

SUPPLEMENTARY FILE

- 1. *CDKN1C* Primer Sequences qPCR and sequencing of cDNA**
- 2. RNA-Seq alignment**
- 3. Haplotype analysis**
- 4. RNA-seq, IGV-view**

Supplementary 1: Primers*CDKN1C* Primer sequences used for RNA-sequencing (cDNA):

CDKN1C-F1	CAGGAGCCTCTCGCTGAC
CDKN1C-F2	TGTCCGGGCCTCTGATCTCCGAG
CDKN1C-F3	TGTCCGGGCCTCTGATCTCCGCTG
CDKN1C-R1	TTTGGGCTCTAAATTGGCTCAC
CDKN1C-R2	ACCGAACGCTGCTCTGCGGCA
CDKN1C-R3	AGCCGCCGC

For analysis of *CDKN1C-201* and *CDKN1C-206* (XM_005252732.4, ENS00000647521.1) that encodes a putative 175aa protein product labeled isoform C (UniProtKB A0A2R8YFP9), we also used the following primers:

CDKN1C-201/206-5UTRex1	GGACAGCCAGCACATCCACGATG
CDKN1C-201/206-5UTR	GACGCAGAAGAGTCCACCACC

RealTime-probes for RNA expression (qPCR):

Hs 00175938_m1	CDKN1C(NM_000076.2)
Hs 00908985_m1	CDKN1C-206
APZTEAZ	CDKN1C(NM_000076.2) specific mutation probe)
Hs 00187842_m1	B2M reference probe
20170628_CDKN1C.sds	Probe specific for 206-transcript: XM_005252732.4

Supplementary 2: Alignment used for generating FASTA files used as designated reference files for mapping the complex delins variant.

The two ref seq files were aligned using EMBOSS NEEDLE to illustrate where the delins is in the sequence (see line # 901 in the alignment file), deleted nucleotides are marked in green in RefSeq, and inserted nucleotides in red). Alignment parameters are included at the top of the file.

```
#####
# Program: needle
# Rundate: Wed 22 Jan 2020 06:51:17
# Commandline: needle
#
# -auto
# -stdout
# -asequence emboss_needle-I20200122-065114-0865-20608599-p2m.asequence
# -bsequence emboss_needle-I20200122-065114-0865-20608599-p2m.bsequence
# -datafile EDNAFULL
# -gapopen 10.0
# -gapextend 0.5
# -endopen 10.0
# -endextend 0.5
# -aformat3 pair
# -snucleotide1
# -snucleotide2
# Align_format: pair
# Report_file: stdout
#####

#=====
#
# Aligned_sequences: 2
# 1: CDKN1C_RefSeq_B38
# 2: CDKN1C_DelIns
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 2802
# Identity: 2798/2802 (99.9%)
# Similarity: 2798/2802 (99.9%)
# Gaps: 1/2802 ( 0.0%)
# Score: 13968.0
#
#
#=====

CDKN1C_RefSeq 1 AGCACAAACAGACTGGAGTTGCAGCATTTTTTCGGCCTCTTTATTTAGAAC 50
|||||
CDKN1C_DelIns 1 AGCACAAACAGACTGGAGTTGCAGCATTTTTTCGGCCTCTTTATTTAGAAC 50

CDKN1C_RefSeq 51 CCGGCGGACGAGGGGCCGGGGCAGTGGTACAGACGGCTCAGGAACCATT 100
|||||
CDKN1C_DelIns 51 CCGGCGGACGAGGGGCCGGGGCAGTGGTACAGACGGCTCAGGAACCATT 100

CDKN1C_RefSeq 101 TAACAGACTTGTCTTCAAGTTTCAGATAAACACAGTCATAATAAGAGAGA 150
|||||
CDKN1C_DelIns 101 TAACAGACTTGTCTTCAAGTTTCAGATAAACACAGTCATAATAAGAGAGA 150

CDKN1C_RefSeq 151 CAGCGAAAGCGCGAAGAGACTGCAAGCTAGATGGGCATGTATGGCAGCTA 200
|||||
CDKN1C_DelIns 151 CAGCGAAAGCGCGAAGAGACTGCAAGCTAGATGGGCATGTATGGCAGCTA 200

CDKN1C_RefSeq 201 CAGCTTGTGAGTGACCCCTTCCCCAGAGTCCGCGATGAAAATAAAGTTA 250
|||||
CDKN1C_DelIns 201 CAGCTTGTGAGTGACCCCTTCCCCAGAGTCCGCGATGAAAATAAAGTTA 250

CDKN1C_RefSeq 251 CACTTGTCAATAACCAGATGTGGGAGATGGAGAGTGCCTTTGGCATAACC 300
|||||
CDKN1C_DelIns 251 CACTTGTCAATAACCAGATGTGGGAGATGGAGAGTGCCTTTGGCATAACC 300

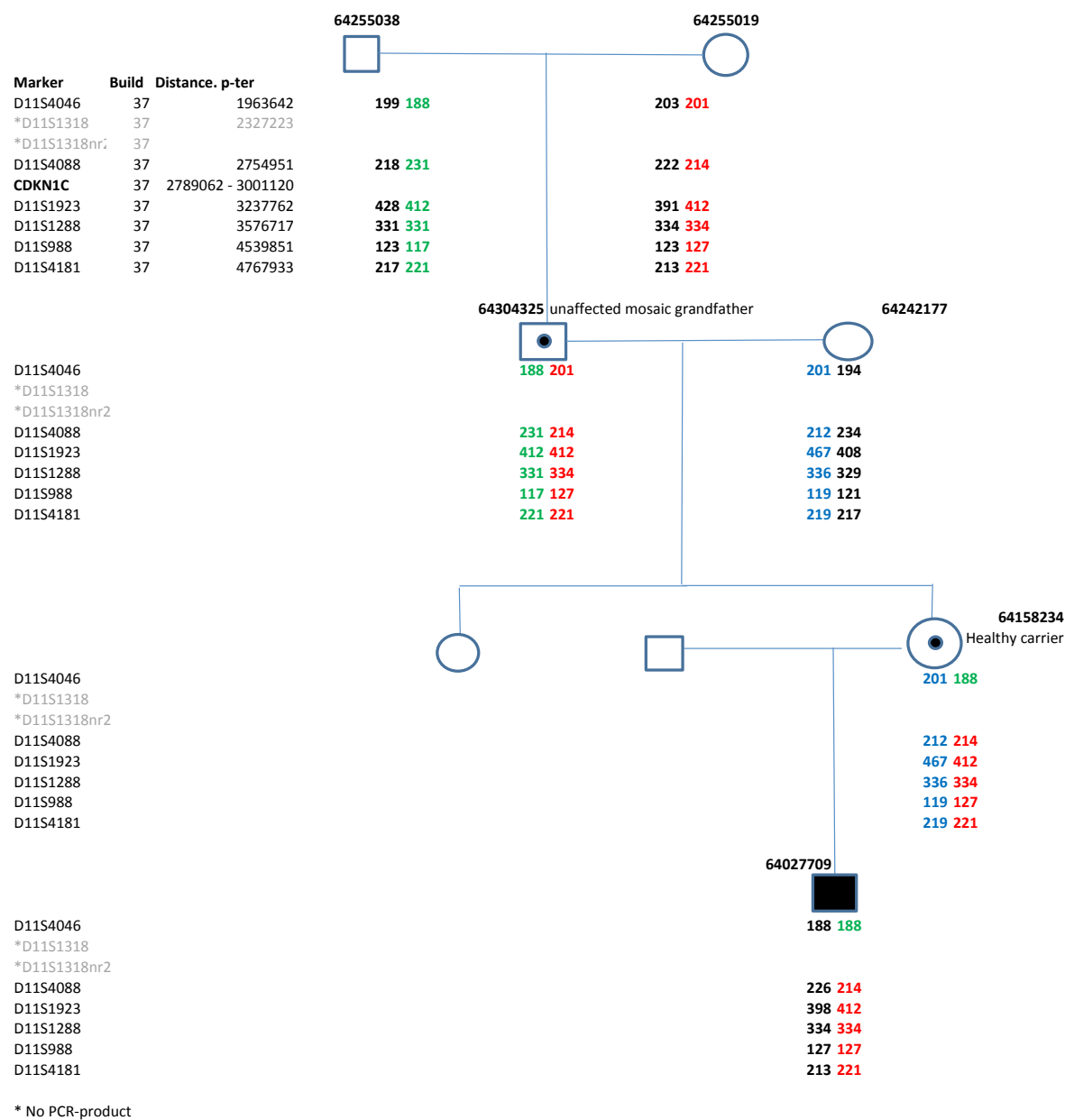
CDKN1C_RefSeq 301 AATAACCGAGCTAGTGCCTGGCAGAGCGGTCCACGCCTGGACATAAATAG 350
|||||
CDKN1C_DelIns 301 AATAACCGAGCTAGTGCCTGGCAGAGCGGTCCACGCCTGGACATAAATAG 350

CDKN1C_RefSeq 351 AAAATATAAGTTAGTATAAAGCTTTAAAAAAGCTTTTGTACAAATATACATGG 400
|||||
```

CDKN1C_DelIns	351	AAAATATAAGTTAGTATAAAGCTTTAAAAACTTTTTGTACAAATATACATGG	400
CDKN1C_RefSeq	401	TTTTTTTATTTTTCTTTTTTTTTCTTTTTCTTTTTTTGCACTGAG	450
CDKN1C_DelIns	401	TTTTTTTATTTTTCTTTTTTTTTCTTTTTCTTTTTTTGCACTGAG	450
CDKN1C_RefSeq	451	TTTCAGCAGAGATTAACATTTTATATAAATGACTCTTAAAGCTTTACAC	500
CDKN1C_DelIns	451	TTTCAGCAGAGATTAACATTTTATATAAATGACTCTTAAAGCTTTACAC	500
CDKN1C_RefSeq	501	CTTGGGACCAGTGTACCTTCTCGTGCAGAATACATTTAGATATAAAAAGA	550
CDKN1C_DelIns	501	CTTGGGACCAGTGTACCTTCTCGTGCAGAATACATTTAGATATAAAAAGA	550
CDKN1C_RefSeq	551	CGTTATTAATACATTGCACAGTTTTCAAATTTAAAAACAAAACCGAACG	600
CDKN1C_DelIns	551	CGTTATTAATACATTGCACAGTTTTCAAATTTAAAAACAAAACCGAACG	600
CDKN1C_RefSeq	601	CTGCTCTGCGGCAGCCGCGCGGTTGCTGCTACATGAACGGTCCAGCC	650
CDKN1C_DelIns	601	CTGCTCTGCGGCAGCCGCGCGGTTGCTGCTACATGAACGGTCCAGCC	650
CDKN1C_RefSeq	651	GAGGCCAGCGCCCTTCCAACGTCCGCTGCCCGGCAGGTTCCCTCGGGG	700
CDKN1C_DelIns	651	GAGGCCAGCGCCCTTCCAACGTCCGCTGCCCGGCAGGTTCCCTCGGGG	700
CDKN1C_RefSeq	701	CTCTTTGGGCTCTAAACTGCGAGGAGAGGGGCGGTCAGCAAAGCCGGCGG	750
CDKN1C_DelIns	701	CTCTTTGGGCTCTAAACTGCGAGGAGAGGGGCGGTCAGCAAAGCCGGCGG	750
CDKN1C_RefSeq	751	GGACCCGGCGGGTCCGGCCCTCCGCGCCCCCAGGTGCGCTGTACTACT	800
CDKN1C_DelIns	751	GGACCCGGCGGGTCCGGCCCTCCGCGCCCCCAGGTGCGCTGTACTACT	800
CDKN1C_RefSeq	801	TGGCTCACCGAGCCTTGTGCGGGGTCTGCTCCACCGAGCCACGCCA	850
CDKN1C_DelIns	801	TGGCTCACCGAGCCTTGTGCGGGGTCTGCTCCACCGAGCCACGCCA	850
CDKN1C_RefSeq	851	GGGGCGGCGCTTGAGAGGGACACGGCGGGGACATCGCCCGACGACTT	900
CDKN1C_DelIns	851	GGGGCGGCGCTTGAGAGGGACACGGCGGGGACATCGCCCGACGACTT	900
CDKN1C_RefSeq	901	CTCAGGCGCTGATCTCTTGGCGTTGGCGA-AGAAATCTGCGGGCAGACG	949
CDKN1C_DelIns	901	CTCAGGCGCTGATCTCTTGGCGTTGGCGAAGCTCTCTGCGGGCAGACG	950
CDKN1C_RefSeq	950	GCGCGCGGCGGTCAGGGCGGGGCCGGCCCGGAGACCCGAGAGGGGGCCG	999
CDKN1C_DelIns	951	GCGCGCGGCGGTCAGGGCGGGGCCGGCCCGGAGACCCGAGAGGGGGCCG	1000
CDKN1C_RefSeq	1000	GGAGAGGGCGCGGGGCGCGGGCCGGCCGGGTGGGGGCGCGTCCCGCC	1049
CDKN1C_DelIns	1001	GGAGAGGGCGCGGGGCGCGGGCCGGCCGGGTGGGGGCGCGTCCCGCC	1050
CDKN1C_RefSeq	1050	CGCGCCGAGTCCGCGGGGTCAGCTTTGTTTACGTGCGCGCAATGT	1099
CDKN1C_DelIns	1051	CGCGCCGAGTCCGCGGGGTCAGCTTTGTTTACGTGCGCGCAATGT	1100
CDKN1C_RefSeq	1100	GCTGTGTAAGCATTTCCCTTTGTCCCGGGCAAGCCCCGGGGCCGCC	1149
CDKN1C_DelIns	1101	GCTGTGTAAGCATTTCCCTTTGTCCCGGGCAAGCCCCGGGGCCGCC	1150
CDKN1C_RefSeq	1150	GCCGCGCTTAACCCCTCCACGCCGTGCTACGGCGCCGGCCGCGCCG	1199
CDKN1C_DelIns	1151	GCCGCGCTTAACCCCTCCACGCCGTGCTACGGCGCCGGCCGCGCCG	1200
CDKN1C_RefSeq	1200	GGGAGGGGCTCCCGCGGCGGGGGCGAAAACTGCGCTCCGGGGGTGCG	1249
CDKN1C_DelIns	1201	GGGAGGGGCTCCCGCGGCGGGGGCGAAAACTGCGCTCCGGGGGTGCG	1250
CDKN1C_RefSeq	1250	GGCCGGGATTACGCTCCACCCCGCCGCGGAAGCCGCTGGAGGGCAC	1299
CDKN1C_DelIns	1251	GGCCGGGATTACGCTCCACCCCGCCGCGGAAGCCGCTGGAGGGCAC	1300
CDKN1C_RefSeq	1300	AACAACGGGGCGGGAGGGGGTAAGGGCGCAGCCGCGCCTGAGCGCTG	1349

CDKN1C_DelIns	1301	AACAACGGGGCGGGAGGGGGTAAGGGCGCAGCCGCGCCCTGAGCGCTG	1350
CDKN1C_RefSeq	1350	CGGGCCCTTAAATGCCACGGGAGGAGGCGGGAACCCAGCGAGGCCCCGA	1399
CDKN1C_DelIns	1351	CGGGCCCTTAAATGCCACGGGAGGAGGCGGGAACCCAGCGAGGCCCCGA	1400
CDKN1C_RefSeq	1400	GGGCTGGGGGACCGGCCGGCCGACAAAGCGGGGCGGGCCGGCCGGG	1449
CDKN1C_DelIns	1401	GGGCTGGGGGACCGGCCGGCCGACAAAGCGGGGCGGGCCGGCCGGG	1450
CDKN1C_RefSeq	1450	GCGGGCCCGTGCGGGGCTACCGGAGATCAGAGGCCGGACAGCTTCTTG	1499
CDKN1C_DelIns	1451	GCGGGCCCGTGCGGGGCTACCGGAGATCAGAGGCCGGACAGCTTCTTG	1500
CDKN1C_RefSeq	1500	ATCGCCGCGCCGTTGGCGCTGGCGGCCGCGGTGCCGGCCGCGGGACGTCC	1549
CDKN1C_DelIns	1501	ATCGCCGCGCCGTTGGCGCTGGCGGCCGCGGTGCCGGCCGCGGGACGTCC	1550
CDKN1C_RefSeq	1550	CGAAATCCCGAGTGCAGCTGGTCAGCGAGAGGCTCCTGGCCGCGCTGCC	1599
CDKN1C_DelIns	1551	CGAAATCCCGAGTGCAGCTGGTCAGCGAGAGGCTCCTGGCCGCGCTGCC	1600
CDKN1C_RefSeq	1600	CCTGGTTCGCGCCCTGCTCGGCGCTCTCTTGAGGCGCCGCTCGGGGCC	1649
CDKN1C_DelIns	1601	CCTGGTTCGCGCCCTGCTCGGCGCTCTCTTGAGGCGCCGCTCGGGGCC	1650
CDKN1C_RefSeq	1650	GGGGCCGGGGCGGGGCCGGGGCCGGGGCCGGGGCCGGGGCTGGGGCCGG	1699
CDKN1C_DelIns	1651	GGGGCCGGGGCGGGGCCGGGGCCGGGGCCGGGGCCGGGGCTGGGGCCGG	1700
CDKN1C_RefSeq	1700	GGCCGCGACTGGAGCCGGGGCCGGAGCCGGAGCCGGAGCCGGGGCCGGGG	1749
CDKN1C_DelIns	1701	GGCCGCGACTGGAGCCGGGGCCGGAGCCGGAGCCGGAGCCGGGGCCGGGG	1750
CDKN1C_RefSeq	1750	CCGGGGCCAGGACCGGACCGGAGCCGGAGCCGGAGCCGGAGCCGGAGCC	1799
CDKN1C_DelIns	1751	CCGGGGCCAGGACCGGACCGGAGCCGGAGCCGGAGCCGGAGCCGGAGCC	1800
CDKN1C_RefSeq	1800	GGAGCCGGAGCCGGGGCCGGGGCTGGAGCCAGGACCGGGACTGGGGGCGG	1849
CDKN1C_DelIns	1801	GGAGCCGGAGCCGGGGCCGGGGCTGGAGCCAGGACCGGGACTGGGGGCGG	1850
CDKN1C_RefSeq	1850	GGTGGACGCCGGGGCCGGGACCGGACACTAGGCAGCTGCTCCGGCGCCT	1899
CDKN1C_DelIns	1851	GGTGGACGCCGGGGCCGGGACCGGACACTAGGCAGCTGCTCCGGCGCCT	1900
CDKN1C_RefSeq	1900	CCTCGAGGCCGTCGAGGGACTCAGCGGCCGGCTCGAGGGGCGGGCTGACA	1949
CDKN1C_DelIns	1901	CCTCGAGGCCGTCGAGGGACTCAGCGGCCGGCTCGAGGGGCGGGCTGACA	1950
CDKN1C_RefSeq	1950	GCCACCGGACCGGACCGGCGCGGCCAGCAGCAGGCGGCAGCGCCC	1999
CDKN1C_DelIns	1951	GCCACCGGACCGGACCGGCGCGGCCAGCAGCAGGCGGCAGCGCCC	2000
CDKN1C_RefSeq	2000	CACCTGCACCGTCTCGCGGTAGAACGCGGGCACCGAGTCGCTGTCCACTT	2049
CDKN1C_DelIns	2001	CACCTGCACCGTCTCGCGGTAGAACGCGGGCACCGAGTCGCTGTCCACTT	2050
CDKN1C_RefSeq	2050	CGGTCCACTGCAGGCGTCCAGGGCCCCGAGCGGCATGTCCTGCTGGAAG	2099
CDKN1C_DelIns	2051	CGGTCCACTGCAGGCGTCCAGGGCCCCGAGCGGCATGTCCTGCTGGAAG	2100
CDKN1C_RefSeq	2100	TCGTAATCCAGCGGTTCTGGTCTCGGCGTTCAGCTCGGCCAGGCGGGC	2149
CDKN1C_DelIns	2101	TCGTAATCCAGCGGTTCTGGTCTCGGCGTTCAGCTCGGCCAGGCGGGC	2150
CDKN1C_RefSeq	2150	CTGCAGCTCGCGGCTCAGCTCCTCGTGGTCCACCGGCCGAAGAGGCTGC	2199
CDKN1C_DelIns	2151	CTGCAGCTCGCGGCTCAGCTCCTCGTGGTCCACCGGCCGAAGAGGCTGC	2200
CDKN1C_RefSeq	2200	GGCAGGCGCTGGTGCACACTAGTACTGGGAAGGTCCACGGGCGACAAGA	2249
CDKN1C_DelIns	2201	GGCAGGCGCTGGTGCACACTAGTACTGGGAAGGTCCACGGGCGACAAGA	2250
CDKN1C_RefSeq	2250	CGCTCCATCGTGGATGTGCTGCGGAGGGACGCGTCGGACATGGCCCGGG	2299

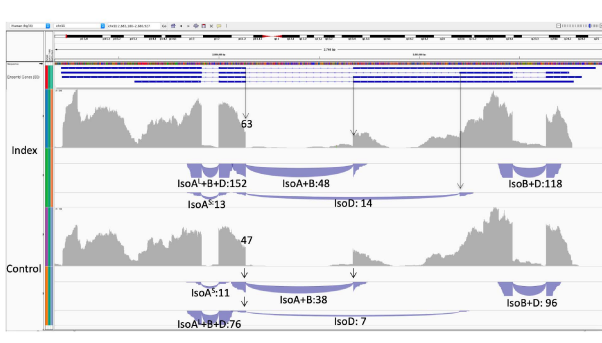
CDKN1C_DelIns	2251	CGCTCCATCGTGGATGTGCTGCGGAGGGACGCGTCGGACATGGCCCGGGG	2300
CDKN1C_RefSeq	2300	CTGCGCAAACGCGGGCAGCGAGAGAGGAGAGGACAGCGAGAAGAAGGGGA	2349
CDKN1C_DelIns	2301	CTGCGCAAACGCGGGCAGCGAGAGAGGAGAGGACAGCGAGAAGAAGGGGA	2350
CDKN1C_RefSeq	2350	AAGGAGAGGAGGAGAGGGCGGAGGCCGGGCGCAAGGGAGACCCCGCGCCG	2399
CDKN1C_DelIns	2351	AAGGAGAGGAGGAGAGGGCGGAGGCCGGGCGCAAGGGAGACCCCGCGCCG	2400
CDKN1C_RefSeq	2400	CCCGACTCTGCGTGTGCGAGGGACGCGGCGGCTACCTGGCTGTCCGGTGG	2449
CDKN1C_DelIns	2401	CCCGACTCTGCGTGTGCGAGGGACGCGGCGGCTACCTGGCTGTCCGGTGG	2450
CDKN1C_RefSeq	2450	TGGACTCTTCTGCGTCGGGTTTCGCCTGTCTCGTCCGGACGGCAGCCGCGC	2499
CDKN1C_DelIns	2451	TGGACTCTTCTGCGTCGGGTTTCGCCTGTCTCGTCCGGACGGCAGCCGCGC	2500
CDKN1C_RefSeq	2500	CCCCTCGATGCCTGTGGCTAGCTCGCTCGCTCAGGCCTGGCCGGCACCC	2549
CDKN1C_DelIns	2501	CCCCTCGATGCCTGTGGCTAGCTCGCTCGCTCAGGCCTGGCCGGCACCC	2550
CDKN1C_RefSeq	2550	CTCGAGCACAGCGCACTTGGCCTGTGGAACGCCAGCCCGCCTGCGCCCC	2599
CDKN1C_DelIns	2551	CTCGAGCACAGCGCACTTGGCCTGTGGAACGCCAGCCCGCCTGCGCCCC	2600
CDKN1C_RefSeq	2600	CTTTATACGCGCGGGCCACCCCGTGCAGCGCGGGCCCGGCCGCGATTA	2649
CDKN1C_DelIns	2601	CTTTATACGCGCGGGCCACCCCGTGCAGCGCGGGCCCGGCCGCGATTA	2650
CDKN1C_RefSeq	2650	GCATAATGTAGTATTTTCAGTTTCAACAACACCACGGCGATTGGCGGCCG	2699
CDKN1C_DelIns	2651	GCATAATGTAGTATTTTCAGTTTCAACAACACCACGGCGATTGGCGGCCG	2700
CDKN1C_RefSeq	2700	CCCCGCTGCCCGCCCGCCGCGGCGCCCGGCCCGCCGCGCACCGCCATTGG	2749
CDKN1C_DelIns	2701	CCCCGCTGCCCGCCCGCCGCGGCGCCCGGCCCGCCGCGCACCGCCATTGG	2750
CDKN1C_RefSeq	2750	CCGCGCGCACACCCACCGGGGGCGGGGCGGGGCGCGGGCCGGGGCGCGC	2799
CDKN1C_DelIns	2751	CCGCGCGCACACCCACCGGGGGCGGGGCGGGGCGCGGGCCGGGGCGCGC	2800
CDKN1C_RefSeq	2800	GG 2801	
CDKN1C_DelIns	2801	GG 2802	

Supplementary 3: *CDKN1C* Haplotype analysis**Result from haplotype analysis**

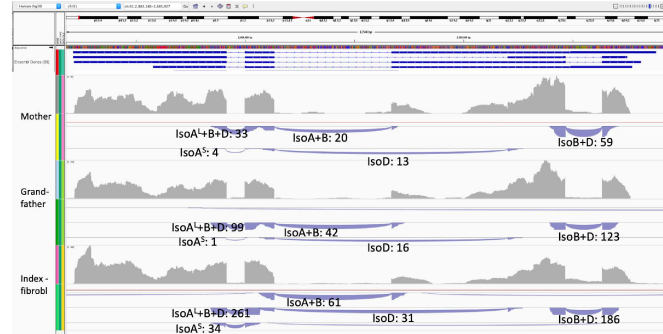
Haplotype analysis in the family with informative (simple tandem repeat) markers (to the left) confirm that the *de novo* *CDKN1C* delins in the maternal grandfather occurred on his maternal allele

Supplementary 4. Results from *CDKN1C* RNA-sequencing displaying Splice Junctions Tracks

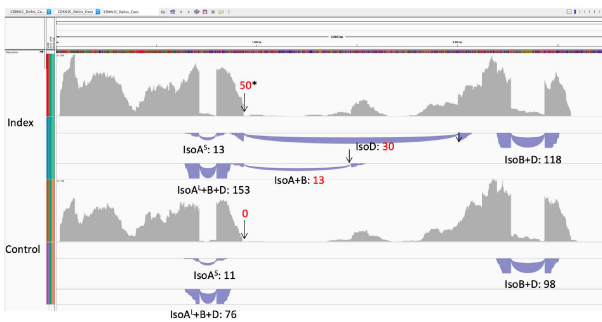
a RefSeq; index boy and healthy control (blood)



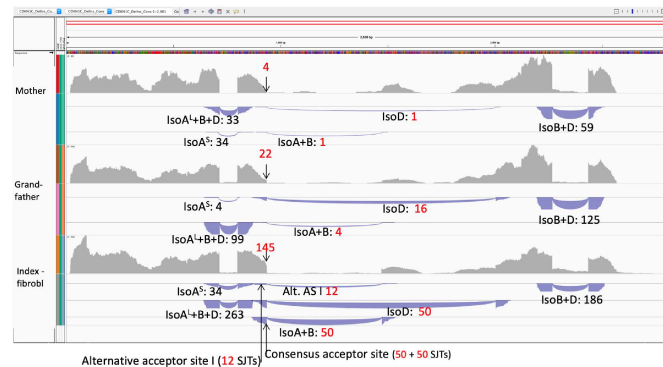
b Ref Seq; mother, maternal grandfather (blood) and index boy (fibroblasts)



c Delins ref; index boy and control (blood)



d Delins ref; mother, maternal grandfather (blood) and index boy (fibroblasts)



All results from RNAseq are presented in IGV (The Integrative Genomics Viewer). (a) and (b) represent mapping against reference sequence genome assembly GRCh38.p11. (c) and (d) were mapped against a FASTA file containing the delins variant, using the consensus splice site as reference.

Panel (a) and (c) show results from analysis of blood-derived RNA from index patient and a healthy control, while (b) and (d) show results from blood-derived RNA from the asymptomatic heterozygous mother, the mosaic grandfather, and at the bottom fibroblast-derived RNA from the index patient.

The four Ensembl transcripts are shown at the top; first *CDKN1C-202* (named IsoA^L in the figure to ease reading), then *CDKN1C-201* (IsoD), followed by *CDKN1C-204* (IsoB) and *CDKN1C-203* (IsoA^S). Isoform A (316aa) is encoded by two transcripts of different UTR-length, *CDKN1C-202* (L for "long") and *CDKN1C-203* (S for "short"). Where the two transcript can not be distinguished, the L/S is omitted. The transcripts are read from the reverse strand (from right to left in the figures). The junctions track displays arcs connecting alignment blocks from a single read. For RNA data these connections normally arise from splice junctions, called Splice Junction Track (SJT). Below each arc, the corresponding isoform (A, B or D) and the total number of (manually checked) SJTs are reported, with delins-containing reads in red. The total number of reads at specific points are marked by arrows. SJTs on the same line do not necessarily correspond to the same transcript.