

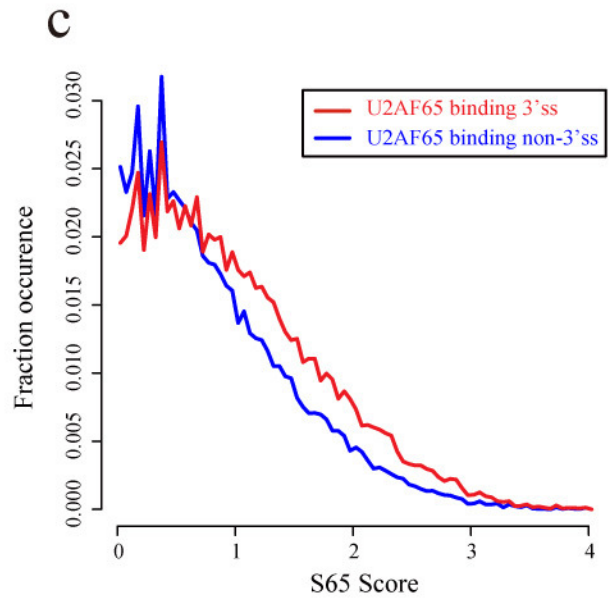
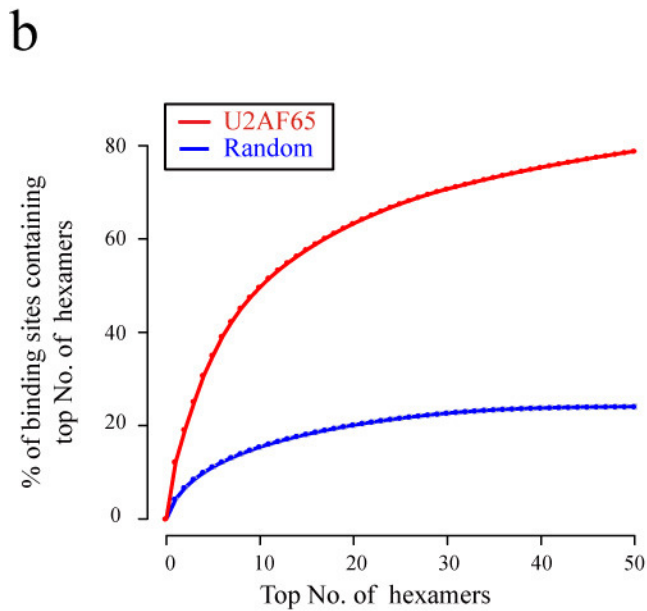
Supplementary Figure 1

Mapping of U2AF65 CLIP tags to the human genome.

(a) Western analysis of the IP efficiency of anti-U2AF65 antibody. (b) Comparison between U2AF65 CLIP-seq data generated in the current study with the published U2AF65 iCLIP-seq data¹⁷. (c,d,e) Profiles of base deletion (c), insertion (d) and substitution (e) detected by CIMS analysis of U2AF65-RNA interactions³⁷. (f) Preferential deletion mutation on uridine residues in CIMS. (g) Footprint of U2AF65 binding on RNA based on CIMS.

a

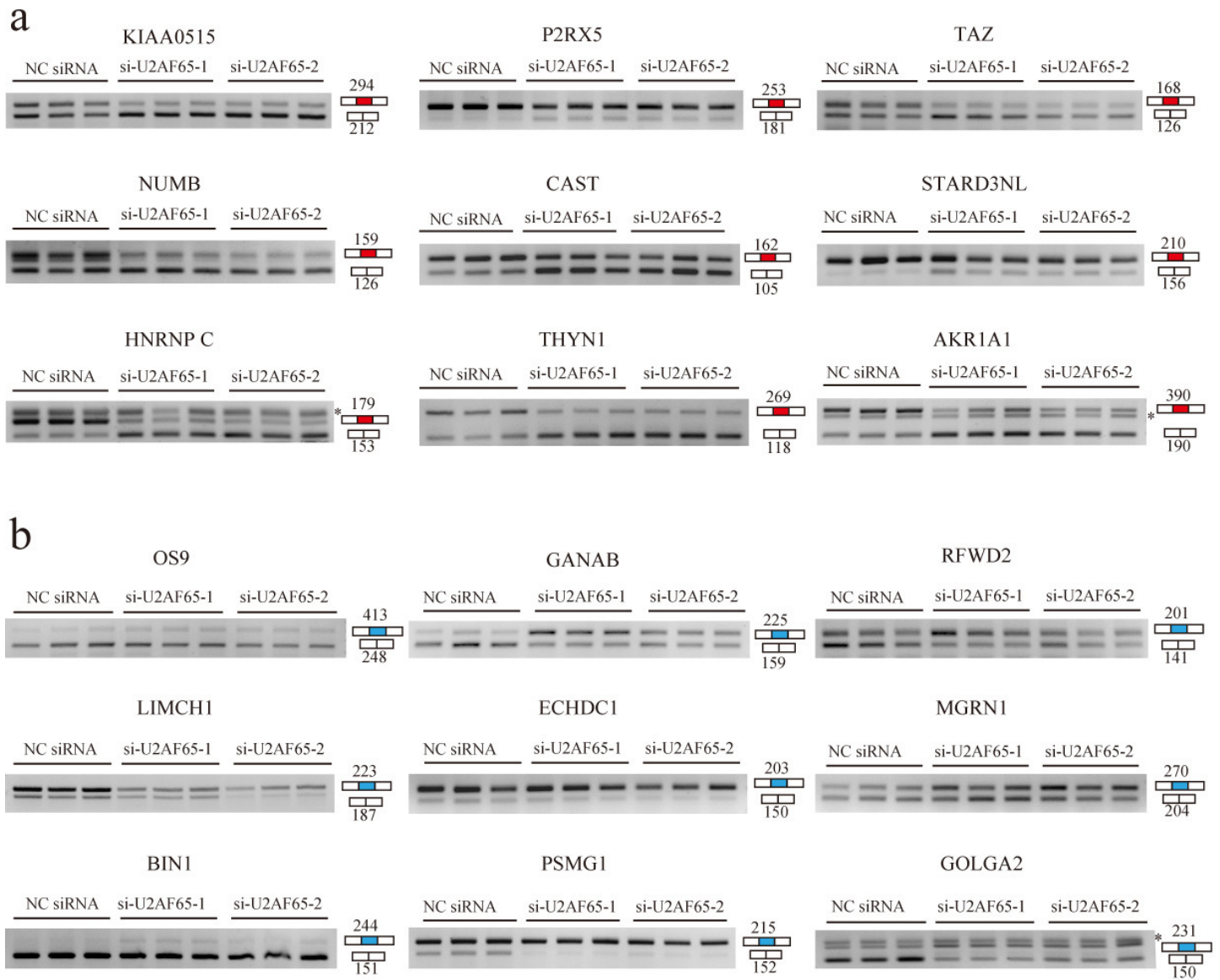
Rank	Motif	Z-Score	Rank	Motif	Z-Score
1	TTTCTT	291.73	11	TTGTTT	218.16
2	TTTTTC	287.13	12	TCTCTT	215.60
3	TTTTCT	284.90	13	CTTTT	205.42
4	TTCTTT	265.92	14	TTTCCT	201.44
5	TCTTTT	248.90	15	TTTGTT	196.16
6	TGTTTT	236.20	16	TCTTTC	183.32
7	TTCCTT	234.80	17	TCCTTT	177.87
8	TTTCTC	226.90	18	CTTTTC	176.71
9	TTCTCT	223.15	19	TTCTTC	165.66
10	TTTTCC	218.24	20	GTTTTT	163.49



Supplementary Figure 2

Enriched motifs in U2AF65-binding sites.

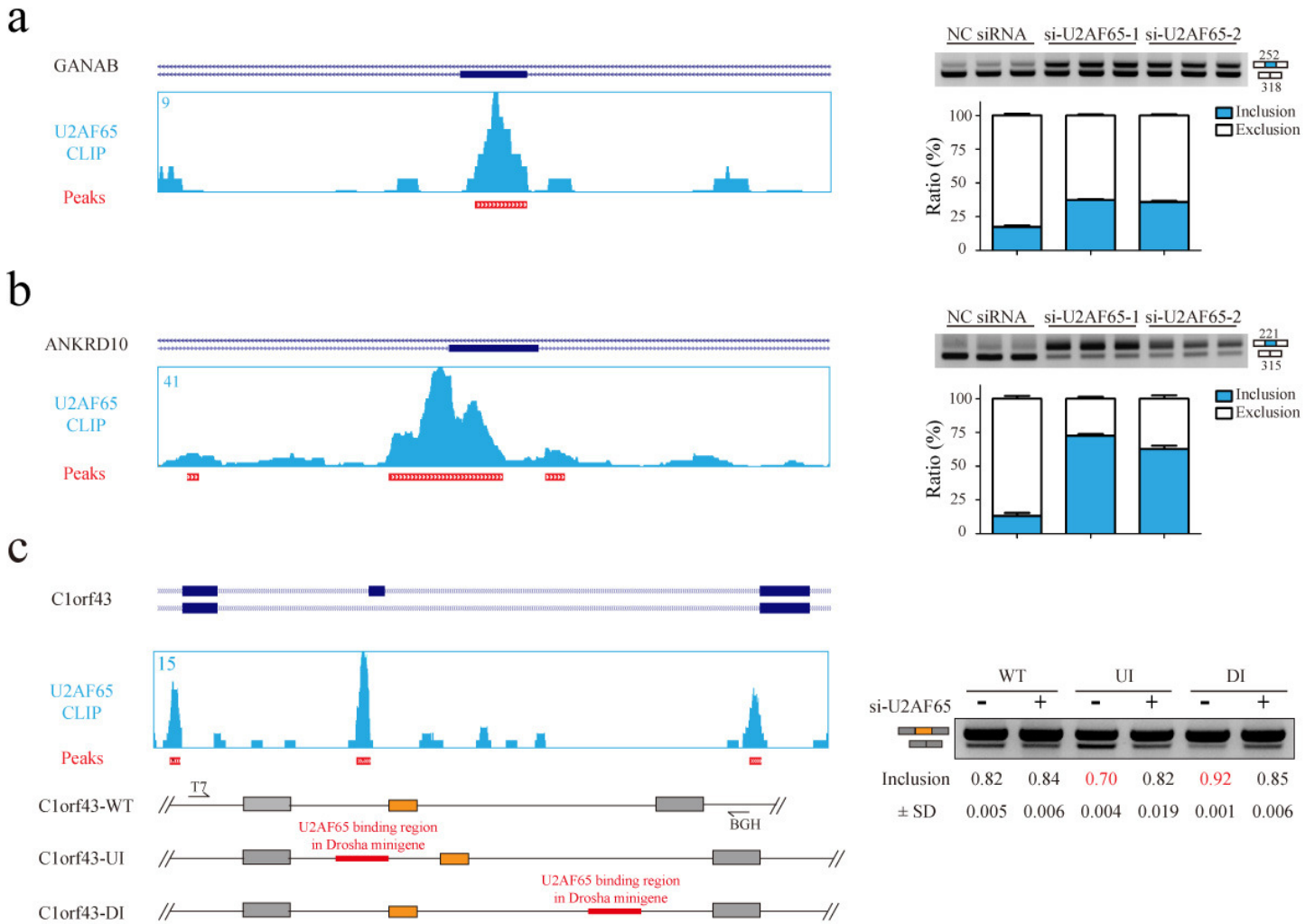
(a) List of top 20 enriched hexamers. (b) Percentage of U2AF65 binding sites that contain one or more top 50 motifs (red), compared with randomly selected 50 hexamers (blue). (c) S65 scores of U2AF65 binding sites in 3' splice sites and non-3' splice sites¹⁸.



Supplementary Figure 3

RT-PCR validation of U2AF65 RNAi-induced alternative splicing.

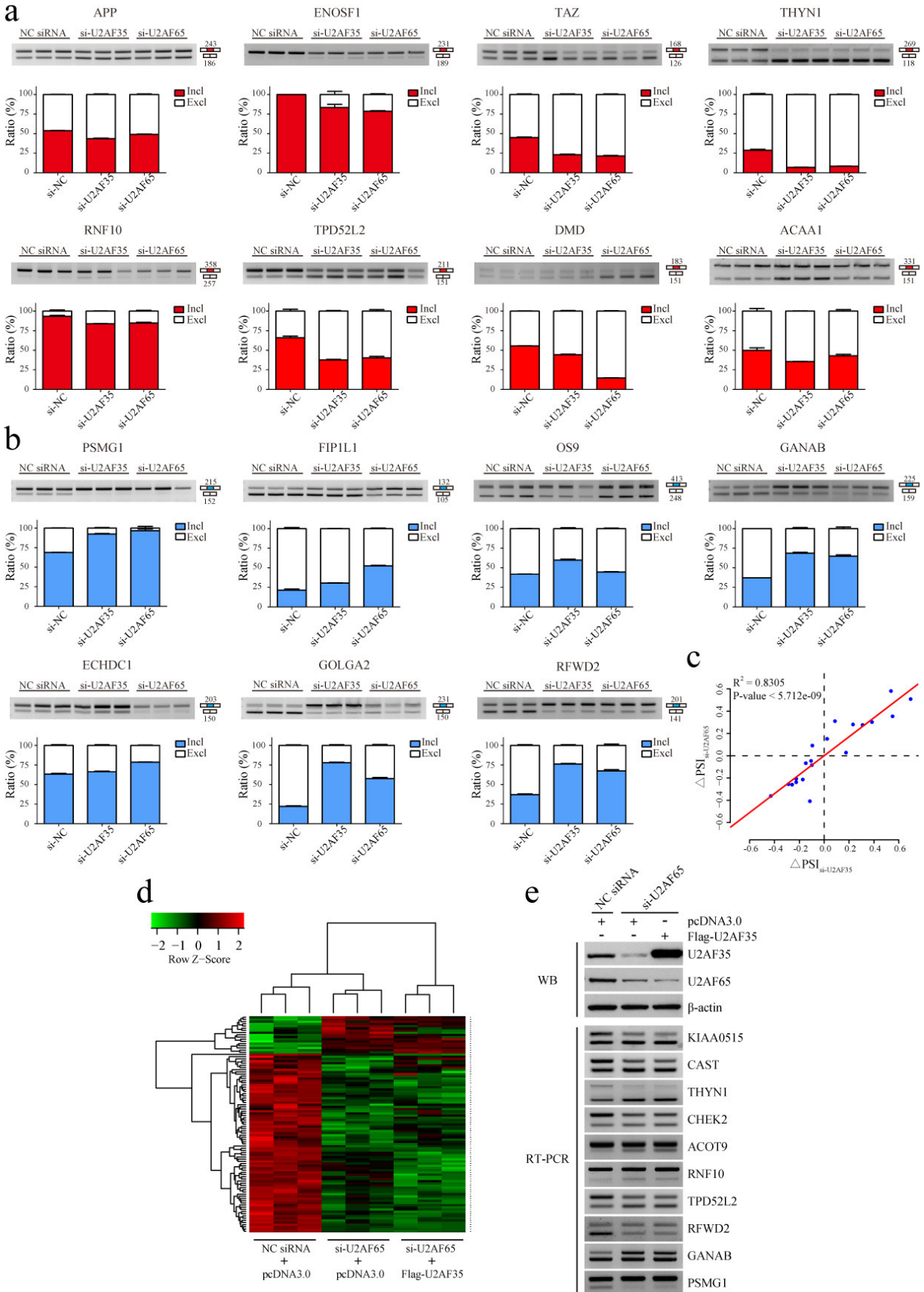
(a) Induced exon skipping events in response to U2AF65 RNAi. (b) Induced exon inclusion events in response to U2AF65 RNAi.



Supplementary Figure 4

U2AF65 binding profile on representative genes and insertion mutational analysis of U2AF65-binding site-induced changes in alternative splicing.

(a) U2AF65 binding on the alternative exon in *GANAB*. (b) U2AF65 binding on the alternative exon in *ANKRD10*. PCR-validated splicing changes are shown in the right. (c) The minigene constructs illustrating the insertion of the U2AF65 binding site from an intronic region in *DROSHA* (see Fig. 4d) into an U2AF65 insensitive gene *C1orf43* either in an upstream or a downstream intronic location. Bottom panel: RT-PCR analysis of wt and mutant minigenes in response to U2AF65 RNAi.



Supplementary Figure 5

Similar effects of U2AF65 RNAi and U2AF35 RNAi on alternative splicing.

(a) Examples of induced exon skipping events in response to U2AF65 or U2AF35 RNAi. (b) Examples of induced exon inclusion events in response to U2AF65 or U2AF35 RNAi. (c) Concordant splicing response to U2AF65 and U2AF35 RNAi determined by RT-PCR. Δ PSI: Difference of Percentage of Splicing In (PSI) between HeLa cells treated with U2AF35 or U2AF65 siRNA and negative control (NC) siRNA. (d) Heatmap showing similar U2AF65 RNAi-induced splicing events with or without exogenously expressed U2AF35. (e) RT-PCR validation of a set of U2AF65 RNAi-induced splicing events in the presence or absence of overexpressed U2AF35.

Supplementary Table 1: Deep sequencing statistics

a: RNA-Seq		
	Ctrl	Knock down U2AF65
total reads	28228751	25595461
mapped reads	14384722	17183922
mapped ratio	50.96%	67.14%
uniquely mapped reads	14119360	16834141
uniquely mapped ratio	91.16%	97.96%

b: U2AF65 CLIP-Seq	
total reads	19513772
mapped reads	12088822
mapped ratio	61.95%
uniquely mapped reads	9329565
uniquely mapped ratio	77.18%
reads with CIMS	1482140

c: RASL-Seq		
	Knock down U2AF35	Knock down U2AF65
total detectable events	1892	
significantly changed events	334	271
co-changed events	208	
events in the same directions	206	
ratio of same direction changes over total	99%	

Supplementary Table 2: List of primers used to express U2AF and its mutants

Expressed gene	Forward Primer (5'--3')	Reverse Primer (5'--3')
U2AF35-WT	CATAAGCTTGCCACCATGGATTACA AGGATGACGACGATAAGGCGGAGT ATCTGGCCTCC	TGAGGATCCTCACAGATCCTCTTCAGAG ATGAGTTTCTGCTCGAATCGCCCAGATC TTTCAC
U2AF35-S34Y	CGTCATGGAGACAGGTGCTATCGG TTGCACAATAAACCG	CGGTTTATTGTGCAACCGATAGCACCTG TCTCCATGACG
U2AF35-S34F	CGTCATGGAGACAGGTGCTTTCGGT TGCACAATAAACCG	CGGTTTATTGTGCAACCGAAAGCACCTG TCTCCATGACG
U2AF35-Q157R	CAGAGAAGCCTGCTGCCGTCGGTA TGAGATGGGAGAATGC	GCATTCTCCCATCTCATAACCGACGGCAG CAGGCTTCTCTG
U2AF35-Q157P	CAGAGAAGCCTGCTGCCGTCGGTA TGAGATGGGAGAATGC	GCATTCTCCCATCTCATAACCGACGGCAG CAGGCTTCTCTG
U2AF35-Del-UHM	CTTCCCAGTCTGCTGACGGTACGGA CTTCAGAGAAGCCTGC	GCAGGCTTCTCTGAAGTCCGTACCGTCA GCAGACTGGGAAG
U2AF65-WT	CATAAGCTTGCCACCATGGATTACA AGGATGACGACGATAAGTCGGACT TCGACGAGTTC	TGAGGATCCTCACAGATCCTCTTCAGAG ATGAGTTTCTGCTCCAGAAGTCCCGGC GGTG
U2AF65-R18W	GCTCAACGAGAATAAACAAGAGTGG GGACAAGGAGAACCGGCATCG	CGATGCCGGTTCTCCTTGTCCCACTCTTG TTTATTCTCGTTGAGC
U2AF65-M144I	GCCCGTGGTCGGGAGCCAGATCAC CAGACAAGCCCGGCCTC	GAGGCGCCGGGCTTGTCTGGTGATCTGG CTCCCGACCACGGGC
U2AF65-L187V	CAGGCCCTGGCAACCCAGTGGTG GCTGTGCAGATTAACCAGG	CCTGGTTAATCTGCACAGCCACCACTGG GTTGCCAGGGGCCTG

Supplementary Table 3: Oligonucleotides for cloning of minigene reporter plasmids and RT-PCR quantification

TPD52L2-WT	Forward	TGACTCGAGGGACCATATGGTCCCACA
	Reverse	TGACCCGCGGAGAGCACCCAGATGTACAG
TPD52L2-Del	Forward	TGCCTGTATCATCAGCACCAACTTTTGTGGAACAGGG
	Reverse	CCCTGTTCCACAAAAGTTGGTGCTGATGATACAGGCA
EIF4A2-WT	Forward	CGCAAGCTTGCTCGCGGGATTGATGT
	Reverse	CCGCTCGAGGTGATCGCCTATTCAGCAAC
EIF4A2-UD	Forward	CAGCATCTTGGCTGTATTGAATTCGAGTGAACCCTGGTTT AGTTATA
	Reverse	TATAACTAAACCAGGGTTCCTCGAATTCAATACAGCCA AGATGCTG
EIF4A2-DD	Forward	GAGCGAGTCGGTATTTATATTTGGATCCAGTTAGAAGCA CGAACTA
	Reverse	TAGTTCGTGCTTCTAACTGGATCCAAATATAAATACCGAC TCGCTC
Drosha-WT	Forward	TGAGGATCCAGAGCGAGAACGGGAGAGAC
	Reverse	TGACTCGAGCACCTGATCCATGGGGTCAC
Drosha-Del	Forward	GAACACAAATGTGTTTCATGATAGCCCGTATTGTAATGTCT GGAAGTTA
	Reverse	TAACCTCCAGACATTACAATACGGGCTATCATGAACACA TTTGTGTTT
C1orf43-WT	Forward	CCCAAGCTTGACTTGAAAGAGGAGATTGATATTCG
	Reverse	ATGCTCGAGCCCTGTCCCATAGCGGGCTGTTC
C1orf43-UP-AgeI	Forward	CAGTCTCAGCTCACTGCAACACCGGTCTCCACCCCTTGT TCAAGC
	Reverse	GCTTGAACAAGGGGGTGGAGACCGGTGTTGCAGTGAGCT GAGACTG
C1orf43-Down-AgeI	Forward	GCATGGTGGTGCACATCTGTACCGGTAATCCCAGCTAAT CAGGAGG
	Reverse	CCTCCTGATTAGCTGGGATTACCGGTACAGATGTGCACC ACCATGC
U2AF65 binding region	Forward	ATAACCGGTATTATTAGTTTTGTTAAATTATGG
	Reverse	ACTACCGGTTGGGAGGATTAGAAAGATTATAC
U2AF65 non-binding region	Forward	GAGAGTTAGAAGCCAGCCACC
	Reverse	CCACCGCCTCCTGGGTT
Quantification primer 1	T7 Promoter	TAATACGACTCACTATAGG
	BGH-Rev-primer	TAGAAGGCACAGTCGAGG
Quantification primer 2	EGFP-Forward	CTGGTCGAGCTGGACGGCGACG
	EGFP-Reverse	TTCTTCTGCTTGTCGGCCAT

Supplementary Table 4: List of primers used to validate U2AF-regulated splicing events

Gene Symbol	Forward Primer (5'--3')	Reverse Primer (5'--3')	Short isoform (nt)	Long isoform (nt)	Validated
EFNA1	GCGCTTCACACCTTTCACC	GCAAGTCTCTTCTCTGTGGA	108	170	Yes
UHL5	TCTGCCTTTCATTATGGAATTG	TGCCTCAGCTATTCAAAAATC TC	199	274	Yes
CD46	TGGAGTTGCAGTAATTTGTGT TG	GCAAACCAGGTTGTGGAATC	156	249	Yes
STAG3L2-E7	CTGGATGGTTTCCATGATCG	AAGGACAGCAGAAGCTGGAA	177	285	Yes
STAG3L2-E4	CCAAGCGGTACATCGTCATA	GAAGCGGCCAGTGAAGAG	187	311	Yes
SEC16A	TAAGCTTGCAGCGTCACTC	CAGCTGAGCAGGGTTGTAGA	143	203	Yes
EIF4G2	GGGACGTCATCGTTCAAATC	TGGTGGTTGTGCACTAGGAG	205	319	Yes
FAM76B	CTCCAAAGAAAAAGCCAAA	AATGGTCTGGTCTCTCTGCTG	167	225	Yes
CCDC53	GGTGTACAGTGATGGCAATAA GA	GAAGATTCGCTATCTGAACTT TCTTC	136	222	Yes
PIGQ	GCTCGTGGACCTCATCAACT	ATGAGCTCCCAAGGAACAG	190	252	Yes
SAT1	TTTGGAGAGCACCCCTTTTA	CTCATCACGAAGAAGTCTCTCA A	158	268	Yes
ANKRD36B	AGCTCTGCTGGATGCTTCAT	TTTCTCAAGCCGCTCAATTT	164	235	Yes
EIF4A2	GCTCGCGGGATTGATGTGC	GAATCCTCTTGTCTTCTTC	154	261	Yes
HNRPDL	AGCACTTATGGCAAGGCATC	TCTTCAATGTGCTCTGCAA	129	234	Yes
MYOF	CTAAAATTGCTGCCTCTGGTG	TGGGAATCCCGTGTACTCTC	128	167	No
NAP1L4	GAAGGTGACGAGGAGGGAGA	CCTCCGCTTCTACTGCTG	100	138	No
ANKRD10	TGAGGGTGAACTCCCATTC	GAACTGGGCACACTCTTGG	161	255	Yes
GIT1	GCTGGCCTTCTACCTCTGTG	TTCTCTTCGATCCACCTCGT	187	214	Yes
ACOT9	CAAAGGGCAGTTACTCCTG	GGCAGTCCATCCTGTGATT	201	228	Yes
KIAA0515	ATGAAAGGCTTCCACTTTGC	TCAGGCCTTACTCTCTCCA	212	294	Yes
ENOSF1	AGCCATTGTGGATTGAGGAG	ACTTTTGGCCATCAGCAAT	189	231	Yes
THYN1	AGAGGCTTACCCAGACCACA	CCTCCAGGCTCAAAACAAA	118	269	Yes
CHEK2	CAGCTCTCAATGTTGAAACAG AA	CTGCACAGCCAAGAGCATC	150	212	Yes
CAST	TGAGAAGAAGTCACAGTCAA CCA	TCAGCTGATCTGGAAACTGCT	105	162	Yes
MGRN1	TGGAGCATGATGAGCACTCT	TGTCCAGGATGTGGTCGATA	204	270	Yes
HNRNPC	GCAGCAGTCGGCTTCTCTAC	CATGGAGCGAGGATCTGTCT	153	179	Yes
TAZ	AGATGGCGTCTACCAGAAGG	AGGATGATGGGGTTGAGATG	126	168	Yes
STARD3NL	CTTCTGGCAGTTTTTCGATTT	CAGGAACCACGTCTCAATCC	156	210	Yes
c16orf35	CAACAATTTGGCAACCAAG	ACGGGACAGGTTATGCAGAC	158	233	Yes
FIP1L1	AGCTCCTCCCACTCACCTTC	CCTGGTGGAGGAGGAAAAC	105	132	Yes
PCBP2	GTTGGCAATGCAACAGTCTC	CGTTTGGAAATGGTGAGTTCAT	110	149	Yes
CALD1	GGAAGAGGAGAAGCCAAAGC	TGTGGGTCATGAATTCTCCA	161	239	Yes
APP	CCGCTGGTACTTTGATGTGA	TTCTCATCCCCAGGTGTCTC	186	243	Yes
SGCE	GGAAGTGATTCTGGAGAGG	GCACTGTGATGGACCAGTTG	204	231	Yes
ZNF207	CCTCCAATGACTCAAGCACA	ACCAACAGGTCCTTGGACAG	156	249	Yes
OS9	TGGGCTGAAGAAGGAGTCAG	TCGTGTCTCATCAGTCAGC	248	413	Yes

PICAML	TGATGTAAATTGGAGTCAACC AG	TCCTATCATGCCTGTTGGTG	151	175	No
FIP1L1	GAGGATACGAATGGGACTTG A	TGGAAGCCCAGTCTTGAACA	160	229	Yes
ECHDC1	AGAGGGGAAAGGCCTCATT	ATTCTGCTCCTCCACCCAAT	150	203	Yes
PSMG1	GTCTGGGAGGAAGTTGGTTG	TGCATGTTCTTCCTTGGACA	152	215	Yes
GOLGA2	CTGCATCGTCTGCTAACCTG	CGAGGATCCCTATGGTCTGA	150	231	Yes
GANAB	CCATTCCGCCTTGACCTACT	ATCCCTGGGTGTTTCTCAG	159	225	Yes
RFWD2	CACATGCAGCCCAACTACAG	TGTGTGATGGAGAAGGAGCT T	141	201	Yes
MYO18A	GCAGGCTGACCTAAAGTTGG	ATCATCAGAAGCTGCCTTGG	202	247	Yes
ADA	GCTACCACACCCTGGAAGAC	CCCGTTTGGTCATCTGGTAA	160	225	Yes
AKR1A1	ATGCTACGACTCCACCCACT	TTGGGGATGCAGATCACTTT	190	390	Yes
CEP78	CCACAATGGCTGGGATAGAT	GGACAGGAAAGGAGTCGAGA	216	264	Yes
TPD52L2	GGGAGCTGAAACAGAACCTG	TCTCCAAGCTTCTGCTGAT	151	211	Yes
TAZ-E5	GCAGACATCTGCTTCACCAA	TGGGAAGATATGCACCCAGT	150	240	Yes
CEP164	TCAGCCTCTTGGGTTTAGGA	GAGGGCTTGCAAGGAGTAG	151	229	Yes
MACF1	CCTACTCGTTCCAGTCCAG	GCAAGGGATGTCCGACTAGA	161	272	Yes
DMD	GCACAGGGTTAGAGGAGGTG	TCTGCTCCTTCTTCATCTGTCA	151	183	Yes
RNF10	CCTGCTCCTCTGATTCTGCT	GCTTGGCTGAAGGAATTTTG	257	358	Yes
UBTF	GAAATCGGACATCCAGAGA	GTGAGGGTGGACTTGGTGAT	165	276	Yes
NUMB	CCTTGGCCATGTAGAAGTTGA	CTGAGTCCATCTGCTGAGACC	126	159	Yes
GSK3B	GAACTCCAACAAGGGAGCAA	GTGGTGTAGTCGGGCAGTT	174	213	Yes
STX16	GCCGATTAAGCAGAAGATG	GCTGGGATCTTTCCTCTCG	155	318	Yes
ACAA1	TCACTGTGACCCAGGATGAG	CTTGCAAAGGCCTCATTGAT	151	331	Yes
BIN1	CAAGCTGGTGGACTACGACA	CTCCTCCTGCAGATCCACAT	151	244	Yes
LIMCH1	GAGGAATACCGCAAGAGCTG	CTTGCCATTTGTCGTCTCT	187	223	Yes
RBPJ	ATAAATGATGGCGCTTCTG	CTGGGACGACACAGAGCATA	152	256	Yes
P2RX5	CCCTGCAGAGTGCTGTCAT	TTTCCAGCTGTAACCGCTTC	181	253	Yes
HMGCR	CGCCGACAGTTACTTTCCA	GAGCCAGGCTTCACTTCTG	186	345	Yes
OSBPL9	AAACTGCATTGTGTTGCTG	ACAGTCTGGGAAGGCATTGT	113	152	Yes
ODF2L	CTGAAATAGTAAAAAGCAGA	GCTGAATAAGACAGTTTTCC	147	306	Yes
IVNS1ABP	ATCAACTGGGTGCAGCGTAG	CATCATCACTGCCAAACACC	151	292	Yes
PCSK6	GGAGCTCTCAGCCCCAGA	TGAAGTGGACGCAGTTCAAG	167	206	Yes
PPP2R5D	CGTGTCTACTTCCCCTTCA	GGAAGAGGGGTTCCATCACT	187	241	Yes
TNFSF13	CTGGGAGAATGGGGAGAGAT	TCCTGGATTCCGACACCATA	150	198	No
RIPK2	CATTCCCTACCACAACTCG	AGGATGCGAAATCTCAATGG	185	339	Yes
FXP1	ACACTGATGCCAGCGAATCT	AGCACTAGTTGGGCGCTTTA	198	290	Yes
SETX	TCACACGAGCCAAGTACAGC	GCACAGGCTTGAGTTTCAGA	171	258	Yes
ASUN	TTGCACGTCCTTAGCAGTTC	TTAGGGCCCTTTCCTCTTGT	245	416	Yes