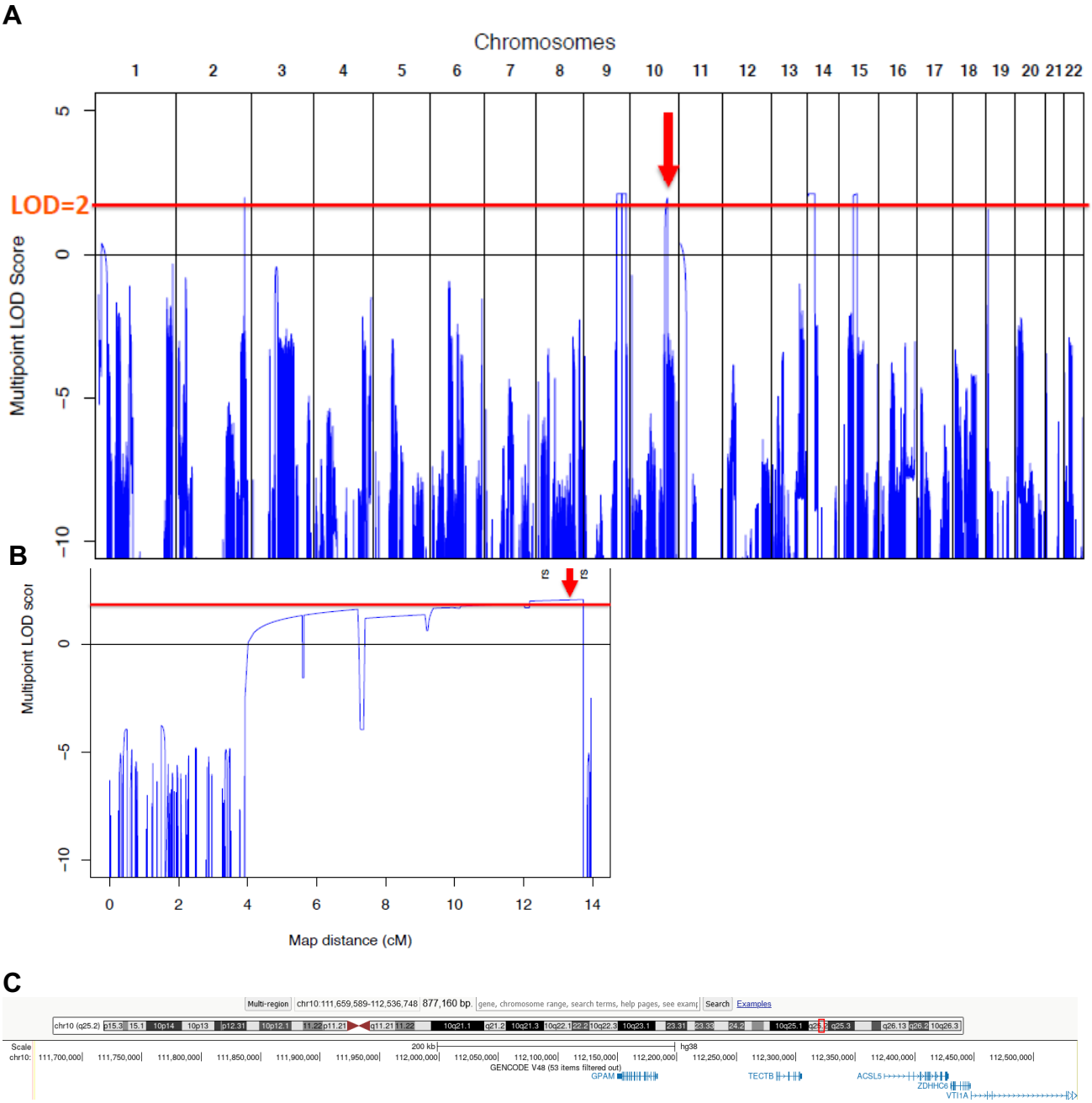
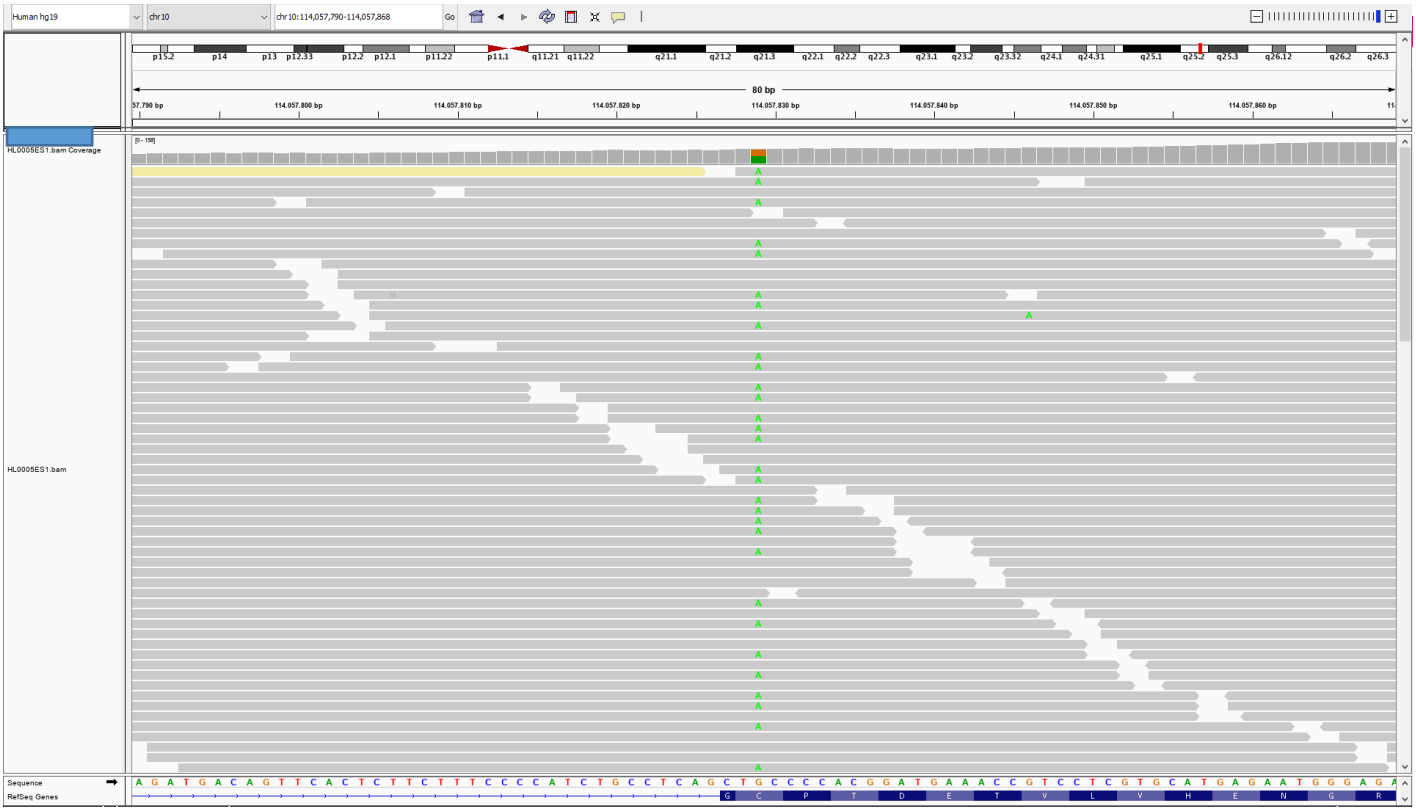
Supplementary Figures

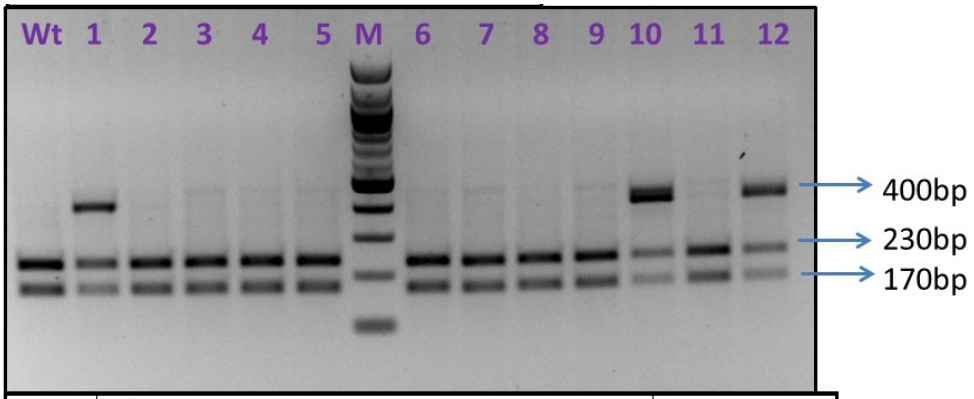


Supplementary Figure 1. Linkage analysis. (**A**) Linkage analysis showing genome-wide linkage intervals reaching a maximum LOD >2 (red line). (**B**) Chromosome 10 linkage map showing the significant interval and location of *TECTB*. (**C**) Gene content of the chromosomal interval on chromosome 10 reaching LOD significance.



Supplementary Figure 2. Exome sequencing visualization of the *TECTB* c.674G>A, p.(Cys225Tyr) variant

A

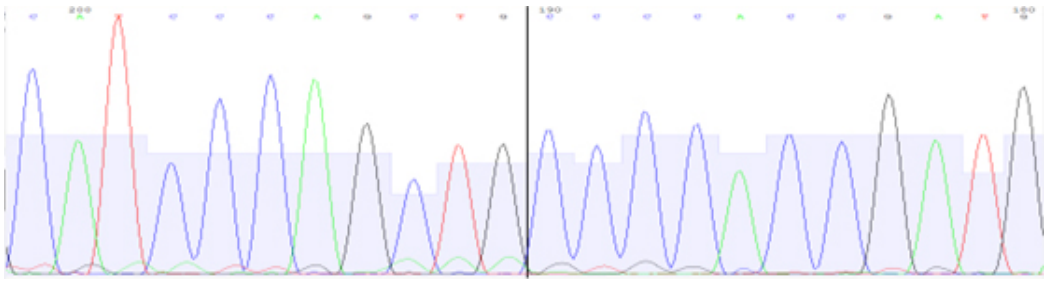


primers	Product size	Wild type after PvuII digestion	Het after PvuII digestion	Homo mutant after PvuII digestion
F1 R1	400	170 + 230	170 + 230 and 400	400

B

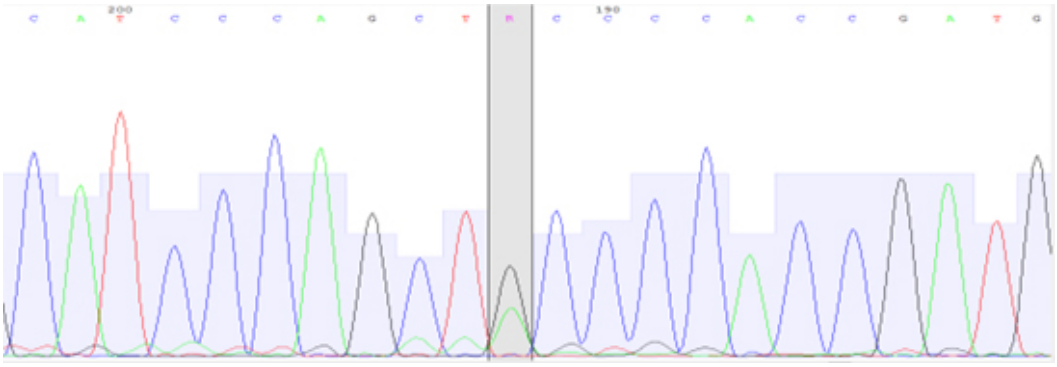
Wild type:

C C C A G C T G C C C C A C C G A T

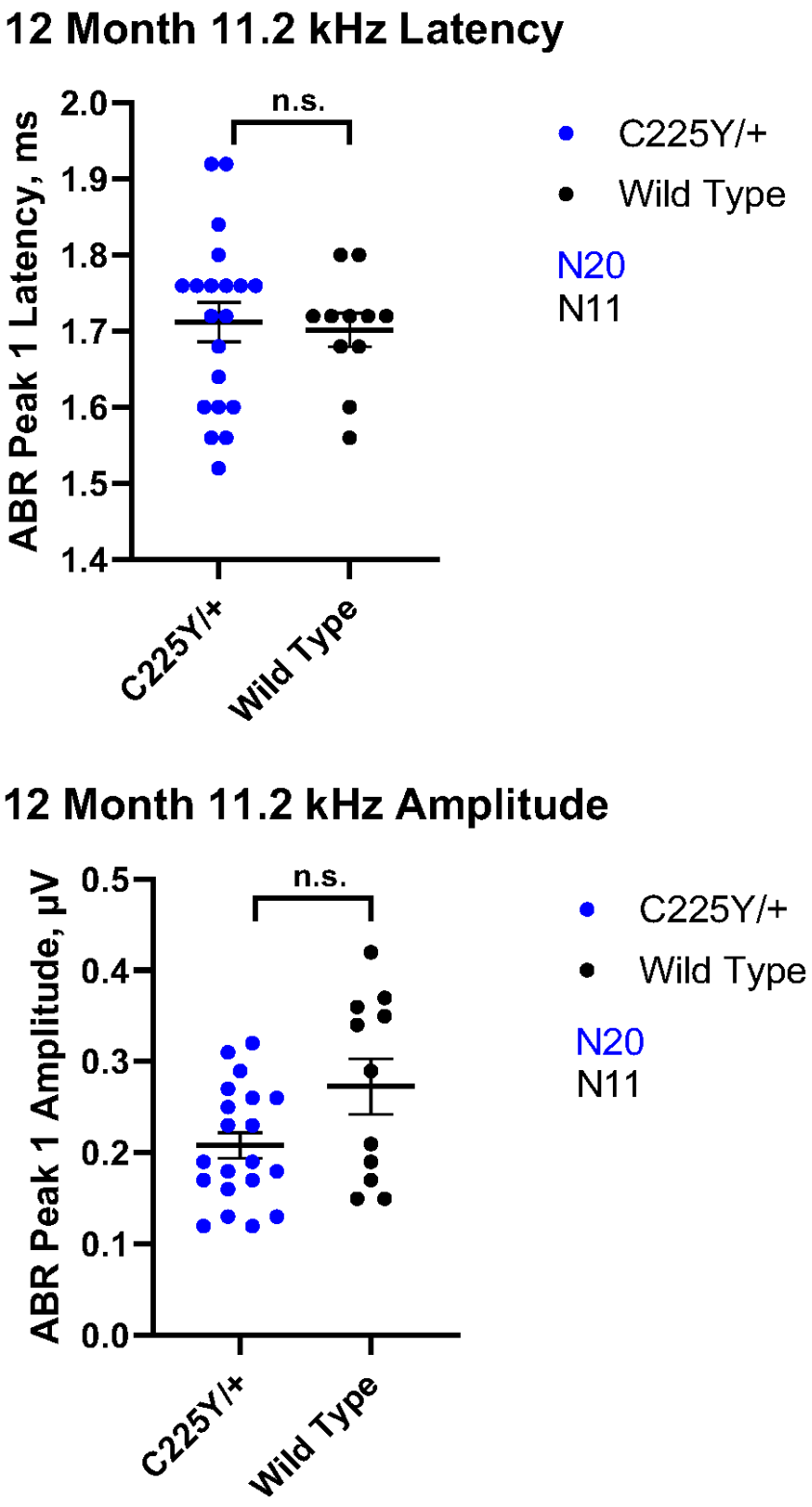


Heterozygous (pup #1):

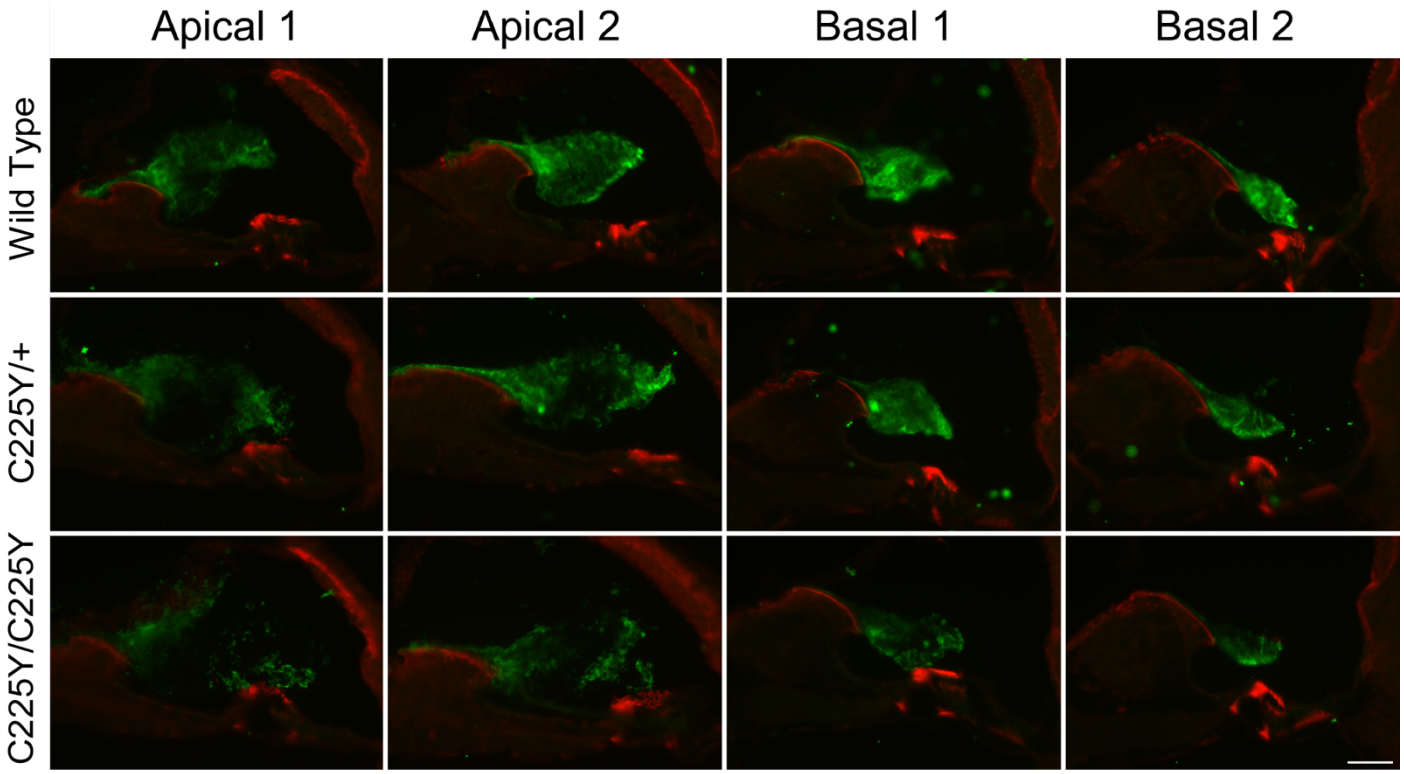
C C C A G C T G/A C C C C A C C G A T



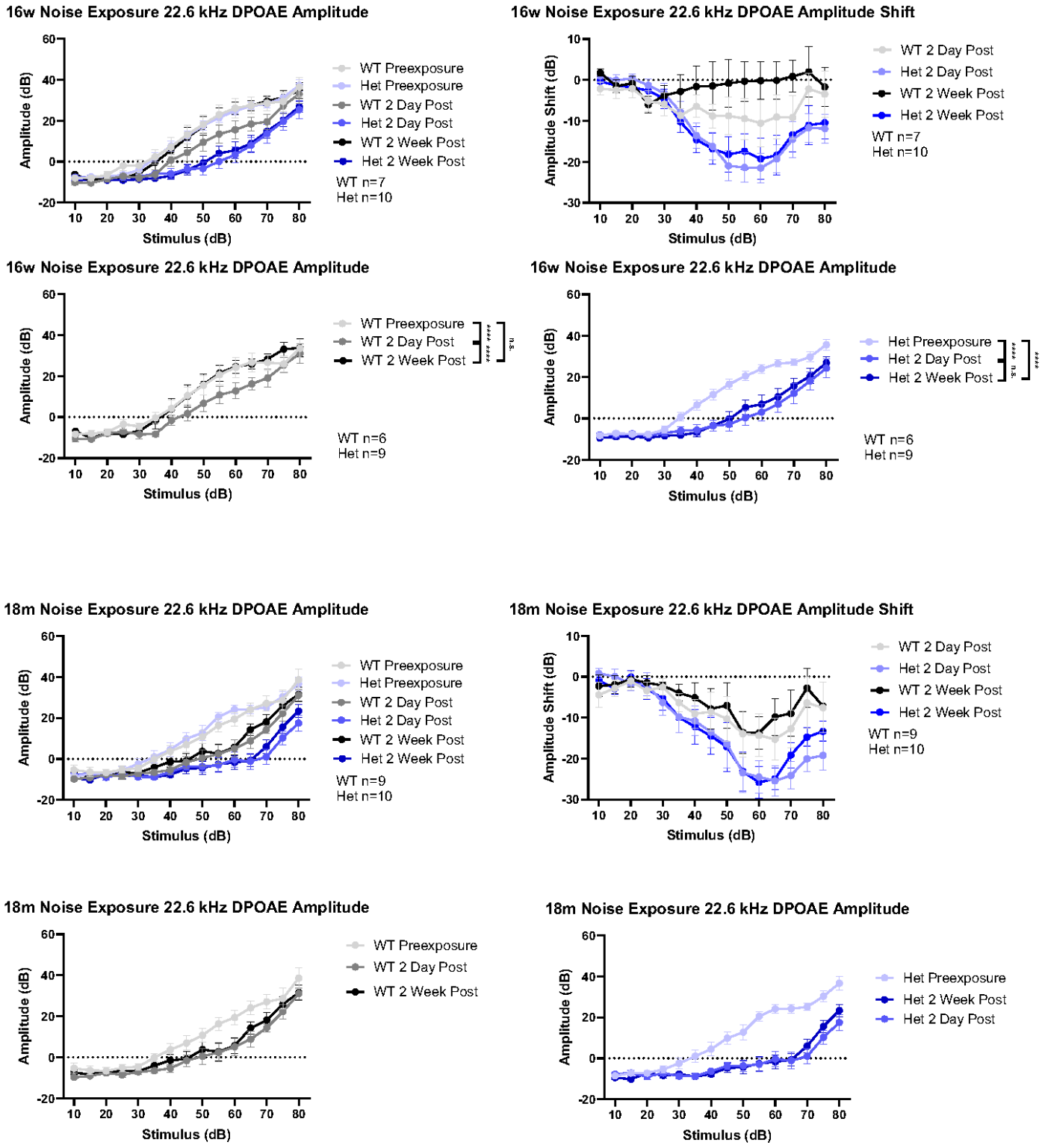
Supplementary Figure 3. (A) Genomic DNA extracted from a wild-type mouse and 12 littermate pups born following CRISPR/Cas-9 mediated genome editing was amplified with primers F1 and R1 and digested with *PvuII* as described in *Material and methods* section. **(B)** Sanger sequencing of F2R2 PCR products from the wild-type and heterozygous (pup #1) littermates shown above in panel *A*. Sequencing confirmed that Pup#1 was correctly modified, while pups #10 and #12 showed evidence of mosaicism.



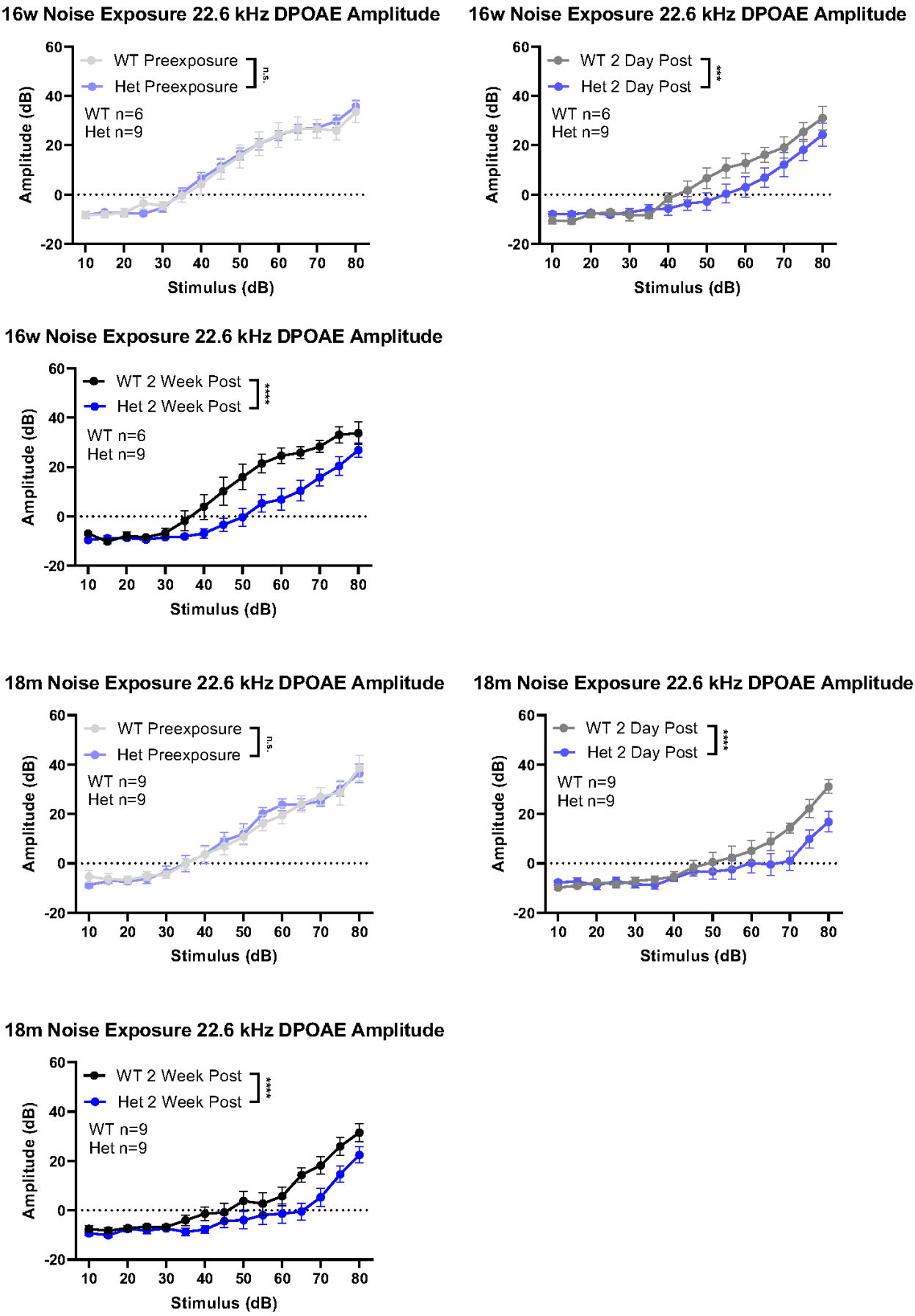
Supplementary Figure 4. ABR peak 1 latency and amplitude measurements in response to 11.2 kHz pure tone stimulation in 12-month-old *Tectb*$^{C225Y/+}$ and wild-type mice. The number of animals per group is indicated within each plot. Data are shown as individual values with the horizontal line representing the mean ± SEM. *Statistical analysis:* Welch's t tests; ns – not significant.



Supplementary Figure 5. TECTB-C225Y protein is detected within the TM. Anti-TECTB (R7) immunolabeling of cochlear cryosections from P80 mice, performed in 4 cochlear locations reveal the presence of anti-TECTB immunoreactivity within the TMs of all three genotypes. These observations indicate that wild-type TECTB and the TECTB-C225Y protein variant are both recognized by the anti-TECTB antibody. *Scale bar* (bottom right), 50 µm.



Supplementary Figure 6. DPOAE peak amplitude growth as a function of sound intensity level for 22.4kHz stimulus measured in WT and *Tectb*$^{C225Y/+}$ mice before noise exposure, 2 days and 2 weeks post-noise. Datasets are grouped to compare the recovery time points. The number of animals per group is indicated within each plot. Data are shown as mean ± SEM. *Statistical analysis:* two-way ANOVA with Tukey's multiple comparisons; ns – not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. For comparison between the genotypes, see *Supplementary Figure 7.*



Supplementary Figure 7. DPOAE peak amplitude growth as a function of sound intensity level for 22.4kHz stimulus measured in WT and *Tectb*$^{C225Y/+}$ mice before noise exposure, 2 days and 2 weeks post-noise, comparing the effect of mouse genotype. The number of animals per group is indicated within each plot. Data are shown as mean ± SEM. *Statistical analysis:* two-way ANOVA with Tukey's multiple comparisons; ns – not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Same data as in *Supplemental Figure 6*.

Supplementary Table 3. List of genes included on the *OtoGenome* hearing loss gene panel

Gene	OMIM #	Gene	OMIM #	Gene	OMIM #	Gene	OMIM #
ACTG1	102560	*GJB2*	121011	*MTTS1*	590080	*SLC17A8*	607557
ADGRV1	602851	*GJB3*	603324	*MYH14*	608568	*SLC26A4*	605646
ATP6V1B1	192132	*GJB6*	604418	*MYH9*	160775	*SLC26A5*	604943
BSND	606412	*GPSM2*	609245	*MYO15A*	602666	*STRC*	606440
CCDC50	611051	*GRHL2*	608576	*MYO1A*	601478	*TECTA*	602574
CDH23	605516	*GRXCR1*	613283	*MYO3A*	606808	*TIMM8A*	300356
CLDN14	605608	*HGF*	142409	*MYO6*	600970	*TJP2*	607709
CLRN1	606397	*ILDR1*	609739	*MYO7A*	276903	*TMC1*	606706
COCH	603196	*KCNE1*	176261	*OTOA*	607038	*TMIE*	607237
COL11A2	120290	*KCNQ1*	607542	*OTOF*	603681	*TMPRSS3*	605511
CRYM	123740	*KCNQ4*	603537	*PCDH15*	604487	*TPRN*	613354
DFNA5	608798	*LHFPL5*	609427	*PDZD7*	612971	*TRIOBP*	609761
DFNB31	607084	*LOXHD1*	613072	*PJVK*	610219	*USH1C*	605242
DIAPH1	602121	*LRTOMT*	612414	*POU3F4*	300039	*USH1G*	607696
ESPN	606351	*MARVELD2*	610572	*POU4F3*	602460	*USH2A*	608400
ESRRB	602167	*MIR183*	611608	*PRPS1*	311850	*WFS1*	606201
EYA1	601653	*MIR96*	611606	*PTPRQ*	603317		
EYA4	603550	*MSRB3*	613719	*RDX*	179410		
GIPC3	608792	*MTRNR1*	561000	*SERPINB6*	173321		

Supplementary Table 2. Other variants identified in the exome sequencing data in the proband.

						Max. MAF gnomAD (v4.1)		All-of-Us							ACMG Criteria/ Classification	In linked interval?	
Gene	Transcript	cDNA (c.)	Protein Change (p.)	Zyg	Read Count (Variant:WT)	AF	AF Population	MAF	SIFT	PolyPhen-2	FATHMM	Mutation Taster	REVEL	CADD			Exclusion criteria
BCS1L	NM_001371449.1	c.670C>T	p.(Arg224Cys)	Het	520 (226:294)	3.1e-5	European (non-Finnish)	8.0e-6	0.00[1]	1.0[1]	-2.61[1]	0.99[1]	0.778[1]	32[1]	PM2_P, PP3_M/ VUS	No	Not in linked interval; Gene is associated with recessive disorders
DIS3L2	NM_152383.5	c.2074A>C	p.(Asn692His)	Het	119 (63:56)	2.2e-5	European (non-Finnish)	Not present	0.03[1]	0.994[1]	0.87[3]	0.33[3]	0.433[2]	28.6[1]	PM2_P/VUS	No	Not in linked interval; Gene is associated with a recessive disorder
GLYR1	NM_032569.4	c.1141C>T	p.(Arg381Cys)	Het	67 (29:38)	3.4e-6	European (non-Finnish)	4.0e-6	0.00[1]	1.0[1]	-0.24[2]	0.80[1]	0.67[1]	32.0[1]	PM2_P, PP3_P/ VUS	No	Not in linked interval; IMPC mouse with normal hearing
ITIH2	NM_002216.3	c.1223T>C	p.(Leu408Ser)	Het	58 (29:29)	2.2e-5	East Asian	4.0e-6	0.03[1]	1.0[1]	-1.49[2]	0.39[3]	0.71[1]	25.4[1]	PM2_P, PP3_P/ VUS	No	Not in linked interval
PLCB2	NM_004573.3	c.431G>T	p.(Arg144Leu)	Het	201 (115:86)	1.7e-4	Admixed American	2.0e-6	0.02[1]	0.954[1]	0.93[3]	0.59[3]	0.30[2]	26.8[1]	PM2_P/VUS	No	Not in linked interval
SLC10A2	NM_000452.3	c.724_725insC	p.(Phe242Serfs*5)	Het	44 (19:25)	0	--	0	--	--	--	--	--	--	PM2_P/VUS	No	Not in linked interval; Gene is associated with a recessive disorder
SMARCA4	NM_001387283.1	c.3355C>T	p.(Arg1119Cys)	Hom	725 (725:0)	8.5e-7	European (non-Finnish)	0	0.00[1]	1.0[1]	-1.07[2]	0.99[1]	0.89[1]	31[1]	PM2_P, PP3_M/ VUS	No	Not in linked interval; Gene is associated with a dominant disorder, phenotype mismatch
STON1	NM_006873.4	c.2167G>A	p.(Gly723Arg)	Het	51 (18:33)	0	--	-0	0.01[1]	1.0[1]	2.94[3]	0.19[3]	0.30[2]	31[1]	PM2_P/VUS	No	Not in linked interval;
*SUPV3L1**	NM_003171.5	c.1925G>A	p.(Ser642Asn)	Het	48 (29:19)	5.0e-5	Admixed American	2.2e-5	0.0[1]	1.0[1]	1.03[3]	0.93[1]	0.47[2]	35[1]	PM2_P	No	Not in linked interval;
TRPV2	NM_016113.5	c.1837C>T	p.(Gln613*)	Het	192 (106:86)	1.7e-5	Admixed American	1.2e-5	--	--	--	--	--	37[1]	PM2_P	No	Not in linked interval; Gene associated with perception to pain caused by heat
UBXN2B	NM_001077619.2	c.274G>A	p.(Ala92Thr)	Het	43 (18:25)	1.7e-5	Admixed American	6.0e-6	0.00[1]	1.0[1]	0.22[3]	0.54[3]	0.55[1]	27.6[1]	PM2_P/VUS	No	Not in linked interval; IMPC mouse with normal hearing
USP3	NM_006537.4	c.11C>G	p.(Pro4Arg)	Het	167 (94:73)	0	--	0	0.00[1]	0.45[3]	-1.81[1]	0.26[3]	0.63[1]	23.8[2]	PM2_P, PP3_P/ VUS	No	Not in linked interval; Unknown function

Pathogenicity is represented as [1]deleterious, [2]neutral, or [3]benign prediction, whereas "--" represents variant not scored.

Abbreviations: IMPC, International Mouse Phenotyping Consortium; VUS, variant of uncertain significance; Zyg, zygosity

*Predicted to cause aberrant splicing

Supplementary Table 1. Risk factor questionnaire

Pedigree ID	Affected Status	Prior Audio	Maternal Infection	Med/Alcohol Exposure	Gentamycin Exposure	Pesticides	Maternal Tobacco	Prematurity	Apnea	Oxygen	Birth Defects	Visual Problems	Hematuria	Neck Mass	Otitis Media	Syncope	Balance Problems	Otorrhea	Treated for Otitis Media	Noise Exposure (TTS)	Occupational Noise
II:1	Unaffected	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	Never	0
II:2	Unaffected	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Never	0
II:3	Unaffected	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	Never	0
II:4	Affected	1	NA	0	NA	0	1	0	NA	NA	0	0	0	0	0	0	1	0	0	Sometimes	0
II:5	Affected	1	NA	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	1	Never	0
III:1	Unaffected	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	Never	1
III:2	Affected	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Never	0
III:3	Affected	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	Never	0
III:5	Affected	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Never	0
III:6	Affected	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	Never	0
III:7	Unaffected	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	Sometimes	0
IV:1	Affected	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*Shorter survey	

Total N	12.0	10.0	12.0	11.0	12.0	12.0	12.0	11.0	11.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	11.0	11.0
Positive	7.0	0.0	0.0	0.0	6.0	1.0	1.0	0.0	0.0	1.0	3.0	0.0	1.0	1.0	1.0	4.0	0.0	1.0	2.0	1.0
Percentage	58.3	0.0	0.0	0.0	50.0	8.3	8.3	0.0	0.0	8.3	25.0	0.0	8.3	8.3	8.3	33.3	0.0	8.3	18.2	9.1

Abbreviations: NA, not answered; TTS, temporary threshold shift