Figure S1

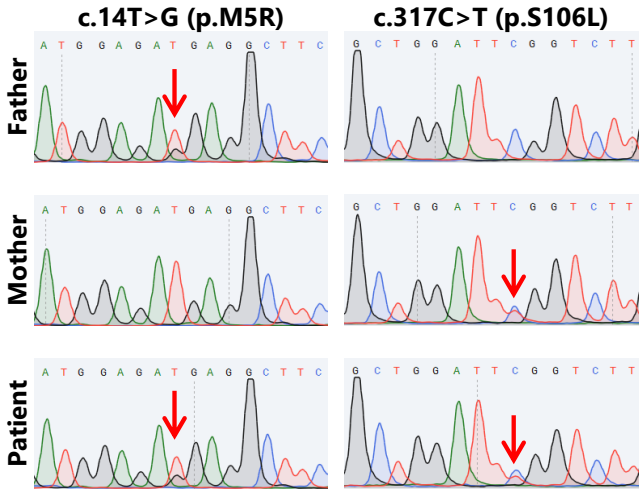

Figure S1. Chromatograms of the direct nucleotide sequencing of EXOSC2. The three left panels exhibit a sequencing for c.14T>G, and the three right panels exhibit a sequencing for c.317C>T. Red arrows indicate the target nucleotide changes.

Figure S2

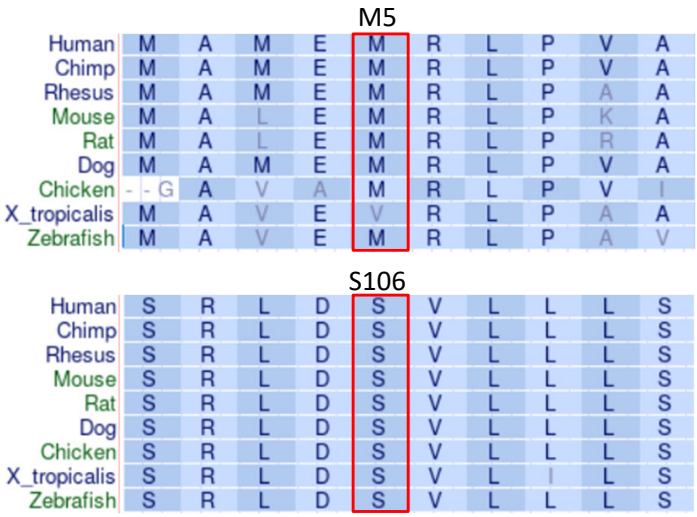

Figure S2. Amino acid sequences around the variants among species from UCSC 100 Vertebrates in the UCSC Genome Browser on Human (GRCh38/hg38). Both M5 and S106 are well-conserved.

Figure S3

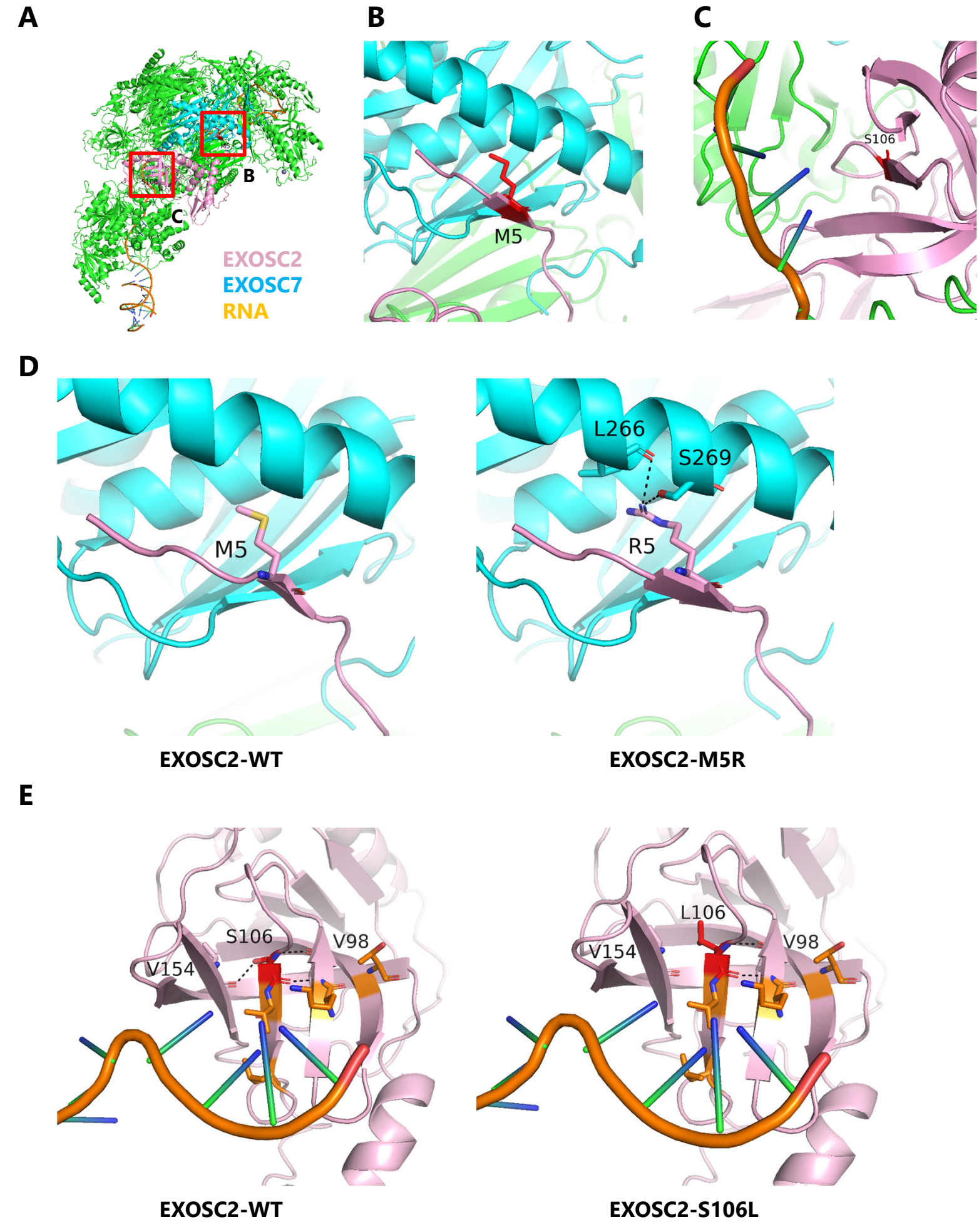
A
B
C
M5
S106
B
C
EXOSC2
EXOSC7
RNA
M5
S106
D
M5
L266
S269
R5
EXOSC2-WT
EXOSC2-M5R
E
S106
V98
V154
L106
V154
V98
EXOSC2-WT
EXOSC2-S106L

Figure S3 (cont.)

Figure S3. Structure of the human nuclear exosome-MTR4 RNA complex (PDB: 6D6Q). A. The whole structure of the exosome complex is shown. EXOSC2, EXOSC7, and RNA are in pink, cyan, and orange, respectively. Red insets indicate the location of zoom-in views shown in B and C. B. p.M5 (red) is located next to an alpha helix of EXOSC7 (cyan). C. p.S106 is located on a beta sheet in a substructure facing RNA. D. The substitution of methionine with arginine added polar bonds between EXOSC2 (pink) and EXOSC7 (cyan). Wild-type (WT) (right) and p.M5R (left) variants are shown. Oxygen, nitrogen, and sulfate were shown in red, blue, and olive, respectively. Polar connections are indicated by black dotted lines. E. S106L mutation is predicted to have lost a polar bond to an adjunct beta sheet, consisting of an RNA-contact structure. Known RNA-interacting amino acids are shown in orange, and WT (left) and p.S106L (right) are shown in red. Polar connections are indicated by black dotted lines.

Figure S4

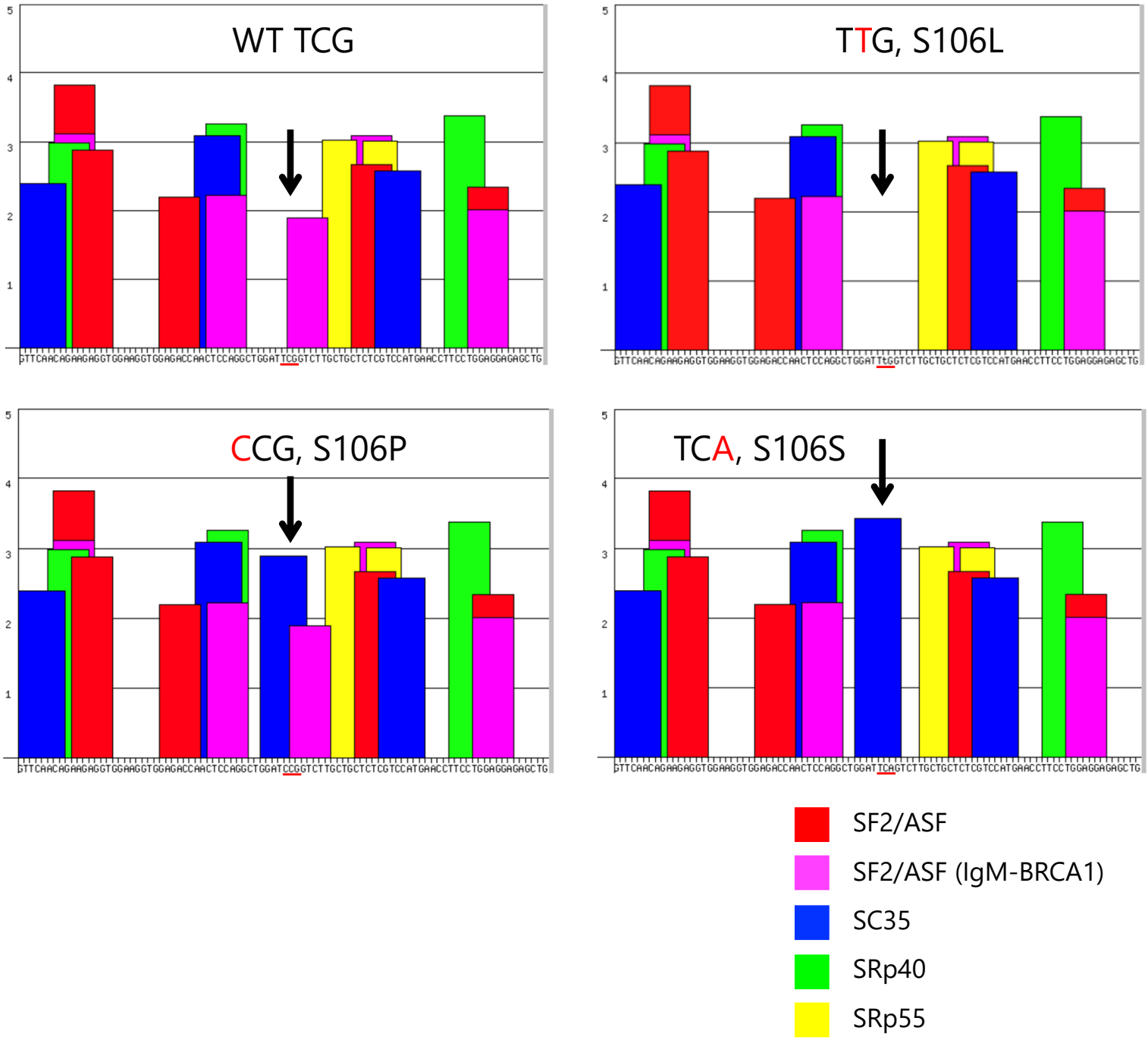

Figure S4. Effect of single nucleotide substitution in exon 4 of EXOSC2 on the Exonic Splicing Enhancers (ESEs) according to the ESE prediction tool. ESEfinder enables the recognition of potential ESE sites. The elevation of the colored bars represents the motif scores, and the girth of the bars indicates the length of the motif. Bars in red, purple, blue, green, and yellow, respectively, indicate potential binding sites for the serine-arginine (SR) proteins SF2/ASF, SF2/ASF (IgM-BRCA1), SC35, SRp40, and SRp55. The black arrow and red underline indicate a position of S106 and the corresponding codon. ESE, Exonic Splicing Enhancers

Figure S5

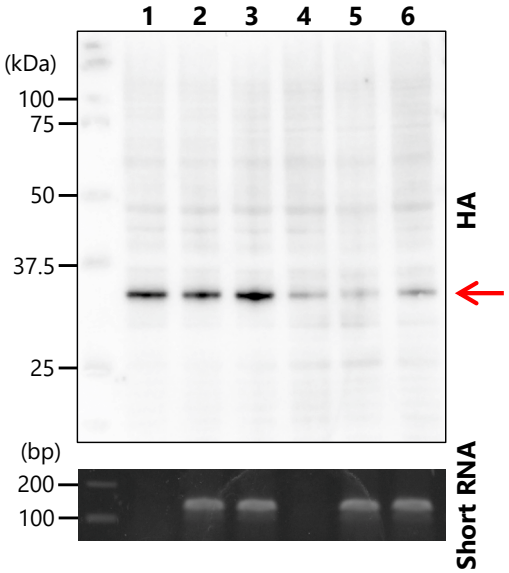

Figure S5. Western blotting for the p.M5R EXOSC2 protein expressed by the EXOSC2-pM5R-HA expression vectors with/without expression of the 78-bp short RNA fragment containing the c.14T>G variant. Result of western blotting (upper panel) and expression of short RNA confirmed by RT-PCR (lower panel). Lane 1: EXOSC2-HA WT and control vector, lane 2: EXOSC2-HA WT and short RNA WT, lane3: EXOSC2-HA WT and short RNA c.14T>G, lane 4: EXOSC2-HA M5R and control vector, lane 5: EXOSC2-HA M5R and short RNA WT, lane6: EXOSC2-HA M5R and short RNA c.14T>G.

Table S1. Primer sets for real-time PCR

Object	Target of amplicication	Forward	Reverse
RT-PCR for EXOSC2	EXOSC2 between exons 1 and 5	CCTGGAGTTGTGGAGTCGGATATCG	AAACCTCTCATTGCAAGCTCATCT
Real-time PCR	EXOSC2 between exons 2 and 4	TTGCATCTGTTGCTGGCTCT	CTCCACCTTCCACCTCTTCTGT
Real-time PCR	TBP between exons 2 and 3	TGGTTTGCCAAGAAGAAAGTG	GGGTCAGTCCAGTGCCATAA
Real-time PCR	HPRT1 between exons 6 and 7	TGACACTGGCAAAACAATGCA	GGTCCTTTTCACCAGCAAGCT
Minigene assay	Exons 3-5 of EXOSC2	AACCGACATCTCAAATGGTGTGTG	ATTTCCCAGTCCCACAGAAAAGCA
Minigene assay	Generating p.S106P variant	ATGAACCTTCCTGGAGGAGAGCTGG	GGACGAGAGCAGCAAGACCG**G**ATCC
Minigene assay	Generating p.S106S variant	ATGAACCTTCCTGGAGGAGAGCTGG	GGACGAGAGCAGCAAGAC**T**GAATCC
Minigene assay	RT-PCR between exons 3 and 5	CATTGGTGAAGTAGGAGACATCGT	AAACCTCTCATTGCAAGCTCATCT
EXOSC2-HA expression vectors	Whole cDNA of EXOSC2	CCTGGAGTTGTGGAGTCGGATATCG	TCTAGATCCCTCCTGTTCCAAAAGCCTC
EXOSC2-HA expression vectors	Generating M5R (ATG>CCG) variant	GAAGCCT**GCG**CTCCATCGGCCATCTT	CAGTGGCTCGCAAGCCTCTTAGCGA
EXOSC2-HA expression vectors	Generating M5R (ATG>TCA) variant	GAAGCCT**TC**TCTCCATCGGCCATCTT	CAGTGGCTCGCAAGCCTCTTAGCGA
RT-PCR for EXOSC2-HA and NeoR/KanR expression	EXOSC2-HA	CATTGGTGAAGTAGGAGACATCGT	AAGCGTAATCTGGAACATCG
RT-PCR for EXOSC2-HA and NeoR/KanR expression	NeoR/KanR	GCTATTCGGCTATGACTGGGCACAA	GCTATGTCCTGATAGCGGTCCGCC

Table S2. Splicing prediction by spliceAI

Δ type	Δ score	
	c.14T>G	c.317C>T
Acceptor Loss	0.00	0.08
Donor Loss	0.00	0.04
Acceptor Gain	0.00	0.00
Donor Gain	0.00	0.00

Note: SpliceAI evaluated the Δscore using 500 bp upstream and downstream from the variants. The Δ score range from 0 to 1 and can be interpreted as the probability of the variant being splice-altering.