

## Dror et al - Supplementary Material

Figure S1. Whole genome linkage analysis of the *loop* mutation. The C57Bl/6 genetic background was used as the outcross strain. A linkage study including low and high resolution mapping was performed on 21 N2 *loop/loop* (C3HeB/FeJ backcross) mutant mice, utilizing polymorphic markers between C57Bl/6 and C3HeB/FeJ inbred strains. Following a whole genome scan, the *loop* mutation was found to be linked to mouse chromosome 12. The linkage interval is flanked by two polymorphic markers *D12Mit215* and *D12Mit112*. The black colored squares refer to linked markers and the white colored squares refer to unlinked markers. N=21.

Figure S2. Pendrin localization in the inner ear is not affected by the S408F mutation. A & B. In the vestibule, pendrin is expressed in the transitional cell layer surrounding the sensory epithelium of the gravity receptors and the cristae. No difference in pendrin expression was found in *Slc26a4<sup>loop</sup>* mutant inner ears. Scale bars equal 25  $\mu$ m in A, B and D. N=4.

Figure S3. Micro-Raman spectroscopy of the aberrant giant minerals in *Slc26a4<sup>loop</sup>* mutants. Normalized Raman spectra of minerals extracted from the saccule of *Slc26a4<sup>loop</sup>* mutants at different developmental stages. A. Spectrum of the mineral extracted from the utricle in newborn *Slc26a4<sup>loop</sup>* mice, showing the typical calcite vibrations at 713 and 1087  $\text{cm}^{-1}$ . B. Spectrum of the mineral extracted from the saccule in *Slc26a4<sup>loop</sup>* mice at 10 months of age. Vibrations at 1478 and 912 are typical of weddelite crystals. N=8.

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