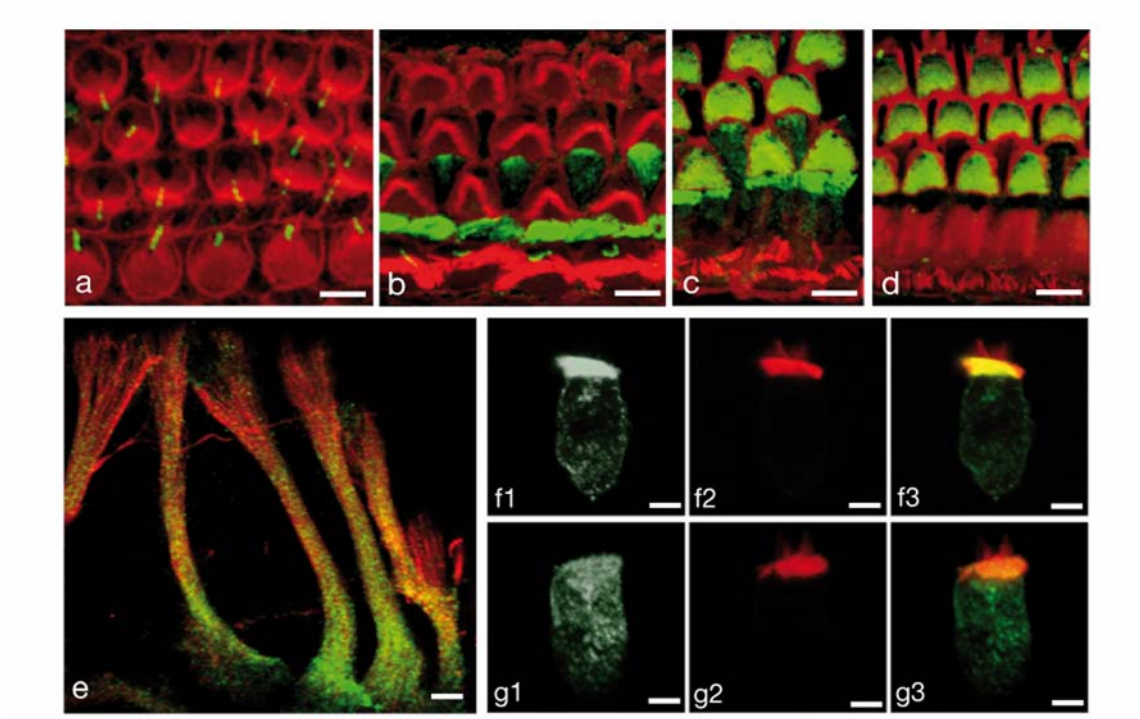

Supplementary Figure 4. Expression of pejvakin in the murine organ of Corti. (**a-d**) Whole-mount preparations of the cochlear duct of wild-type mice at postnatal stages P4 (**a**), P8 (**b**), P12 (**c**), and P30 (**d**), stained for pejvakin (green) and actin (red). In developing IHCs and OHCs, pejvakin staining first appeared in the kinocilium (**a,b**). In OHCs, intense pejvakin staining was apparent from P12 onward in the cuticular plate, the apical filamentous network which supports the hair bundle (**c,d**). Pejvakin staining in IHCs was similar to that observed in the OHCs, but weaker and transient (around P12). Scale bars: 4 μm. (**e**) Pillar cells imaged in a whole-mount preparation of the organ of Corti of P10 wild-type mice, stained for pejvakin (green) and alpha-tyrosinated tubulin (red). Pejvakin expression in both inner and outer pillar cells was detected from P1 onwards. Pejvakin labeling was concentrated in the apical surface and the stalk portion of both pillars, co-localizing with the characteristic array of long, transcellular microtubules spanning all the distance from the apex to the basal footplate. Scale bar: 2.5 μm. (**f-g)** Isolated OHCs from P12 *Dfnb59*$^{+/+}$ (**f1-f3**) and *Dfnb59* $^{tm1Ugds/tm1Ugds}$ (**g1-g3**) mice, stained for pejvakin (green) and actin (red). Note the punctate pejvakin labeling in the bodies of isolated OHCs (**f**). In knock-in mice, pejvakin labeling in the cuticular plate of OHCs (co-localizing with actin) is weaker, which, combined with a brighter pejvakin signal in the cytoplasm, suggests that mutant pejvakin is mislocalized. Scale bars: 2.5 μm.

Figure 4. CONSEQ conservation analysis of human pejvakin based on the alignment of the 53 full-length members of the pejvakin-DFNA5-gasdermin-MLZE family known to date. Colors indicate the degree of conservation of each residue. The putative nuclear localization signal (residues 249-258) and zinc-binding motif (residues 305-331) are underlined. Abbreviations used: b, buried; e, exposed; f, functional; s, structural. Arrowheads indicate the two residues (Thr54 and Arg183) mutated in DFNB59 auditory neuropathy subjects.