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Supplemental information

AON-based degradation of c.151C>T mutant *COCH* transcripts associated with dominantly inherited hearing impairment DFNA9

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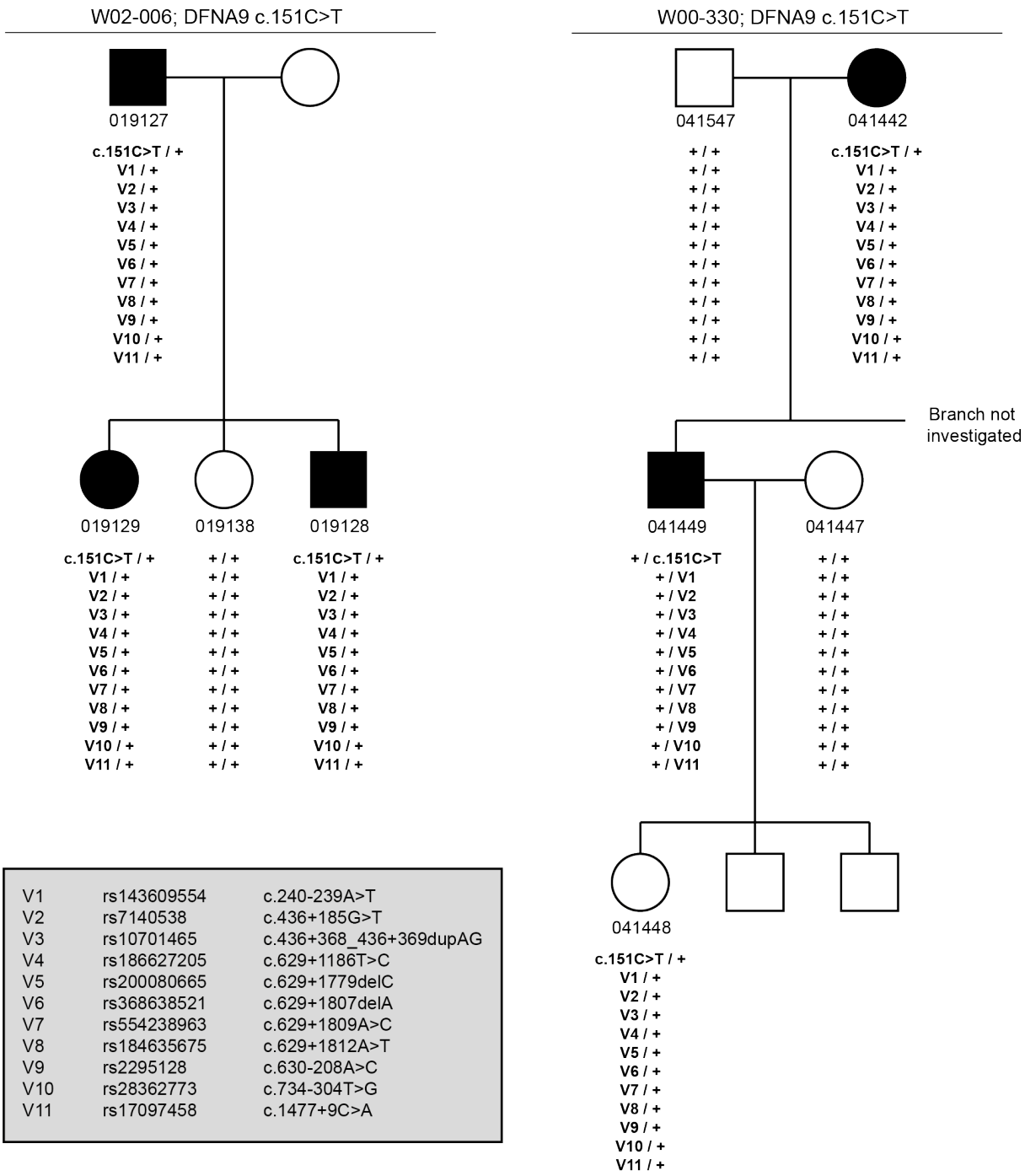

Figure S1. Segregation analysis of haplotype-specific variants. Small branches from the pedigrees of two large Dutch DFNA9 families (W02-006 and W00-330) were investigated to confirm co-segregation of the haplotype-specific variants with the c.151C>T mutation. Numbers below each individual depict the internal identifier of the DNA samples. Individual 041448 was not clinically affected at the time of sample collection. V1-V10: *COCH* variants (see grey box); +: wildtype; square: male; circle: female; open symbol: clinically unaffected; closed symbol: clinically affected.

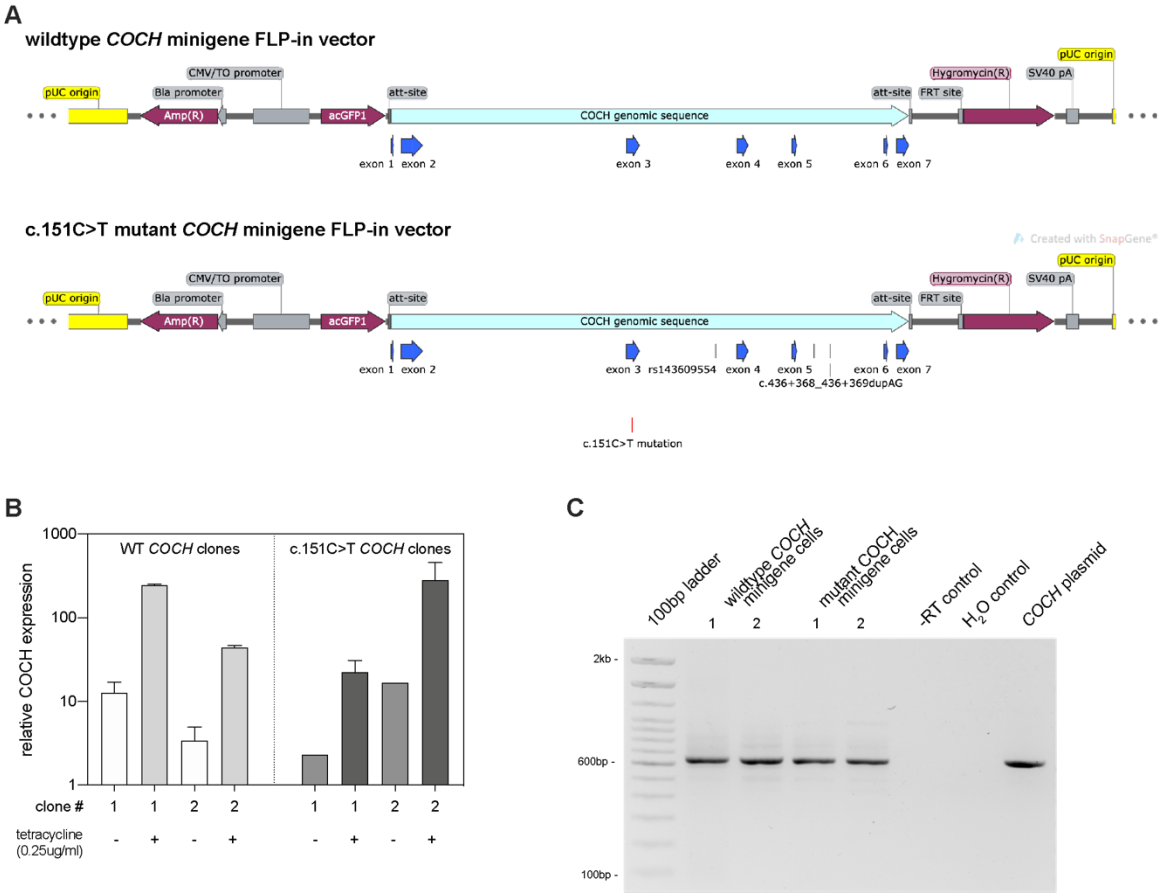

Figure S2. Inducible *COCH* minigene T-REx 293T cells. A) schematic overview of the wildtype and mutant COCH vectors that were used to establish the COCH minigene T-REx 293T cells. **B)** Measurement of *COCH* expression upon overnight induction with tetracycline. Two clones of wildtype *COCH* minigene-expressing transgenic cells, and two clones of mutant *COCH* minigene-expressing transgenic cells were investigated. Wildtype clone 2, and mutant clone 1 were selected for experiments based on the relatively similar levels of *COCH* expression upon tetracycline treatment. Note that uninduced cells always show a certain level of background *COCH* expression. As the Taqman™ probe for the mutant *COCH* transcript is highly specific, it appears that the transcriptional activity of the tetracycline promotor is not completely off in uninduced cells. Data shown as mean ± SD. **C)** RT-PCR analysis of *COCH* transcripts in tetracycline-treated mutant and wildtype *COCH* minigene-expressing cells. For each cell line, two replicate samples are shown. Sanger sequencing of the amplicons confirmed correct splicing of the minigene *COCH* transcripts. The positive control is a plasmid containing the coding sequence of *COCH* that was amplified from fetal cochlear cDNA.

Table S1: AON sequences and chemical and thermodynamic properties. Phosphorothioate links in the AON sequences are indicated by the asterisks between bases. Variant c.436+368_436+369dupAG is abbreviated in the table as dupAG. The 2'-O-methyl-RNA bases are placed between brackets. The allele-discriminating variants are indicated by bold underlined fonts. Secondary structure and free energy predictions are done with the RNAstructure webserver (http://rna.urmc.rochester.edu/RNAstructureWeb/). The RNAstructure webserver can not take the chemical modification or gapmer composition into account. Therefore, gapmers were analysed as both DNA and RNA molecules. # AONs were investigated in gapmer chemistry and complete PS-DNA chemistry

			AON secondary structure free energy (kcal/mol)		**self dimerisation (kcal/mol)**		**free energy of bimolecular structure (kcal/mol)**		**Difference in free energy between mutant and wildtype bimolecular structure (kcal/mol)**
AON name		**AON sequence**	*as DNA*	*as RNA*	*as DNA*	*as RNA*	*mutant COCH*	*wildtype COCH*	*delta*
c.151C>T	AON-A	(C*C*C*)T*G*<u>**A**</u>*G*C*A*G*A*G*G*A*C*A*T*(C*U*G)	-1,9	-3,0	-7,1	-13,4	-41,0	-36,2	4,8
	AON-B	(C*U*G*)<u>**A**</u>*G*C*A*G*A*G*G*A*C*A*T*C*T*(G*C*U)#	-5,3	-7,5	-12,6	-18,0	-39,4	-34,6	4,8
	AON-C	(C*C*C*C*)T*G*<u>**A**</u>*G*C*A*G*A*G*G*A*C*(A*U*C*U)	1,4	-3,0	-7,1	-14,2	-41,7	-36,9	4,8
	AON-D	T*G*A*G*C*A*G*A*G*G*A*C*A*T*C*T*G*C*T*T	-5,3	-	-12,6	-	-37,8	-33,0	4,8
	AON-E	(A*G*C*C*)C*C*C*T*G*<u>**A**</u>*G*C*A*G*A*G*(G*A*C*A)#	1,4	-3,0	-6,3	-14,6	-44,5	-39,7	4,8
	AON-F	(C*C*U*G*)<u>**A**</u>*G*C*A*G*A*G*G*A*C*A*T*(C*U*G*C)	-4,4	-5,7	-9,9	-14,4	-41,1	-36,3	4,8
	AON-G	G*C*A*G*C*C*C*C*C*T*G*<u>**A**</u>*G*C*A*G*A*G*G*A	-0,8	-	-7,2	-	-46,1	-41,3	4,8
dupAG	AON-A	(U*C*A*U*)A*G*C*T*A*G*A*C*<u>**C*T*C*T**</u>(G*U*C*U)	-1,6	-1,6	-8,8	-9,1	-36,1	-27,0	9,1
	AON-B	(A*U*A*G*)C*T*A*G*A*C*<u>**C*T*C*T**</u>G*T*(C*U*A*A)#	-2,2	-3,7	-8,8	-10,6	-33,9	-24,8	9,1
	AON-C	(A*U*C*A*)T*A*G*C*T*A*G*A*C*<u>**C*T*C*(U**</u>G*U*C)	-0,8	1,4	-8,8	-8,9	-35,6	-26,6	9,0
	AON-D	C*A*U*C*A*T*A*G*C*T*A*G*A*C*<u>**C*T*C*U**</u>G*U	1,5	-	-7,8	-	-35,3	-27,5	7,8
	AON-E	A*G*C*T*A*G*A*C*<u>**C*T*C*T**</u>G*T*C*T*A*A*A*A	-2,2	-	-8,8	-	-33,3	-24,2	9,1
	AON-F	(U*A*G*C*)T*A*G*A*C*<u>**C*T*C*T**</u>G*T*C*(U*A*A*A)	-2,2	-3,7	-8,8	-10,6	-33,7	-24,5	9,2
	AON-G	C*A*T*A*G*C*T*A*G*A*C*<u>**C*T*C*T**</u>G*T*C*T*A	-2,1	-	-8,8	-	-35,5	-26,9	8,6
scrambled AON		(G*C*T*A*)T*C*G*A*T*T*A*C*A*C*T*A*(T*C*G*A)	-2,3	-3,6	-7,4	-9,4			

Table S2 - primer list for segregation analysis of identified allele-specific variants

variant	forward primer	reverse primer	sequencing primer
c.151C>T	CACTGTAGTCTCCCCACCAC	CAGATGGGTAAAGCAGGAAAG	
c.240-239A>T	TCACACCTGTAATCCCACCA	CCACACTTTTTCAGGGCATC	
c.436+185G>T	ACAAGCAGTGTCCACAGCAC	CTGAACTTTGGGAGGCTGAA	CCCGGCACAGCATTTGGAAG
c.436+368_436+369dupAG			
c.629+1186T>C	AGACCATCCTGGCTAACACG	TTTTCAAGCTTTCTATAATGAGCA	
c.629+1779delC c.629+1807delA c.629+1809A>C c.629+1812A>T	CCTGGCCCCTTCAGTATTTT	CTGAGCAGCTGGCACTACAG	
c.630-208A>C	GCTGTGTTTCATCAGGCAAA	TTGGAATTACCCCCTCTGAA	
c.734-304T>G	GAATGCAGATGTGGCAGAAA	GATGCATCAGCTGGGAAAGT	
c.1477+9C>A	TGGTGGAACAGCTACTGGTG	TGGTGGAACAGCTACTGGTG	