Legends to Supplementary Figures

Supplementary Figure 1. Audiograms of six hearing-impaired members of family 4009. O, solid lines, right air conduction; ×, solid lines, left air conduction; <, broken lines, right bone conduction, unmasked; >, broken lines, left bone conduction, unmasked; ], broken lines, left bone conduction, masked. Line colors match those in Fig. 1A-1B. Ages at time of audiometric testing are indicated per individual.

Supplementary Figure 2. Human ADCY1 sequence aligned with (A) similar proteins from 52 non-human species and (B) eight other human adenylate cyclase proteins. Unchar, uncharacterized protein with similar homology to ADCY1. Both alignments show positions 1037-1053 of human ADCY1, a segment which includes identical (*) or highly conserved (: ) residues. The arginine residue at position 1038 and lysine at 1044 in human ADCY1 are identical in all aligned sequences.

Supplementary Figure 3. Comparison of FM1-43 labeling between adcy1a morphant and wildtype zebrafish revealed no obvious difference in dye uptake. The adcy1a morphant zebrafish has severe morphological defects, including axis curvature and reduced eye and brain size.

Supplementary Figure 4. (A) Western blot, using anti-ADCY1 antibody and protein extract from P17 C57BL/6 mouse inner ear (50 μg), showed a band corresponding to the protein of the predicted size for full-length ADCY1 isoform (~123 kDa). Upper band could represent the ADCY1 protein with secondary modification or additional isoforms of ADCY1. (B) COS-7 cells
transfected with an expression vector containing a cDNA construct for full-length human ADCY1 epitope tagged with myc (red) were immunostained with ADCY1 antibody (green), which revealed complete colocalization (last panel). DAPI (blue) was used to label cell nuclei. Scale bar: 10 μm. (C) The localization of wildtype GFP-tagged ADCY1 using espin-mediated microvillar elongation. COS-7 cells were co-transfected with GFP-tagged ADCY1 and tdTomato-tagged espin constructs. Wildtype ADCY1 was efficiently targeted to the espin-rich microvillar actin bundles at the cell surface. Scale bar: 10 μm.
Wildtype

adcy1a morphant