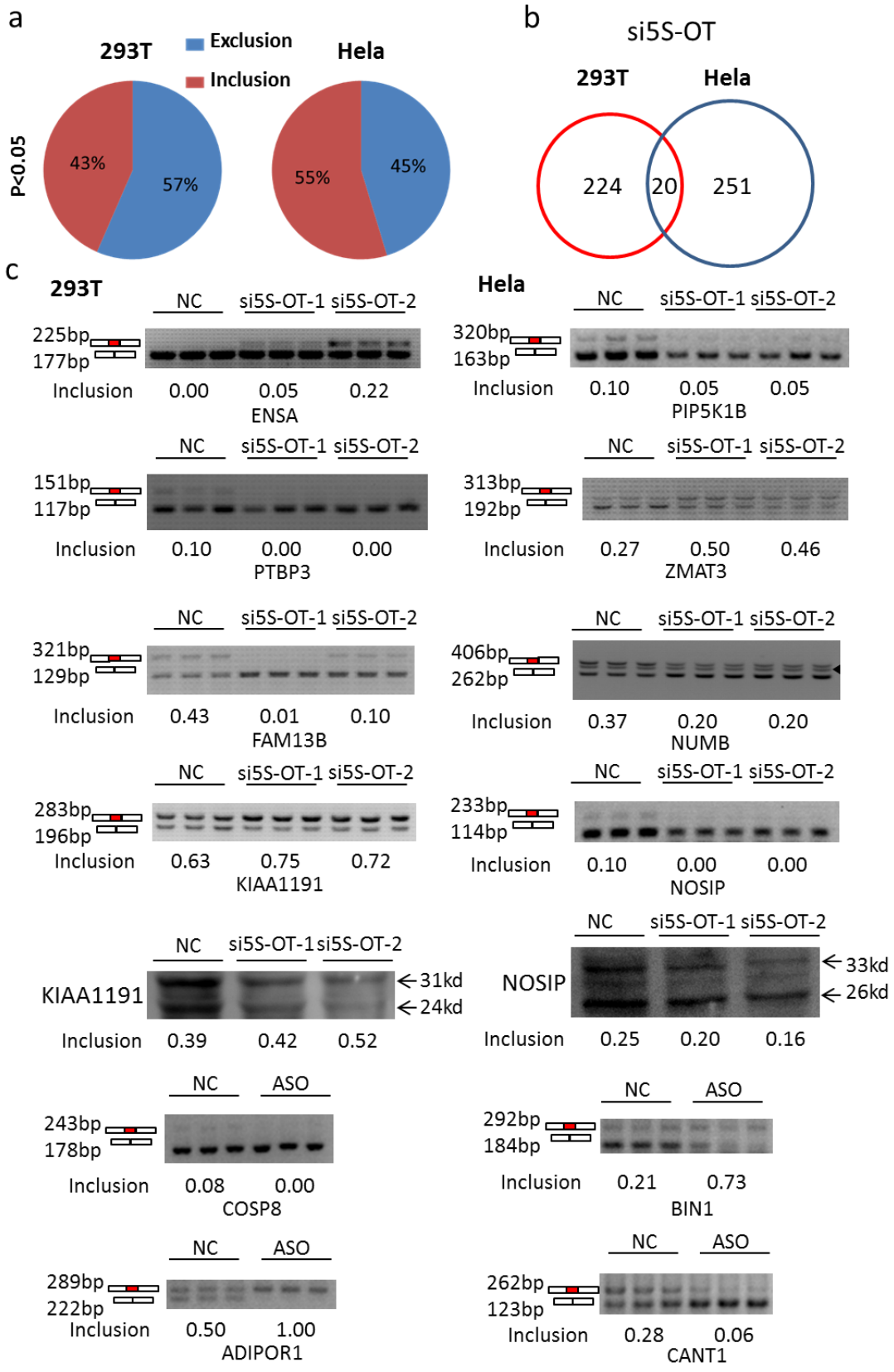
c, Nuclear run-on assays showing the decrease in the transcription of 5S but not the 5S-OT after 5S-OT knockdown using two individual siRNAs in 293T and 3T3, respectively. NC, siRNAs with scrambled sequences. U6 snRNA, another pol III transcript, negative control for 5S rRNA.

d, Knocking down 5S-OT in human (Hela and 293T) cells with ASO did not affect the total levels of 5S rRNA, but decreased the transcription of 5S but not the 5S-OT. NC-ASO, ASO with scrambled sequences. U6 snRNA, another pol III transcript, negative control for 5S rRNA.

e, Efficiency of RNA pulldown in ChIRP shown in Fig. 1f.

Error bars, s.e.m. from triplicate experiments. *P < 0.05; **P < 0.01; ***P < 0.001 by two-tailed Student's t test.

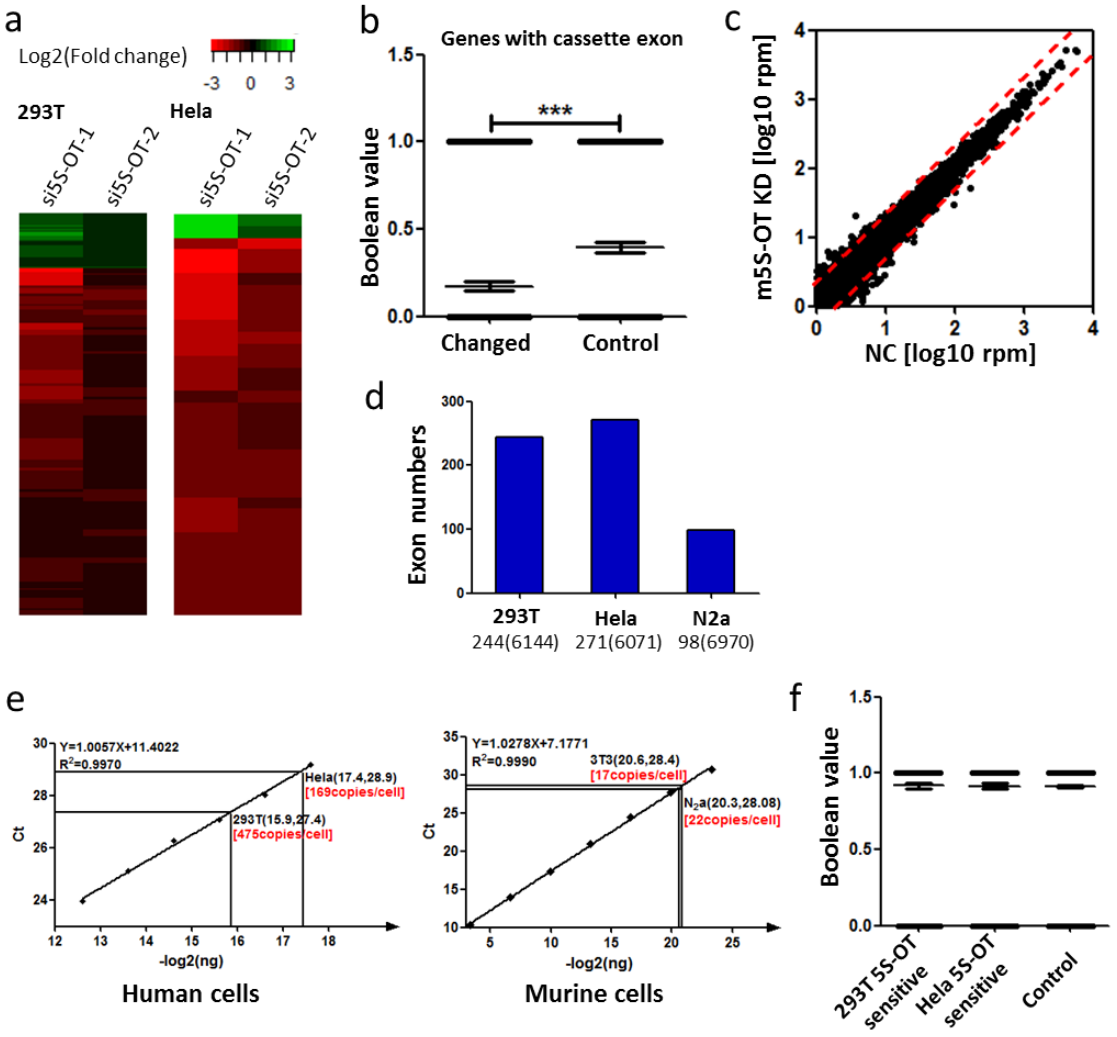
Supplementary Figure3



Supplementary Figure 3

h5S-OT knockdown leads to changes in alternative splicing.
a, Percentage of inclusion or exclusion (p<0.05) of h5S-OT sensitive exons in Hela and 293T cells.
b, Overlapped h5S-OT sensitive exons in 293T and Hela cells.
c, RT-PCR validation of 5S-OT sensitive exons in 293T and Hela cells with or without siRNA / ASO knocking down. For the RT-PCR gel of NUMB, an unspecific band was also amplified (indicated with a triangle). For KIAA1191 and NOSIP, western blots were performed to examine the protein levels of the corresponding isoforms. NC, negative control with scrambled sequences.

Supplementary Figure4



Supplementary Figure 4

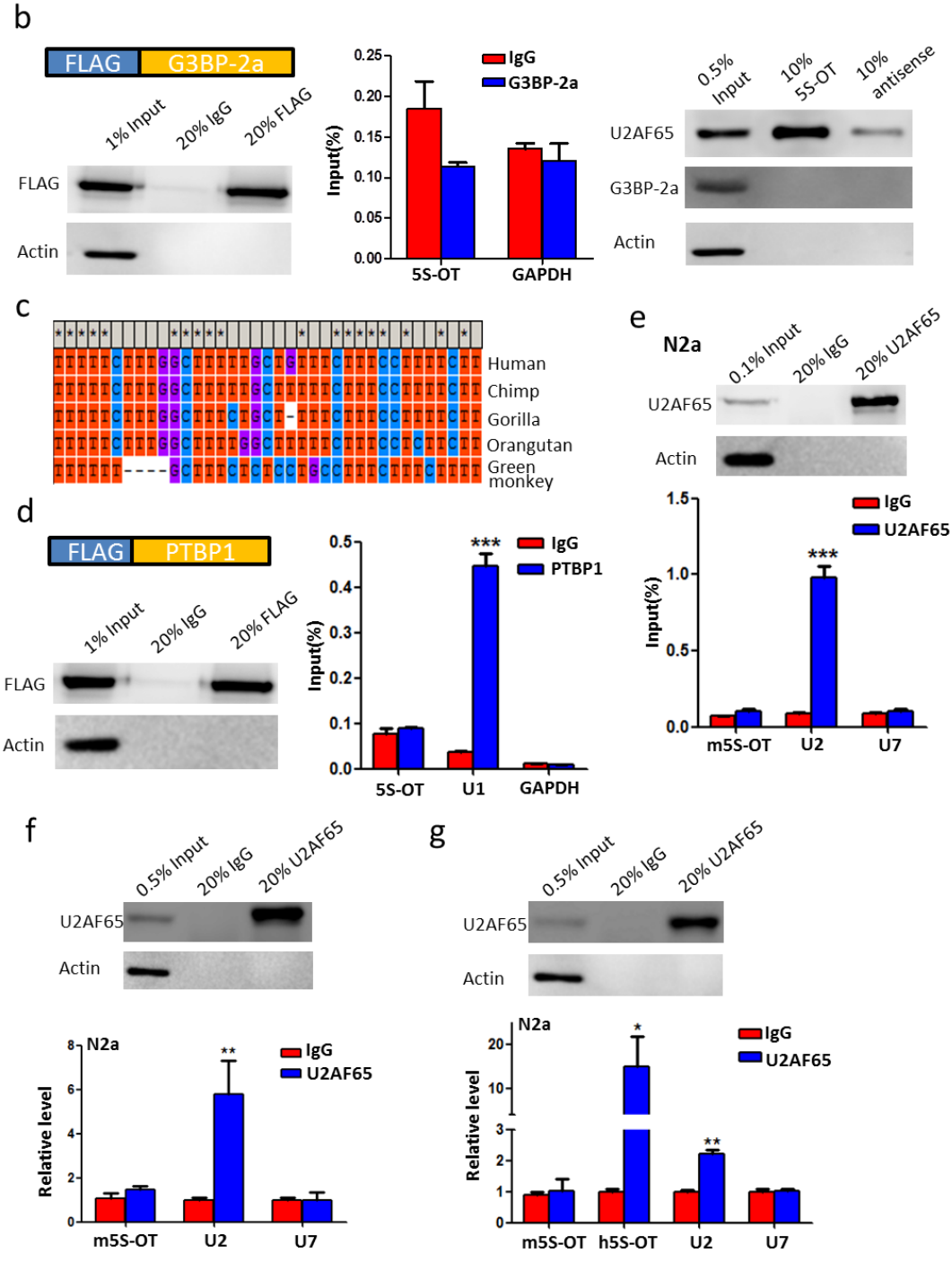
Comparison of effects of h5S-OT and m5S-OT on gene expression and alternative splicing, 5S-OT copy numbers, and possible indirect effects of h5S-OT in alternative splicing.
a, Heatmaps of gene expression (≥two fold change and p<0.01, genes with RPKM≥1 counted as expression) upon h5S-OT knockdown in 293T and Hela cells. siRNAs with scrambled sequences were used as negative control.
b, Boolean distribution of cassette exon in genes showed significant changes in expression levels upon h5S-OT knockdown. Control, 300 randomly chosen genes. Without cassette exon (0), with cassette exon (1).
c, Plot of gene expression upon m5S-OT knockdown in murine N2a cells.
d, Number of exons showed significant (p<0.01) changes in alternative splicing upon knocking down of 5S-OT in human (293T and Hela) and murine N2a cells. Total number of cassette exons detected in each cell line is indicated.
e, Plot of 5S-OT copy numbers in human and mice cell lines.
f, Boolean distribution of sense Alu sequences in full length pre-mRNA of h5S-OT sensitive genes. Control, all genes with cassette exons detected in 293T and Hela. No sense Alu sequences (0), with sense Alu sequences (1).
g, Splicing factor genes sensitive to h5S-OT. Overlaps between h5S-OT sensitive genes and PTBP3 RIP data (Brazão, et al., 2012) from 293T cells are shown. Isoform specific knocking down of PTBP3 affected the splicing of PUM2 (One of the 31 overlapped genes). Interestingly, there is no sense Alu within 2 knt upstream or downstream of the cassette exon in PUM2.

Brazão, T. F. *et al*. A new function of ROD1 in nonsense-mediated mRNA decay. *FEBS Lett* **586**, 1101-10 (2012).

Supplementary Figure5

a

Hits	Protein Mass	No. of Peptide	Protein	Description	Relative Abundance
1	53809.36	4	U2AF65	RNA binding protein	91.2%
2	61807.69	3	MCCC2	Mitochondrial protein	5.4%
3	54134.83	1	G3BP-2a	RNA binding protein	1.9%
4	54331.32	1	NONO	RNA binding protein	1.5%

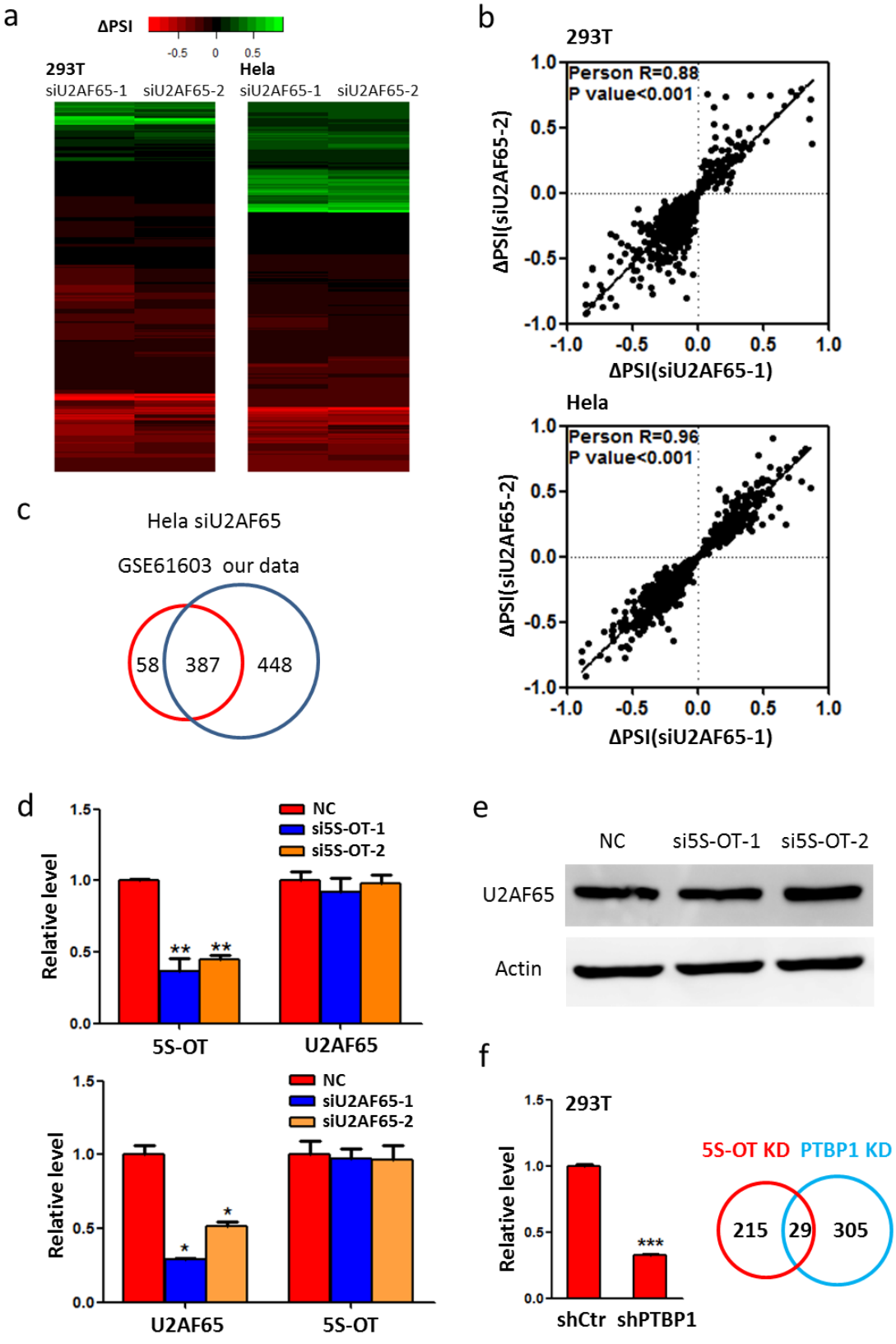


Supplementary Figure 5

Examination of the h5S-OT *trans* effect, interacting proteins, the Py site, and interaction between 5S-OT and splicing factors.

a, Mass spectrometry result of the h5S-OT specific band shown in Fig. 4a.
b, G3BP-2a and h5S-OT has no interaction with each other. RIP with FLAG antibody for FLAG-G3BP-2a did not pulldown h5S-OT; h5S-OT RNA pulldown did not co-pulldown G3BP-2a (U2AF65, positive control; Actin, negative control).
c, Alignment of Py site in the 5S-OT of Human, Chimp, Gorilla, Orangutan and Green monkey.
d, PTBP1 has no interaction with h5S-OT. RIP with FLAG antibody targeting the overexpressed FLAG-PTBP1 did not pull down h5S-OT. GAPDH mRNA was a negative controls, and U1 snRNA was a positive control.
e, Pulldown of U2AF65 protein did not pulldown m5S-OT. Western blots showing efficient pulldown of U2AF65 with Actin as a negative control. U2 snRNA, known to interact with U2AF65, was a positive control for m5S-OT, and U7 snRNA as a negative control.
f, Pulldown of U2AF65 protein did not pulldown m5S-OT even when it was overexpressed. Western blots showing efficient pulldown of U2AF65 with Actin as a negative control. U2 snRNA, known to interact with U2AF65, was a positive control for m5S-OT, and U7 snRNA as a negative control.
g, Pulldown of U2AF65 protein co-pulled down h5S-OT when it was artificially expressed in murine cells. Western blots showing efficient pulldown of U2AF65 with Actin as a negative control. U2 snRNA, known to interact with U2AF65, was a positive control, and U7 snRNA as a negative control.
Error bars, s.e.m. from triplicate experiments. *P < 0.05; **P < 0.01; ***P < 0.001 by two-tailed Student's t test.

Supplementary Figure6

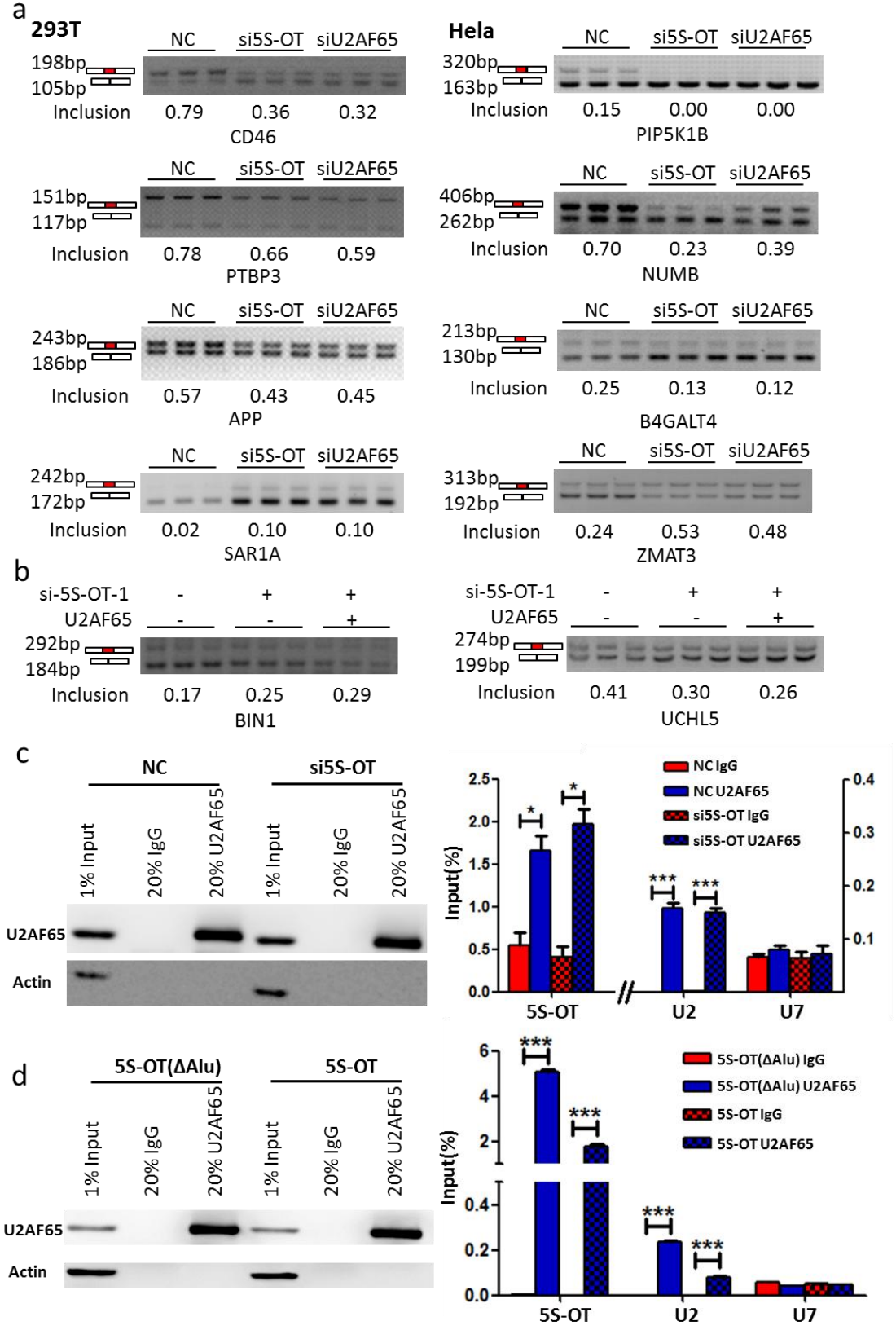


Supplementary Figure 6

U2AF65-sensitive exons and h5S-OT/U2AF65 and h5S-OT/PTBP1 relationships.

a, Heatmaps of ΔPSI upon knockdown of U2AF65 in 293T and Hela cells with two independent siRNAs. siRNAs with scrambled sequences were used as negative control. Cassette exons with significant (*P* <0.01) changes in PSI are shown. *P* values were generated by two-tailed Mann-Whitney U test.
b, Correlation plot of ΔPSI from two independent siRNAs in human 293T and Hela cells, respectively.
c, Venn diagrams showing the overlap of U2AF65 sensitive exons in Hela cells between our data and GSE61603[18]. We counted exons with significant (*P*<0.01) changes in ΔPSI, whereas the Ref. 16 also applied a cutoff of ΔPSI≥0.15 besides *P*<0.01.
d, Real-time PCR showing that knocking down either h5S-OT or U2AF65 did not change the expression level of the other.
e, Western blots showing that protein level of U2AF65 did not change when knocking down h5S-OT. Actin, loading control. NC, negative control with scrambled sequences.
f, Venn diagram demonstrating the overlap between h5S-OT and PTBP1 sensitive exons in human 293T. Knocking down efficiency of PTBP1 is shown in the bar figure. Vector (shCtr) of the shRNA construct was used as a control.
Error bars, s.e.m. from triplicate experiments. *P < 0.05; **P < 0.01; ***P < 0.001 by two-tailed Student's t test.

Supplementary Figure7

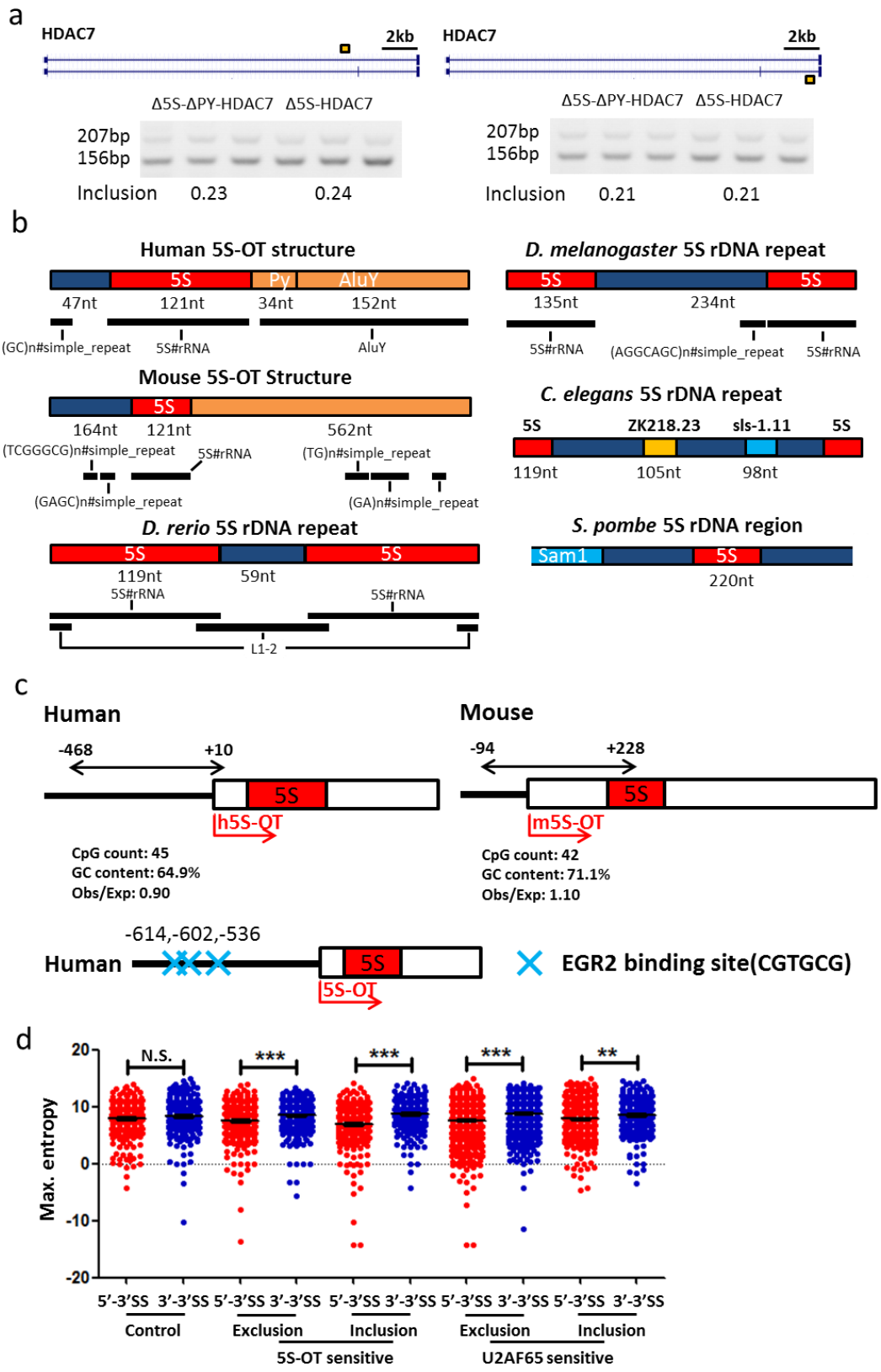


Supplementary Figure 7

Validation of h5S-OT- and U2AF65-sensitive exons and RIP efficiency of U2AF65.

a, RT-PCR validation of h5S-OT and U2AF65 sensitive exons in 293T and Hela cells.
b, Overexpression of U2AF65 did not compensate for h5S-OT knockdown in the effect of alternative splicing in two examples examined with 293T cells.
c, Pulldown of h5S-OT in RIP with an antibody against U2AF65 with or without the knocking down of h5S-OT. Western blots showing efficient pulldown of U2AF65 with Actin as a negative control. U2 snRNA, known to interact with U2AF65, was a positive control for h5S-OT, and U7 snRNA as a negative control.
d, Pulldown of h5S-OT in RIP with an antibody against U2AF65 upon the overexpression of h5S-OT. h5S-OT without the antisense Alu (ΔAlu) was used as a comparison. Western blots showing efficient pulldown of U2AF65 with Actin as a negative control. U2 snRNA, the positive control; U7 snRNA, the negative control.
Error bars, s.e.m. from triplicate experiments. *P < 0.05; ***P < 0.001 by two-tailed Student's t test.

Supplementary Figure8



Supplementary Figure 8

Examples of gene-specific h5S-OT that did not affect alternative splicing, 5S-OT structure in model organisms, the h5S-OT/m5S-OT promoter, and the strength of flanking 3' SSs of h5S-OT- and U2AF65-sensitive exons.

a, Examples of "gene specific" h5S-OT failed in affecting the alternative splicing. Target upstream (5') 3'SS or downstream (3') 3'SS with "gene specific" h5S-OT did not change the splicing pattern of the corresponding cassette exon.
b, Human and mouse 5S-OT structure, 5S rDNA region of model organisms, and annotation of repeats.
c, CpG island in the promoter proximity of h5S-OT and m5S-OT. No TATA box is present in either promoter. With the results shown in Sfig. 1e, it seems that both h5S-OT and m5S-OT promoters are "broad peak promoter", which often associates with ubiquitously expressed genes (Müller, et al., 2007). Shown below are EGR2 binding sites in the promoter of h5S-OT detected with MEME tool. There is no extra transcription factor binding site in the m5S-OT promoter region as against h5S-OT promoter.
d, Both h5S-OT and U2AF65 sensitive exons tend to have a relatively stronger 3' SS for their immediate downstream introns than the 3' SS of their immediate upstream introns. Control, 300 randomly chosen human cassette exons. N. S., not significant; **, P<0.01; ***, P<0.001; *P* values were generated by two-tailed Mann-Whitney U test.

Müller, F., Demény, M. A. & Tora, L. New problems in RNA polymerase II transcription initiation: matching the diversity of core promoters with a variety of promoter recognition factors. *J Biol Chem* **282**, 14685-14689 (2007).

Supplementary Table 1 Oligos used in the study

h5S-OT-F	GCCAGGCGCCTCCTTCAG	For human 5S-OT RT-PCR	Fig1a, sFig1a,b
h5S-OT-R	CTGCACTCCAGCCTGGGCGAC		
GAPDH-F	CTTCATTGACCTCAACTACATGG	For GAPDH RT-PCR of human and mouse	Fig1a,c
GAPDH-R	CTCGCTCCTGGAAGATGGTGAT		
m5S-OT-F	CAGCAGGCTCTTGGGGCTTG	For mouse 5S-OT RT-PCR	Fig1a, sFig1a
m5S-OT-R	GTATTCCCAGGCGGTCTCC		
m5S-OT-1-F	AAGG GAGAGAGAGACAGAG	For mouse 5S-OT RT-PCR	sFig1,c
m5S-OT-1-R	CGGGCGGGCGAGTGAGCGAG		
Ce-5S-OT-F	TGTAACTGACATGGGACTTGTC	For *C. elegans* 5S-OT RT-PCR	Fig8a,b
Ce-5S-OT-R	AAGTACTAACTGGACTCAAC		
Ce-Gad-1-F	GATCCAATGACATTTGATCTTG	For *C. elegans* GAPDH RT-PCR	Fig8a
Ce-Gad-1-R	CGTTTGATCGTAGTACACGTG		
Dm-5S-OT-F	CTGAATACATCGGTTCTCGTCC	For *D. melanogaster* 5S-OT RT-PCR	Fig8a,b
Dm-5S-OT-R	CTGATTTGTAGCCAATAATGC		
Dm-GPDH-F	GATCTGATCACGACGTGTTACG	For *D. melanogaster* GAPDH RT-PCR	Fig8a
Dm-GPDH-R	CTTCAGCATGTAGTTGACCTC		
Zebra-5S-OT-F	ATGTTCTCAACACGTTAGCTC	For *D. rerio* 5S-OT RT-PCR	Fig8a,b
Zebra-5S-OT-R	CCATGTGGTCTCTTATCCAG		
Zebra-GAPDH-F	GTCATTCCTGAGCTTAACGG	For *D. rerio* GAPDH RT-PCR	Fig8a
Zebra-GAPDH-R	CCTTCTTGACAGACTCCTTG		
S-yeast-5S-OT-F1	CTTCCATATGTACGGCTGC	For *S. cerevisiae* 5S-OT RT-PCR (No product)	Fig8a
S-yeast-5S-OT-R1	CATATCTACCAGAAAGCACC		
S-yeast-5S-OT-F2	CTGAGTTTCGCGTATGGTCAC		
S-yeast-5S-OT-R2	TGTGCGTGTGCGTGTGCTAAGAC		
S-yeast-TDH1-F	GTCGATGTTTCCGTTGTTGAC	For *S. cerevisiae* GAPDH RT-PCR	Fig8a
S-yeast-TDH1-R	CATCGAAGATGGAAGCGTGAGTG		
F-yeast-5S-OT-F	GATCACTGCAGTTAAGCGTCTG	For *S. pombe* 5S-OT RT-PCR	Fig8a,b
F-yeast-5S-OT-R	GGAAATCTAACTCGCATGAG		
F-yeast-TDH1-F	CATGTACGTTGTCGGTGTCAAC	For *S. pombe* GAPDH RT-PCR	Fig8a
F-yeast-TDH1-R	ACCGTCAACGGTCTTTTGGGTG		
Adaptor-primer	GCGCTGACAACGCTCCCGCTGAATT GGAATTTTTTTTTTTTTTTTTTTT	For 3'RACE in both human and mouse	Fig1c
Outer-primer	GCGCTGACAACGCTCCCGCT		
Inner-primer	CGCTCCCGCTGAATTGGAAT		
hGSP2	CTTTTCTTCCAGACGGAGTC	For 3'RACE in human	
hGSP1	GTTAGTACTTGGATGGGAGAC		
h5-GSP-1	GCGGTGGCTCGCGCCTGTAATCCCAG	For 5'RACE in human	
h5-GSP-2	GGGCGACAGGGCGAGACTCCGTCTGG		
mGSP-1	CTGGTTAGTACTTGGATGGG	For 3'RACE in mouse	
mGSP-2:	GACGGGAGAGCCTCTCTCCA		

m5-GSP-1	CAAATGGAGAGAGGCTCTCCCG	For 5'RACE in mouse	
m5-GSP-2	TGGGGTTTGGATGGCGCTGGCC		
Q-5S-R	CCTACAGCACCCGGTATTC	For 5S qPCR in both human and mouse	Fig1d,e, sFig2a,b,c,d,
Q-5S-F	CTACGGCCATACCACCCTG		
Q-h5S-OT-F	TCTTTGGCTTTTTGCTGTTTC	For 5S-OT qPCR and Northern and FISH probes in human	Fig1b,d,e,Fig2d Fig4c,Fig6a,b et al
Q-h5S-OT-R	AAGTAGCCGGGCGTGGTGG		
Q-m5S-OT-F	TAAGACAGGGCACACCACG	For 5S-OT qPCR and Northern and FISH probes in mouse	Fig1b,d,e,Fig2d sFig1d etal
Q-m5S-OT-R	TGGATGAGGATGGAGTGTTC		
Q-18S-F	CGGCGACGACCCATTCGAAC	For 18S qPCR in human and mouse	Fig1d, sFig1d
Q-18S-R	GAATCGAACCCTGATTCCCCGTC		
Q-28S-F	GAGAGTTCTCTTTTCTTTGTG	For 28S qPCR in human and mouse	Fig1d sFig1d
Q-28S-R	GTTCACCTTGGAGACCTGCT		
Q-U6-F	CGCTTCGGCAGCACATATAC	For U6 qPCR in human and mouse	Fig1e, sFig2c,d
Q-U6-R	TTCACGAATTTGCGTGTCAT		
Q-U2-F	CTCGGCCTTTTGGCTAAGAT	For U2 qPCR in human and mouse	Fig4b,sFig5e,f,g sFig7c,d
Q-U2-R	TATTCCATCTCCCTGCTCCA		
Q-human-U7-F	CAGTGTTACAGCTCTTTTAG	For U7 qPCR in human	Fig4b, sFig7c,d
Q-human-U7-R	AGGGGCTTTCCGGTAAAAAG		
Q-ACTB-F	CCAACACAGTGCTGTCTGG	For actin qPCR in human and mouse	Fig6a, sFig1b,c
Q-ACTB-R	GAGTACTTGCGCTCAGGAG		
Q-CD11b-F	ACTCCGACTTTCTGGCTGAG	For CD11b qPCR in human	Fig6a
Q-CD11b-R	TCTCAGCTGTGCTCACGATC		
Q-mouse-U7-F	AAGTGTTACAGCTCTTTTAG	For U7 qPCR in mouse	sFig5e,f,g,
Q-mouse-U7-R	AGGGGTTTTCCGACCGAAGT		
Q-U1-F	GATACCATGATCACGAAGGTG	For U1 qPCR in both human and mouse	sFig5d
Q-U1-R	CTACCACAAATTATGCAGTCG		
Q-U2AF65-F	GAGTGTGGGAGCCAAGAATGC	For U2AF65 qPCR in human and mouse	sFig6d
Q-U2AF65-R	AGGCAGCACCATGTTCATG		
Q-PTBP3-Q-1-F	CAGATCTTATAACAGTCGGT	For PTBP3 qPCR	sFig4g
Q-PTBP3-Q-1-R	TCTTGCTGTCATTCCCATTA		
P-h5S-OT-F1	TCGTCCTTGCTCTCCTTCAAG	For 5S rDNA loci in human	Fig1f
P-h5S-OT-R1	CACCAGGAGCAAATCCACTC		
P-h5S-OT-F2	GAGGGATCCAAAACGCTGCCT		
P-h5S-OT-R2	TCCCGAGCTTCCACCACATC		
P-m5S-OT-F1	GTATCCATGTATCCATCCAC	For 5S rDNA loci in mouse	Fig1f
P-m5S-OT-R1	CCAGCTTCTGGAATCCTGAC		
P-m5S-OT-F2	GAGGAGGGGAGATGTGTGT		
P-m5S-OT-R2	TCACGCTGCCTACAACCTC		
A(human)-F	TCGTCCTTGCTCTCCTTCAAG	For 5S rDNA loci in human	sFig1e
A(human)-R	CACCAGGAGCAAATCCACTC		
B(human)-F	GGGTCTTGGTCGGGACAAGC		

B(human)-R	TCTTGCCCCACCCACCCAGA		
C(human)-F	GAGGGATCCAAAACGCTGCCT		
C(human)-R	TCCCGAGCTTCCACCACATC		
D(human)-F	CGGTGTCGGCTGCAATCC		
D(human)-R	GCGTTCAGGGTGGTATGG		
E(human)-F	TCGGGCCTGGTTAGTACTTG		
E(human)-R	TCCGTCTGGAAGAAAAGGAA		
F(human)-F	GCTCACTGCAAGCTCCGCCTC		
F(human)-R	CAGGGTGAAAGCCCGTCTCTAG		
A(mouse)-F	GCAAGCTGCAGTGCCCTGAC	For 5S rDNA loci in mouse	sFig1e
A(mouse)-R	CACGCACAGCCACACCCATC		
B(mouse)-F	GTATCCATGTATCCATCCAC		
B(mouse)-R	CCAGCTTCTGGAATCCTGAC		
C(mouse)-F	GAGGAGGGGAGATGTGTGT		
C(mouse)-R	TCACGCTGCCTACAACCTC		
D(mouse)-F	GTGTCGTGTGGGTGGGGCT		
D(mouse)-R	GACGACGACGACGAGCTCAC		
E(mouse)-F	AGCAGGCTCTTGGGGCTTGT		
E(mouse)-R	CAAGTACTAACCAGGCCCGA		
F(mouse)-F	GTAGGCTTTTTGGACTCCCC		
F(mouse)-R	TGTGAGAGGACGAGGGTGG		
P-hsa-U6-F	CTGCTCTACAGTTCTTGCA	For U6 promoter in human	Fig1f
P-hsa-U6-R	GGTTGTGGAATCTAAGATC		
P-mus-U6-F	GTCATGCAGACAAGTTGGAAG	For U6 promoter in mouse	Fig1f
P-mus-U6-R	GACATGTCTGCCAAGATCTC		
h3'-1-F	TCCTAGAGACGGGCTTTCAC	Human control primers	Fig1e,1f
h3'-1-R	GCCACAAAAGCCTACAGCAG		
h3'-2-F	AGCTCCTGACCTCGTGATCC		
h3'-2-R	CAGGGGAGGTTGGGTAGCAT		
m3'-1-F	TGAGGGTAGGGGCAGGTGTG	Mouse control primers	
m3'-1-R	AGGGCACTGCAGCTTGCGCA		
m3'-2-F	GCAGCCTGCGAGTGAGTGGT		
m3'-2-R	CACGGAAACAGACACATGTC		
h5S-OT oligo1	CTGAAGGAGGCGCCTGGCTGCCCCAAGAGC	human 5S-OT antisense oligo with 5'biotin labeled	Fig1f,Fig3h,i, Fig4c,sFig2e
h5S-OT oligo2	GGCGACAGGGCGAGACTCCGTCTGGAAGAA		
h5S-OT oligo3	AGGCGGAGCTTGCAGTGAGCCGAGATGGC		
Srcamble	TTCTCCGAACGTGTCACGTTCGAACGTGTC	Control oligo with 5'biotin labeled	Fig1f,Fig3h,i Fig4c,sFig2e
m5S-OT Oligo1	CTGGCTGATTGAGCAAGCAAG	mouse 5S-OT antisense oligo with 5'biotin labeled	Fig1f sFig2e
m5S-OT Oligo2	CAGGTGAATTAGCAGAAGGCA		
m5S-OT Oligo3	GTGTTGGATGAGGATGGAGTG		
m5S-OT Oligo4	GTTGCTAGCTAACTCAGCAG		
m5S-OT Oligo5	AAAGGGAGAGAGAGACAGAGG		

m5S-OT Oligo6	ACGACGACGACGAGCTCACAC		
si-NC	UUCUCCGAACGUGUCACGU ACGUGACACGUUCGGAGAA	Negative control	Fig1e,Fig5g, Fig6b et al
si-h5S-OT-1	UUUGCUGUUUCUUUCCUUU AAAGGAAAGAAACAGCAAA	siRNAs of h5S-OT	Fig1e,Fig5g et al
si-h5S-OT-2	CUUUUUCUUUGGCUUUUUGC GCAAAAAGCCAAAGAAAAAG		
si-U2AF65-1	GCACGGUGGACUGAUUCGU ACGAAUCAGUCCACCGUGC	siRNAs of U2AF65 in human	Fig5a,b,c, sFig6
si-U2AF65-2	GCAAGUACGGGCUUGUCAA UUGACAAGCCCGUACUUGC		
si-m5S-OT-1	CUCCAUUUGCACCGAGCCC UU GGGCUCGGUGCAAAUGGAG	siRNAs of 5S-OT in mouse	Fig1e,sFig2b,c,d sFig4c,d
si-m5S-OT-2	GGAGAGCCUCUCUCCAUUU AAAUGGAGAGAGGCUCUCC		
NC-ASO	mU*mU*mC*mU*mC*CGAACGTGTCmA*mC*mG*mU*mU*	ASO of h5S-OT in human	sFig2d, sFig3c
H5S-OT-ASO	mG*mC*mA*mA*mA*AAGCCAAAGAmA*mA*mA*mA*mG*		
FISH-human-5S-F	FAM-TTTTCCTAGAGACGGGCTTTCAC	For DNA probe of 5S promoter in human	Fig2d
FISH-human-5S-R	FAM-TGCTTTGGAGGTGGGTTTCGTAG		
FISH-mouse-5S-F	FAM-TGTGGTGTGTGCTTGCGCTCTGGAG	For DNA probe of 5S promoter in mouse	Fig2d
FISH-mouse-5S-R	FAM-GACCCTGCTTAGCTTCCGAGATCAG		
Full-h5S-OT-F	GGCCGGGCCGGGCCGGGCTC	Plasmid for full length 5S-OT in human	Fig1c
Full-h5S-OT-R	AAGTAGCCGGGCGTGGTGG		
Full-m5S-OT-F	GCTGTGTGGTGTGTGTCTGTCG	Plasmid for full length 5S-OT in mouse	Fig1c
Full-m5S-OT-R	TTTTTACTGGCTGATTGAGCAAGC		
Tru-5S-AS-F	GCAGCCAGGCGCCTCCTTCAG CAAAGCCTACAGCACCCGG	Plasmid for 5S-OT replaced 5S with 5S inverted in human	Fig4d
Tru-5S-AS-R	GAAACAGCAAAAAGCCAAAGAAG TCTACGGCCATACCACCC		
T7-5S-F	TAATACGACTCACTATAGGTC TACGGCCATACCACCC	Plasmid for 5S insertion in human	Fig4d
T7-5S-R	AAAGCCTACAGCACCCGG		
Tru-5S-F	TTCTTTGGCTTTTTGCTGTTTC	Plasmid for 5S-OT with 5S deletion in human	Fig4d
Tru-5S-R	GCTGAAGGAGGCGCCTGGCTGC		
Tru-Py-F	CCAGACGGAGTCTCGCCCTG	Plasmid for 5S-OT with Py deletion in human	Fig4d
Tru-Py -R	GCCTACAGCACCCGGTATTC		
Py-F	AAGTGCCACCTGATGCGGTGTG	Plasmid for Py insertion in human	Fig4d
Py-R	AAGAAAAGGAAAGAAACAGCAAAAAGCC AAAGAAAAAGCCCTATAGTGAGTCGTATTA		

P-h5S-OT-F1	AGTAATCAATTAGTTATTAATTG TACATTGGTCAGGAAGAAC	Plasmid for overexpression 5S-OT	Fig5h,Fig7a,b,c sFig7d
P-h5S-OT-R1	CCAGGTAAGTATGAGATCTTC		
P-h5S-OT-F2	GAAGATCTCATACTTACCTGGG GCCGGGCCGGGCCGGGCTC		
P-h5S-OT-R2	GCGGTACCGTCGACTGCAGAAT TCAAGTAGCCGGGCGTGGTGG		
P-vect1-F	GAATTCTGCAGTCGACGGTAC	Plasmid for M-gene construction in human	Fig7a,b,c
P-vect1-R	GAGACTCCGTCTGGAAGAAA		
P2RX5-F	TTTCTTCCAGACGGAGTCTCGGCGGTCCCTCTGCCTGGGTT	Plasmid for M-P2RX5 construction in human	Fig7a
P2RX5-R	GTACCGTCGACTGCAGAATTCGGGCAGTTGGGGTTTGTGAG		
P-Δ5S-R	TGAAGGAGGCGCCTGGCTGC	Plasmid for Δ5S construction in human	
P-Δ5S-F	GCAGCCAGGCGCCTCCTTCACTTTTTCTTTGGCTTTTTGC		
P-Δ5SΔpy-R	TGAAGGAGGCGCCTGGCTGC	Plasmid for Δ5S Δpy construction in human	Fig7a, sFig8a
P-Δ5SΔpy-F	GCAGCCAGGCGCCTCCTTCATCTTCCAGACGGAGTCTC		
HMGCS1-AS-F	TTTCTTCCAGACGGAGTCTC AGTGAAAGGTTCAGTCCTGG	Plasmid for Δ5S Δpy construction in human	Fig7a, sFig8a
HMGCS1-AS-R	GTACCGTCGACTGCAGAATTCCCTTGGTTTAAAGCCTCAATTGG		
FAM96A-AS-F	TTTCTTCCAGACGGAGTCTC TGCGAAGCACCTAACTTAG		
FAM96A-AS-R	GTACCGTCGACTGCAGAATTC ACTGCTTTCTTACAGCTGGC		
TAZ-AS-F	TTTCTTCCAGACGGAGTCTCATCTCTGTGATCCTCCCTG		
TAZ-AS -R	GTACCGTCGACTGCAGAATTCTCCATGGTGGCAGTAGCATG		
PRR3-AS -F	TTTCTTCCAGACGGAGTCTCGTGCACAACCCAGTTCTCAC		
PRR3-AS -R	GTACCGTCGACTGCAGAATTCACCTATGGTGTCAGGATAG		
BTBD10-AS -F	TTTCTTCCAGACGGAGTCTCCTGGAATAACGTGTCTTTG		
BTBD10-AS -R	GTACCGTCGACTGCAGAATTCAAGCTCTTGCCTTCTCTTC		
NFIC-AS-F	TTTCTTCCAGACGGAGTCTCTGAAAGGCCCAAGGTCCTTG		
NFIC-AS-R	GTACCGTCGACTGCAGAATTCTGTGAATTACAGAGCCATCC		
HDAC7-AS-up-F	TTTCTTCCAGACGGAGTCTCCAAGCTTGAGCACGCCAAAG		
HDAC7-AS-up-R	GTACCGTCGACTGCAGAATTCGCTGTAGTCTGAGTTCAAGTC		
HDAC7-AS-down-F	TTTCTTCCAGACGGAGTCTCCTCAGGCTCACTTGACAATG		
HDAC7-AS-down-R	GTACCGTCGACTGCAGAATTCGATACAGTGTTACTGTGCAC		
HDAC7-check-F	TCTCACAGTCGCTCTGCAG		
HDAC7-check-R	GCCTGGTGTGTCTGCACAG		
NFIC-check-F	GCTCAAAGATCTTGTCTCGC		
NFIC-check-R	GATCCCTTGGTCCTAATCC		
BTBD10-check-F	ATCTGGCTGAGGAGGAAGTG		
BTBD10-check-R	CACTAGCACCATGTAGACTC		
TAZ-check-F	AGACATCTGCTTCACCAAGG		

TAZ-check-R	GAGCTTCTCCAAAATGAAGTC		
PRR3-check-F	GAAGAGACTGGAGATGAGGAG		
PRR3-check-R	CTCAGCAGTGGTGGAATCAG		
FAM96A-check-F	ACGCTGAGCAGAGTCCTGT		
FAM96A-check-R	GAGACCACTTCCAGTTCTTC		
HMGCS1-check-F	ACTGTCCTTTCGTGGCTCAC		
HMGCS1-check-R	GCAAGCTTCTGCATTCAAAGG		
3XFLAG-PTBP-F	GATGACAAGCTTGCGGCCGCCATGGACGGCATTGTCCCAGAT	Plasmid for overexpression of PTBP1	sFig5d
3XFLAG-PTBP-R	TTTTTGTTCGGATCCTCTAGACTAGATGGTGGACTTGGAGAAG		
3XFLAG-G3BP2-F	GATGACAAGCTTGCGGCCGCCATGGTTATGGAGAAGCCCAG	Plasmid for overexpression of G3BP-2a	sFig5b
3XFLAG-G3BP2-R	TTTTTGTTCGGATCCTCTAGATCAGCGACGCTGTCCTGTGAAG		
pET-U2AF65-F	AAGAAGGAGATATACATATGTCGGACTTCGACGAGTTCG	Plasmid for U2AF65 protein in pET22b vector	Fig5i
pET-U2AF65-R	TGGTGGTGGTGGTGCTCGAGCTACCAGAAGTCCCGGCGG		
KIAA1191-F	AGGGTATCATCATAGCTGAC	For RT-PCR check of splicing	sFig3 sFig4g sFig7a,b
KIAA1191-R	TCACAATGGCTTGGGTCCAG		
PTBP3-F	CCGCCTGCTTCCTCTGCTC		
PTBP3-R	GTCCGTTAATGATGCCAGAAG		
FAM13B-F	TGCTTGCTCAGTGAGGGTAG		
FAM13B-R	AAGCCACTCCACTGTCTCAG		
ENSA-F	TGCCTGAGAGAGCTGAAGAG		
ENSA-R	GTCCTGCACTTGGCAGCTG		
ZMAT3-F	AAGTTGCTCCGAGAAGAGGC		
ZMAT3-R	GTGTTGCAAGAGGATCATTGG		
PIP5K1B-F	TTCGCTGTGGGGAAGCGACAAC		
PIP5K1B-R	GGCAAGTCATCTGTAGTTAGTAG		
NOSIP–F	CACAGTTGAAGAAGCGACCG		
NOSIP–R	CTGTGTCCTTCTTCTTCTCG		
NUMB-F	CTGTGCTCACAGATCACCAATG		
NUMB-R	GCTGCAGAGGAGCAGCTGAGG		
CD46-F	TGGAGTTGCAGTAATTTGTGTTG		
CD46-R	GCAAACCAGGTTGTGGAATC		
C1orf43-F	GTTCAGGATATCAAGTATGAGC		
C1orf43-R	TTTCGCAGATCCAGCAGGTAG		
SAR1A-F	ACGTACATCCGGCGAGTAGC		
SAR1A-R	ACTGGAGCACACTGCTGAAG		
APP-F	CCGCTGGTACTTTGATGTGA		
APP-R	TTCTCATCCCCAGGTGTCTC		
PUM2-F	GGTCAACCTGGAAGTACATC		
PUM2-R	GCTGCTGGAGCTAAATAGAC		
COPS8-F	ACAGTCTGGGGTTTGGCTGTC		

COSP8-R	GCTAGAAGCTGACCATACAC		
BIN1-F	ACGACAGCAGGAAGAGAGCTG		
BIN1-R	TCAAGCAGGAGCAGATCCTC		
ADIPOR1-F	TCCAGCGCCGCGGGATGTA		
ADIPOR1-R	AGCTTCCCTGTTACTGGCAG		
CANT1-F	AAGCTGAGTGAGGAAGGAAG		
CANT1-R	TTAGCCCAGCCAAGCCCAG		
UCHL5-F	TCTGCCTTTCATTATGGAATTG		
UCHL5-R	TGCCTCAGCTATTCAAAAATCTC		
CHIRP-DCLRE1C-F	GTAACAGTATCTAACCTAGG	Primers for ChIRP assay	Fig3f,g Fig5g,h
CHIRP-DCLRE1C-R	GGATAATGGTCTCTATGGTCC		
CHIRP-BIN1-F	GTGCTGGAACCAAGTTACTC		
CHIRP-BIN1-R	GAGCATTTAGAGCACCTACTG		
CHIRP-MTO1-F	CCAACATGGTGAAACTCTGTC		
CHIRP-MTO1-R	GTAGCGTGATCTCAGCTCAC		
CHIRP-ASL-F	CATGTGTCAGGAGACAAGTG		
CHIRP-ASL-R	CGCACACACAGAGAACCAAG		
CHIRP-ATF2-F	AGTGGCTCACGCCTGTAATC		
CHIRP-ATF2-R	TTACAGGTGTGCACCACCAC		
CHIRP-GPBP1-F	CTGAGTGCAGTGGCTCACTC		
CHIRP-GPBP1-R	TGATCATTATTGGCCAGGCTG		
CHIRP-MDM1-F	GACATTGCACAAATACAGCC		
CHIRP-MDM1-R	ATGCACAGTCTGATGACTG		
CHIRP-CHEK2-F	AGGCTAGAGTGCAATGGTGC		
CHIRP-CHEK2-R	CTGTAATCCCAGCTACTCAG		
CHIRP-YTHDF3-F	GCTTCGGCTCGGCATCAGAG		
CHIRP-YTHDF3-R	CATGGCATATTTAACAGTGG		
CHIRP-SEPN1-F	TCAAGACCAGCCTGGTCAAC		
CHIRP-SEPN1-R	TACAGGTGTGCACCACCAC		
CHIRP-FAM13B-F	TCCGCCTCGCAGGTTCAAGC		
CHIRP-FAM13B-R	GCTCACACCTGTAATCTCAGC		
pre-GAPDH-F	CTGCTCACATATTCTGGAG		
pre-GAPDH-R	GTTAAAAGCAGCCCTGGTG		