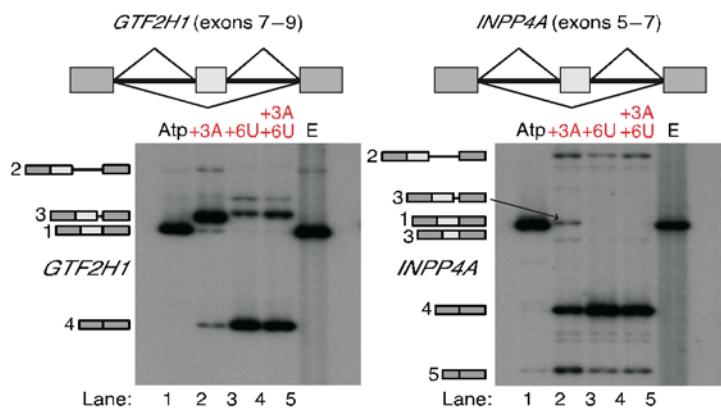


## SUPPLEMENTARY MATERIAL

TITLE: Recognition of atypical 5' splice sites by shifted base-pairing to U1 snRNA

AUTHORS: Xavier Roca and Adrian R. Krainer

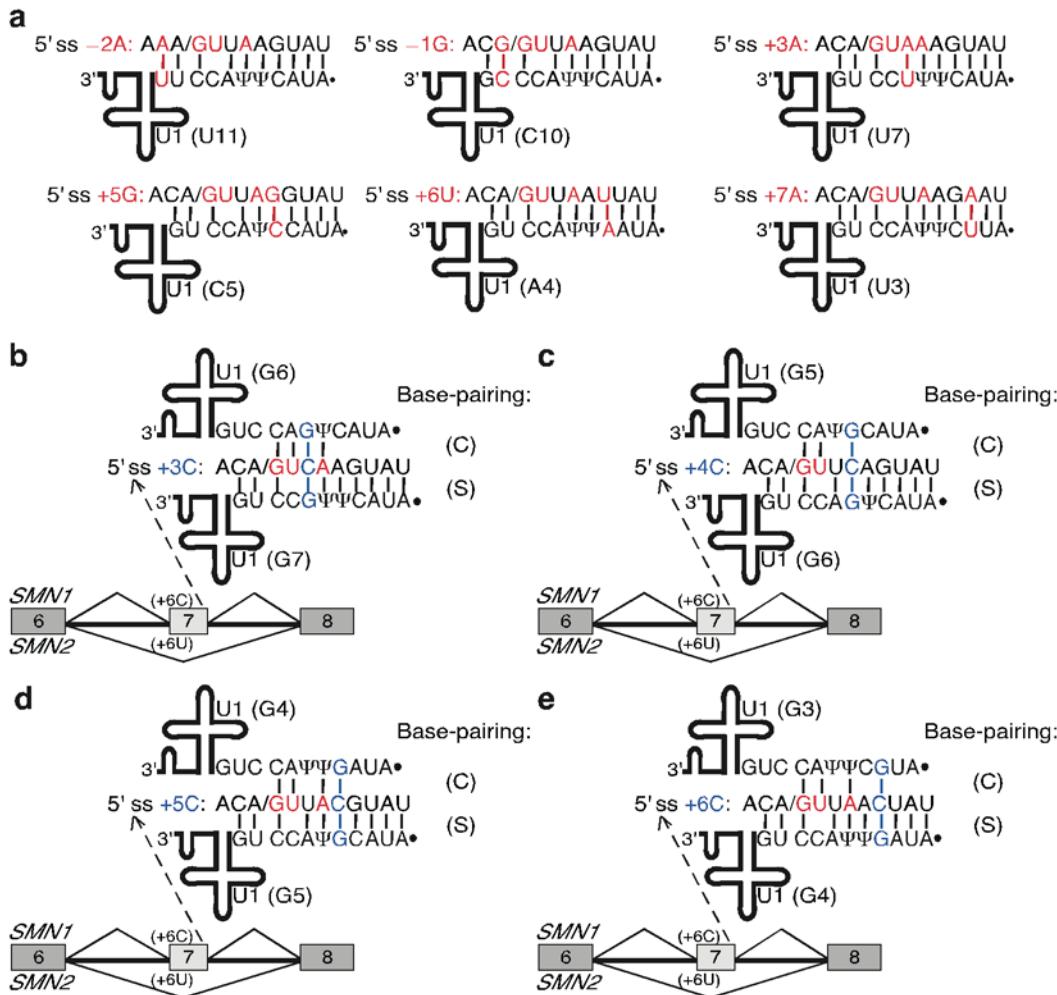
## SUPPLEMENTARY FIGURES



**Supplementary Figure 1 Detection of endogenous and transfected *GTF2H1* and *INPP4A* mRNAs.**

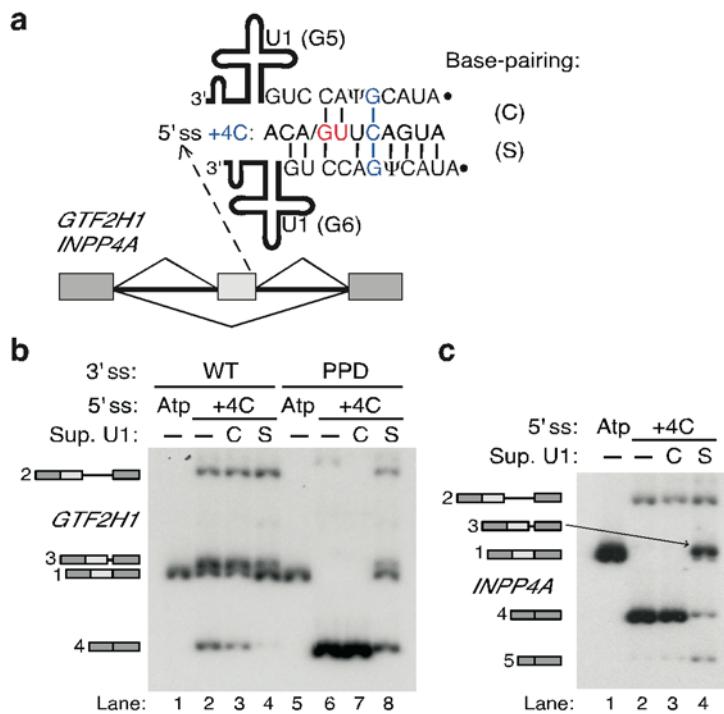
cDNAs from HeLa cells transfected with the various minigenes (lanes 1-4) or mock-transfected (lane 5) were amplified using primers located in the exons flanking the exon containing the atypical 5' ss. The RT-PCR products from the mock-transfected samples correspond to the endogenous *GTF2H1* and *INPP4A* transcripts (E, lane 5), confirming the efficient use of the atypical 5' ss. A small amount of intron 8 retention was seen for endogenous *GTF2H1* (left panel, lane 5). Although the primers can amplify the transcripts from both transfected and endogenous genes, in transiently transfected cells the RT-PCR products are derived mostly from the minigenes, which are expressed at higher levels. In the mock-transfected samples (E, lane 5), ~ 50-fold more total cDNA template was added to the reaction. The identity of the RT-PCR products, schematically depicted on

the left of each panel, is described in the **Fig. 1** legend. For the *GTF2H1* minigene, the mutations at the exon 8 5' ss activated cryptic 5' ss at positions +20 (GAG/GUGCAG) and +25 (GCA/GUAACU) in intron 8 (band #3). For the *INPP4A* minigene, the analogous mutations activated a cryptic 5' ss 31 nucleotides upstream of the 5' ss in exon 6 (CGU/GUAUGA), and to a small extent another cryptic 5' ss at position +176 (AGG/GUAAGA) in intron 6. The arrow indicates a cryptic 5' ss at position +5 (AAA/GUAAUG) of *INPP4A* intron 6 that was activated only in the context of the +3A mutation. In addition, band #5 in *INPP4A* corresponds to an mRNA species generated by skipping of exon 6 and use of a cryptic 5' ss (GCG/GUGAGU) 63 nucleotides upstream of the 5' ss of exon 5.

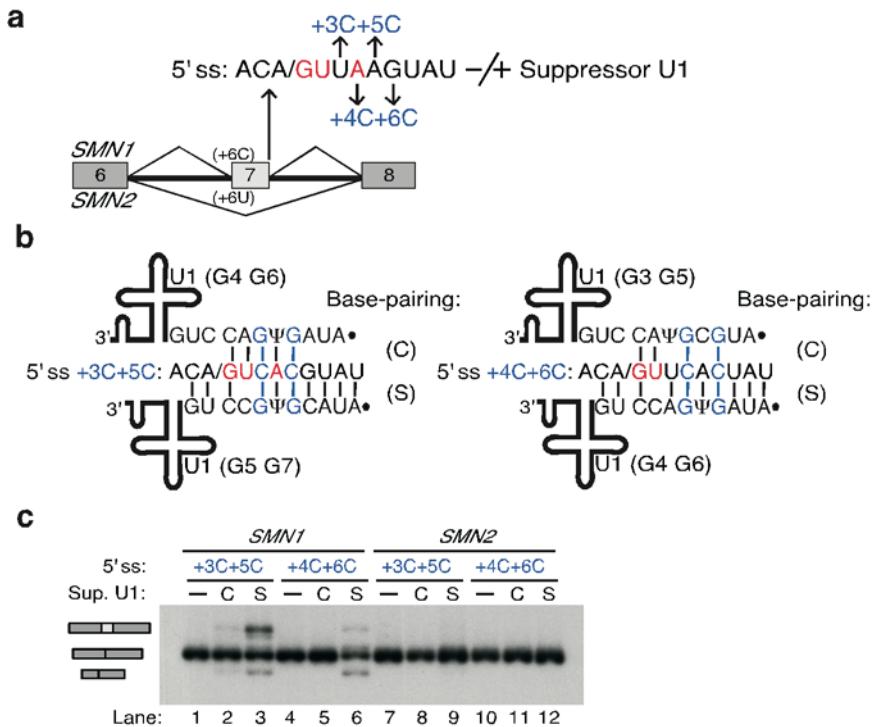


**Supplementary Figure 2 Schematic of the base-pairing profiles for the mutant 5' ss used in Figs. 2 and 3.** **a**, Base-pairing profiles of mutants -2A, -1G, +3A, +5G, +6U and +7A with their corresponding suppressor U1 snRNAs restoring base-pairing in the shifted register (**Fig. 2**). Mutations at the 5' ss or U1 are highlighted in red. For these mutant 5' ss, there is no suppressor U1 in the canonical register, because the mutant nucleotide already base-pairs to endogenous U1 in the canonical register. **b-e**, Diagrams show the atypical 5' ss carrying the +3C (**b**), +4C (**c**), +5C (**d**) and +6C (**e**) mutations, along with the corresponding suppressor U1 snRNAs in the canonical (C) or shifted (S) base-pairing registers (**Fig. 3**). The 5' ss mutations and the compensatory U1 mutations are highlighted in blue. Note that some of the suppressor U1 snRNAs were used for more than one 5' ss

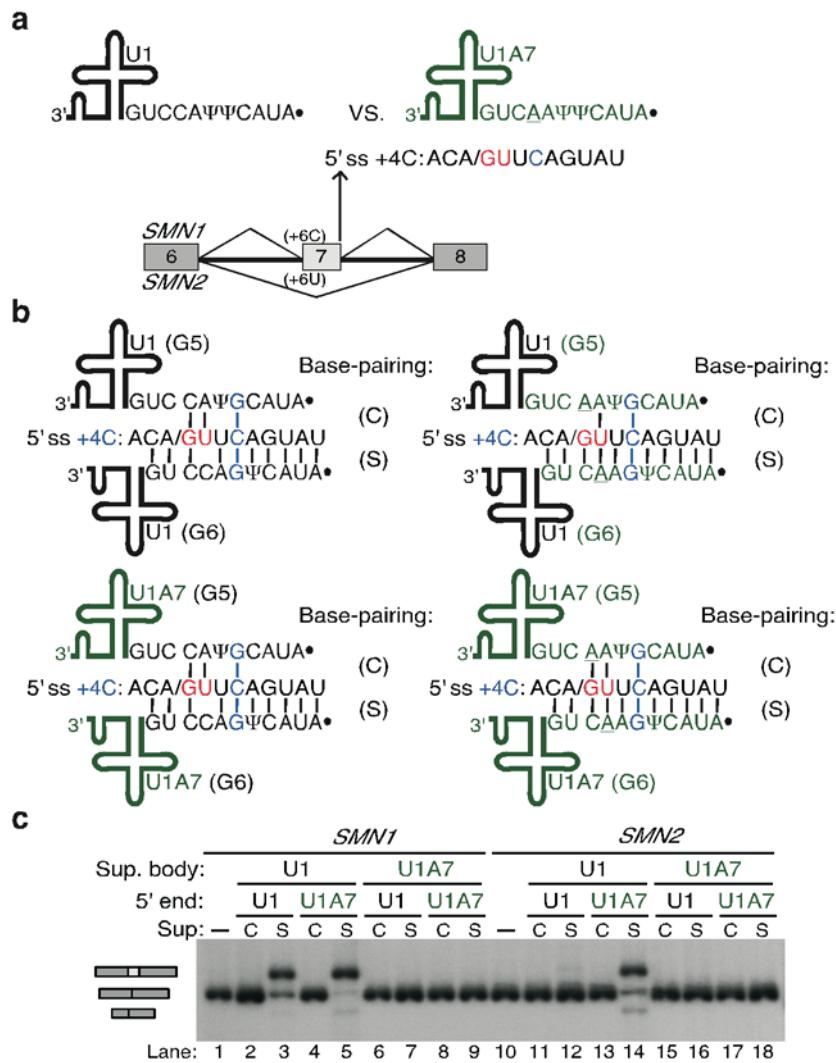
mutation: U1 (G5) compensates for 5' ss mutation +4C in the canonical register, and for mutation +5C in the shifted register. **Fig. 3** shows that U1 (G5) rescued exon 7 inclusion for mutant +5C but not for +4C, and that U1 (G6) rescued mutation +4C (shifted register) but not +3C (canonical register). These results indicate that the distinct effects of the suppressor U1s are not due to their relative expression levels, and further strengthen the demonstration of shifted base-pairing between the atypical 5' ss and U1.



**Supplementary Figure 3 Suppressor U1 snRNA analysis for atypical 5' ss in their natural context.** **a**, Base-pairing profiles of the +4C mutant 5' ss in the *INPP4A* and *GTF2H1* minigenes with the corresponding suppressor U1 snRNAs carrying compensatory mutations in the canonical (C) or shifted (S) registers. Mutant nucleotides and compensatory U1 mutations are highlighted in blue. **b**, Suppressor U1 analysis of the +4C mutant 5' ss in the *GTF2H1* minigene. The top labels indicate the version of the 3' ss upstream exon 8, the version of the atypical 5' ss, and the mock or suppressor U1 snRNA used. In lanes 5-8, the 3' ss upstream of exon 8 was weakened by a point mutation at the polypyrimidine tract ('polypyrimidine tract down mutation' or PPD), so as to compromise exon 8 inclusion. Suppressor U1 in the shifted but not in the canonical register rescued exon 8 inclusion in both the wild-type and the PPD minigenes (lanes 2-4, 6-8). **c**, Suppressor U1 analysis in the *INPP4A* minigene. Whereas the suppressor U1 in the canonical register did not rescue exon 6 inclusion (lane 3), the suppressor in the shifted register did, albeit weakly (lane 4).

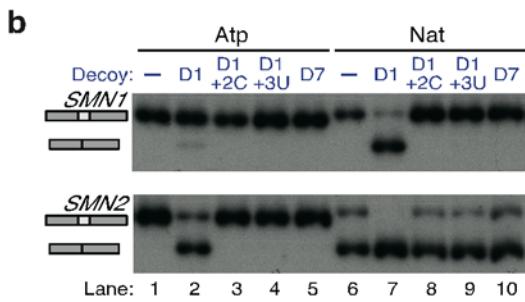
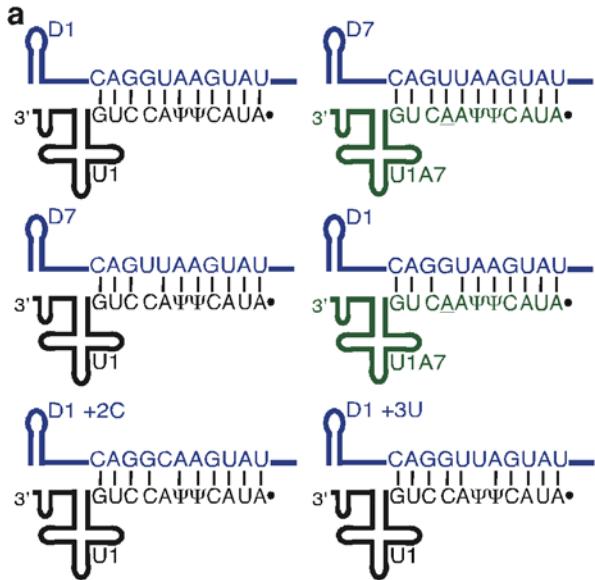


**Supplementary Figure 4 Analysis of double mutations at atypical 5' ss.** **a**, Schematic of the +3C+5C and +4C+6C 5' ss mutations in the *SMN1/2* context. Mutant nucleotides are shown in blue. **b**, Base-pairing profiles of the two double-mutant 5' ss with the corresponding suppressor U1 snRNAs carrying compensatory mutations in the canonical (C) or shifted (S) registers. Compensatory U1 mutations are indicated and highlighted in blue. **c**, Analysis of the mutant 5' ss. The top labels indicate the minigene (*SMN1* or 2), the mutant 5' ss in exon 7, and the mock or suppressor U1 snRNA used. In this case, the suppressor U1 snRNAs rescued exon 7 inclusion only in the *SMN1* context (lanes 1-6). For +3C+5C (lanes 1-3), both suppressors rescued splicing, but the suppressor U1 (G5 G7) in the shifted register was more effective. For +4C+6C (lanes 4-6), only U1 (G4 G6) in the shifted register had an effect. In this experiment, aberrant splicing products were also seen upon use of suppressor U1s, for unknown reasons.



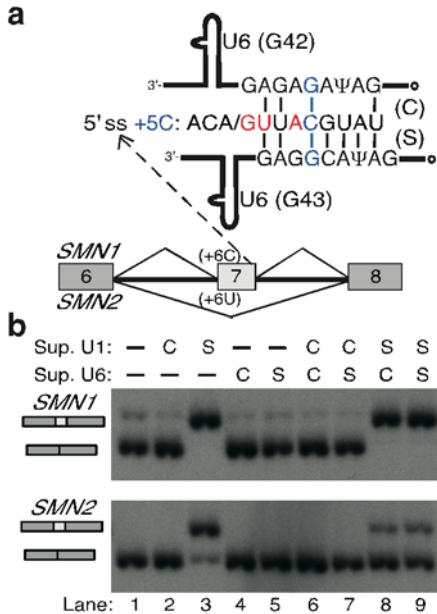
**Supplementary Figure 5 Use of U1 and U1A7 snRNA suppressors to rescue splicing via mutant atypical 5' ss.** **a**, Schematic of the U1 (black) and U1A7 (green) snRNAs and the mutant atypical (+4C) 5' ss in the *SMN1/2* context. U1 and U1A7 differ by one nucleotide at their 11-nucleotide 5' ends (underlined in U1A7), and have several additional nucleotide differences in the snRNA body. **b**, Base-pairing of the four different combinations of suppressor U1/U1A7: the U1 snRNA body with the U1 snRNA 5' end; the U1 body with the U1A7 5' end; the U1A7 body with the U1 5' end; and the U1A7 body with its 5' end. For each combination, the compensatory mutation was introduced in the canonical (G5, (C)) or shifted (G6, (S)) register, indicated in blue. **c**, Analysis of the

+4C mutant 5' ss with U1/U1A7 suppressors. The top label indicates the minigene (*SMN1* or 2), the suppressor body (U1 or U1A7), the 5' end (U1 or U1A7), and the compensatory mutation (control (-), C or S). None of the suppressors with the U1A7 body rescued exon 7 inclusion, but both 5' ends of U1 and U1A7 in the U1 body did.



**Supplementary Figure 6 Specificity of the U1/U1A7 snRNA decoys.** **a**, Base-pairing profiles of the different decoys used with the 5' end of U1/U1A7. The D1 and D7 decoys have perfect (11 bp) complementarity to their cognate snRNAs (top diagrams). In addition, the D1 decoy has one mismatch (10 bp) to the U1A7 snRNA in the canonical register, and the D7 decoy has one less potential base pair (10 bp) to U1 in the shifted register (middle diagrams). To assess the specificity of the D1 and D7 decoys, two additional decoys were constructed, D1+2C and D1+3U, which can have the same number of base pairs (10 bp) with U1 as the D7 decoy (bottom diagrams). **b**, Decoys need perfect complementarity (11 bp) to their cognate snRNA to have a strong effect. The top label indicates the 5' ss in *SMN1/2* exon 7 and the decoy used. As in b, expression of the D1 decoy resulted in exon 7 skipping. In contrast, the D1+2C, D1+3U and D7 decoys, which have one nucleotide

mismatch (10 bp) when base-pairing with U1, did not affect recognition of the natural or the atypical 5' ss. These results indicate that the snRNA decoys need perfect complementarity to their cognate snRNA, and explain why the D7 decoy does not significantly affect exon 7 inclusion.



**Supplementary Figure 7 U6 snRNA does not base-pair to atypical 5' ss in a shifted register.** **a**, Schematic of the suppressor U6 experiment. In this case, the suppressor U6 snRNAs carry only a point mutation to compensate for the +5C mutation in the atypical 5' ss. The U6 G42 and G43 mutations rescue base-pairing to the +5C 5' ss in the canonical (C) or shifted (S) register, respectively. Note that the G43 suppressor U6 has much stronger predicted base-pairing to the mutant 5' ss than the G42 suppressor. **b**, RT-PCR analysis of co-transfections of the *SMN1/2* constructs carrying +5C 5' ss and the various suppressor U1 and U6 snRNAs. The suppressor U1s (C or S) used are shown in **Supplementary Fig. 2d** online. As in **Fig. 3**, the suppressor U6s alone did not show any effect on exon 7 inclusion. Combined with suppressor U1, none of the suppressor U6 enhanced the levels of exon 7 inclusion.

## SUPPLEMENTARY TABLES

	<i>H. sapiens</i>	<i>M. musculus</i>	<i>D. melanogaster</i>	<i>C. elegans</i>	<i>A. thaliana</i>
Search					
NNHGTYRAGT <sup>i</sup>	40	35	10	11	55
NYGGTYRAGT	7	5	3	4	17
NYAGTRRAGT	4	5	0	3	5
NYAGTYYAGT	1	2	1	1	7
NYAGTYRBGT	1	3	2	14	4
NYAGTYRAHT	1	1	2	23	9
NYAGTYRAGV	4	5	2	7	18
Homology <sup>ii</sup>	1	3			
Total	59	59	20	63	115
Human-mouse orthologous 5' ss					
Conserved	27	27			
Not conserved	13	8			
Intron not in ortholog <sup>iii</sup>	1	7			
No ortholog <sup>iv</sup>	10	0			
Not in databases <sup>v</sup>	8	17			
Total	59	59			

**Supplementary Table 1 Summary of the in silico searches for atypical 5' ss.** <sup>i</sup>See Methods for details about the SpliceRack<sup>1</sup> searches. <sup>ii</sup>Number of 5' ss that were added based on homology to the gene from the other mammalian species. <sup>iii</sup>The other mammalian counterpart does not have an intron mapped at the same location. <sup>iv</sup>Genes for which we were not able to find an ortholog in the other mammalian species. <sup>v</sup>Genes that we were unable to find in the ENSEMBL or UCSC genome resources, and that correspond to putative genes awaiting confirmation. See Supplementary Table 3 for a complete list of atypical 5' ss in all five species.



	Search	NYAGTYYAGT	1				
H52	NM_006256	PKN2	7	1280	CTTGTCCAgttcagtaaccagattttaaaatcatgt	M46	
	Search	NYAGTYRBGT	1				
H53	NM_139025	ADAMTS13	25	466	GCAGTCCAgttatgtctgtcccttcgtcaggca	NO, NM_001001322	AGAGCATGgttagtttccctgtttccgtggggaa
	Search	NYAGTYRAHT	1				
H54	XM_370939	LOC388221	5	3534	AATTGTAGttaatttgcctcgtgactaca	NOT FOUND IN ENSEMBL, UCSC	
	Search	NYAGTYRAGV	4				
H55	NM_004476	FOLH1	8	882	TCTACACAgtaagagacttttaattttactttt	M52	
H56	NM_153696	PSMAL	3	887	TCTACACAgtaagagacttttaattttactttt	NO ORTHOLOG	
H57	NM_024721	ZFHX4	4	9276	GAGCAGCAggttagatcgaccctcaggtaatggctta	NO, NM_030708	GGCCTCAGgttaatgttcccttcagaagctggcc
H58	XM_375358	Klkb14	5	1216	CATCTGCAgtaaggatcccttcggcagaaggct	YES, XM_134585	CGTCTGCAgtaatgcattcccttcgtggaggct

## Manually added human/mouse orthologs

H59	NM_015199	ANKRD28 (KIAA0379)	21	5662	CTACAATGgttaagtatacaaacacaaatgcataatcat	M39	
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MOUSE	<i>Mus musculus</i>						
Search	NNHGTYRAGT	35					
Accession	Gene Name	Int. #	Int. Length	Sequence	Orthologs		
M1	NM_030266	Inpp4a	4	260	AGGGAACAgtaagtatcgctgcgtggccaaacagtct	H21	
M2	NM_007495	Astn1	17	8557	CATCATTAGtcaagtcagaatgtctccctctcttt	Intron not in human ortholog, NM_004319	
M3	NM_007622	Cbx1	5	1420	TTAGCCCTgttgatcaccggccctgcacctctgact	Intron not in human ortholog, NM_006807	
M4	NM_008379	Kpnbl	19	697	CGTACACCgttgatatacgccaggccatccaaatcc	H14	
M5	NM_134037	Acly	5	447	AAGAAAGAggttagttgttagggaaacggcggtggca	H13	
M6	NM_008747	Ntsr2	2	1495	GGTTCTCAggttaagtctaccacaaataccctgggg	H20	
M7	NM_007960	Etv1	9	1873	GAACAGAAgtaagtctttataagattgtttataa	H29	
M8	NM_030225	Dlst	4	113	TGTGTGCAgtaagtaccctgttttgtaatggact	NO, NM_001933	TGTATGCAgtaagtaccctgttttgtaatggaaatggatt
M9	NM_032465	Cd96	10	1677	CAAGAACTgttgatattgtcatggctccattcat	H25	
M10	NM_009985	Ctsw	6	86	CAACAAACgttgatccaggccctgtggggatcgat	NO, NM_001335	CAACAAACgttgatgcactgccccccacttggacat
M11	NM_028412	Ciz1	1	507	CAGGATTAGttaatggcggttgcattatcgaggg	NO, NM_012127	CCGCAGAGgttgatgtgtgtgtggggggggccgtggcc
M12	NM_175184	2610528K11Rik	1	12860	GGGGAACAgtaagtggggccgggggggggggggg	H40	
M13	NM_010406	Hc	14	1361	TGCAAACAGgttaagtgtatccgtgtggatgggg	H30	
M14	NM_011978	Slc27a2	5	2964	TACAAAAGgttaagtccggaaatgtggatggatgt	NO, NM_003645	TACAGAAAgttaagtatggaaaaatggagacatacg
M15	NM_009797	Capzal	5	4924	TCTGTACTgttaagtatccgtgtccctcgggagaaca	H5	
M16	NM_181406	Rarsl	2	4784	AAAAAAAGAgtaagtatgttttttttttttttttttt	H28	
M17	NM_019481	Slc13a1	11	2557	AGAAATTAGtttgatatttttttttttttttttttttt	H45	
M18	NM_019715	Kcmf1	3	3286	CCTAGAAAAGtttgatgttttttttttttttttttttt	Intron not in human ortholog, NM_020122	
M19	NM_008186	Gtfzh1	8	103	AGGAAAACgttaagttagatgttttttttttttttttt	H9	
M20	NM_133214	BC017612	2	20057	TGCATACAggttgatggccattaatgttttttttttt	NO, C11orf75	GCGGGCAGgtggctgggggggggggggggggggggg
M21	NM_144935	BC018242	7	329	CTCGAAAAGgttaagtgtggctcggatgggggggg	H17	
M22	NM_025417	Commd4	1	227	CCAAAATTgttaagtgttttttttttttttttttttt	H43	
M23	NM_023605	Fbxo9	3	1684	GAAGAAAAAGtttgatgttttttttttttttttttt	H27	
M24	NM_133984	Hemk1	2	338	CCAAAACAgtaatgttttttttttttttttttttttt	H24	
M25	NM_029413	Morc4	12	498	AAGCAACAggttgatataccgggggggttttttttt	H31	
M26	XM_484060	Ppm1e	1	109399	TACAAATAgtaatgttttttttttttttttttttttt	H15	
M27	XM_484173	9030205A07Rik	21	9472	CAAAATCCgttgatcttttttttttttttttttttt	H11	
M28	XM_488661	NO NAME	5	3011	CCAGCACTgttgatgttttttttttttttttttttt	NOT FOUND IN ENSEMBL, UCSC	
M29	XM_358492	NO NAME	1	15613	GGCATTCAggttaagttaatttttttttttttttttt	NOT FOUND IN ENSEMBL, UCSC	
M30	XM_485305	NO NAME	2	205	CCAAAACAgtaatgttttttttttttttttttttttt	NOT FOUND IN ENSEMBL, UCSC	
M31	XM_485339	1700008P02Rik	4	1296	AAATCAATgttgatttataatataatataatataat	Intron not in human ortholog	

FRUITFLY		<i>Drosophila melanogaster</i>			
Search	NNHGTYRAGT	10			
Accession	Gene Name	#	Int.	Int. Length	Sequence
NM_057884	<i>ppk</i>	2	185	GATGAGGA	Gtgtagttttcagcatatactttatag
NM_137551	<i>CG15117</i>	4	61	GATA	CAGAGtgtggattttccgacacctcgaaaggatt
NM_139436	<i>CG15822</i>	1	1762	AAAACCA	AAGtcgaggtaactccatggaaacaacgcga
NM_168190	<i>NO NAME</i>	1	283	GCCAAAGC	gtcaagtgcgtggccgtacgtcaaccatgt

D5	NM_140439	<i>CG5842-RA (nan)</i>	4	60	CGAATACAgtaagtattgtccttatattaatataca
D6	NM_169384	<i>CG31386</i>	3	6050	AAAAACCAAGtgtgagttataatgtactgcacaacaaa
D7	NM_142173	<i>CG4210-RA CG7985-RA</i>	1	63	TGATTCAAAGtgtgagttataatgtactcattttaata
D8	NM_142432	<i>(CG7985) CG1786-RA</i>	1	11970	CTTTAACAGttaagtgcggaaatgttgaaaaaaccaat
D9	NM_167318	<i>(Cyp318a1) CG3291-RA</i>	1	63	TCCGAACAGtgtgagttgttagactgcaggccattagg
D10	NM_078684	<i>(pcm)</i>	5	84	CAGGAACAGtgtgagttgtggccgcgtcgccctgc
	Search	NYGGTYRAGT	3		
D11	NM_166710	<i>CG32013 CG4795-RA, (Cpn)</i>	1	89	CGGCGGTGgtcgagtcggcacccgacgtcaacggggct
D12	NM_169454		2	67	AAACTACGtgtgagttctctgttagccggaaaaatctctg
D13	NM_169605	<i>CG3631-RB</i>	1	72	AACAAACGtgttaagtcatggaaacttttgtgccta
	Search	NYAGTRRAGT	0		
	Search	NYAGTYYAGT	1		
D14	NM_167187	<i>CG17754-RC</i>	6	1279	GTGCTACAGtttagccaaacccaatccactttcggtt
	Search	NYAGTYRBGT	2		
D15	NM_166504	<i>CG11474-RB</i>	1	71	GGTGGGTAgttgttgtgcactttaccttgacccttgage
D16	NM_130679	<i>CG3526-RB</i>	1	1038	TGCGTTTAgtgttgttgtatgtctaatatgcgtcatt
	Search	NYAGTYRAHT	2		
D17	NM_168054	<i>CG32259-RB</i>	4	308	CGGCGCCAAGttaattgcggaaactgtctgatccccat
D18	NM_170040	<i>CG31139-RA</i>	1	56	CTCAAATAgtaactatataatataatataatataaa
	Search	NYAGTYRAGV	2		
D19	NM_141447	<i>CG14609-RA</i>	6	58	GGAGAAATAgttgaggcgacgtctacaattaaaaagttagc
D20	NM_133036	<i>CG15373-RA</i>	5	109	TCGCACCAAGtgtgaggatgtttatataagatagaaga

**WORM** *Caenorhabditis elegans*

*menorhabditis elegans*







A103	NM_129500	AT2G39420	3	97	CATGAACAggttagatacttcggccaaacttcatgttt
A104	NM_113609	AT3G26950	6	92	CAGTCTCAgtaagatcgtttttagtttcctcaacac
A105	NM_117800	AT4G16970	11	89	CCTGAACAgtaagatctgacttgaatgagtacacat
A106	NM_119057	ATHXK1	3	86	AAGAAGCAGttaagctcgatttcgttcaactattca
A107	NM_119554	AT4G33940	3	210	TCTTGCAgtaagaacatcttaccctgaaatttacat
A108	NM_120026	AT4G38650	3	87	CTTCTCCAgtaagcatttcatgccttcatctctaga
A109	NM_120494	AT5G04120	3	108	CTGTTGCAgtaagatccaaecctttaatccctgt
A110	NM_120572	AT5G04900	12	114	TATTCTCAgtaagacgatataatgcctcgtagaaag
A111	NM_122051	AT5G20440	3	93	CATGAACAgtaagacacaatcattatcatccatagtt
A112	NM_122301	AT5G23960	5	78	AGAAATCAgtaagacaaaaggaaactttaaagcat
A113	NM_148058	AT5G38386	1	105	AGAAACTAgttaagtcattatctggatttgatattc
A114	NM_125499	AT5G61060	8	217	CTACTCCAggttggacccctttttgttaccattt
A115	NM_125736	AT5G63410	5	192	ATAACACAgtaagtcgtttatgttgttttc

**Supplementary Table 2 Complete list of predicted atypical 5' ss in five species.** Each atypical 5' ss is labeled with the initial of the species where it was found, and a number. For each 5' ss, we provide the accession number, the gene name, the intron number and length, and a sequence that includes the atypical 5' ss (8 nucleotides in the exon, 30 in the intron). The query sequence used to obtain each hit is also shown. For the human and mouse collections, the orthologous 5' ss in the other species is indicated, whether it is conserved or not. Cases for which the orthologous intron or gene could not be found are also indicated.