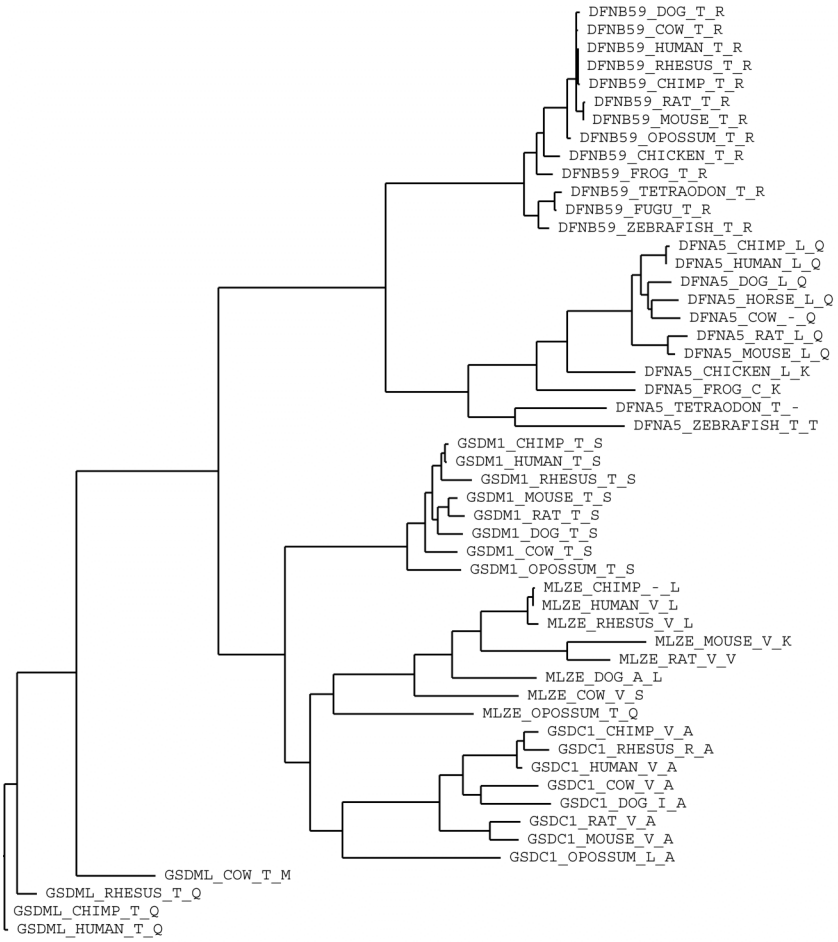

Supplementary Figure 2. Phylogenetic tree showing the evolutionary relationships between pejvakin and the remaining members of the DFNA5-gasdermin-MLZE protein family. Capital letters after the sequence names denote the residues of each protein that align with Thr54 and Arg183 of human pejvakin. None of the 53 pejvakin orthologs harbors a tryptophan in the position equivalent to Arg183 in human pejvakin; similarly, only canine GSDC1 harbors isoleucine in the position equivalent to Thr54.

Figure 2. Audiological characterization of patients with mutations in the *DFNB59* gene. (**a**) Mean audiometric hearing thresholds for all tested members of families 312 (blue circles) and 705 (purple squares). The binaural mean pure-tone averages of thresholds for air conduction at frequencies 0.5-1-2 kHz ($PTA_{0.5,1,2\ kHz}$) are 84.7±10.3 dB (family 312) and 102.2 ±7.4 dB (family 705). (**b**) Click-evoked ABR waveforms at different sound intensities recorded in subject VI-6 of family 312 (black), compared to a control individual (red). The peaks of ABR waves I, III and V are indicated. Note the increased latency of wave V, the only wave that could be recognized in the patient. (**c**) SSOAE records at 3 kHz for patients V-9 of family 705 (top), VI-3 of family 312 (middle), and a control individual (bottom). All of them show preserved SSOAEs. SSOAEs are spontaneous, long-lasting (up to 20 ms) ringing sounds emitted by the cochlea that are synchronized to an external stimulus and recorded by using time-averaging procedures. SSOAEs are so stable in amplitude (up to 20 dB SPL) and frequency that repeated recordings overlap almost completely (blue and red traces in each record). Preserved SSOAEs, regardless of their amplitude, indicate normal function of OHCs responding to the frequencies revealed in the SSOAEs.