

SUPPLEMENTARY ONLINE DATA

CNGA3 is expressed in inner ear hair cells and binds to an intracellular C-terminus domain of EMILIN1

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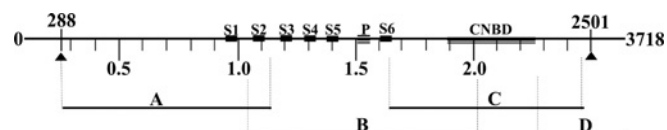


Figure S1 CNGA3 primer positions

Trout hair-cell CNGA3 cDNA sequences were identified through sequential PCR for positions A–D, with nucleotides 288 and 2501 referring to the beginning and end of the start and stop codons respectively. See Table S1 for detailed information on forward and reverse degenerate primers. The PCR products for primer sets CNGA3-A, CNGA3-B, CNGA3-C and CNGA3-D corresponded to Δ of 851 bp (A), 950 bp (B), 816 bp (C) and 740 bp (D), respectively.

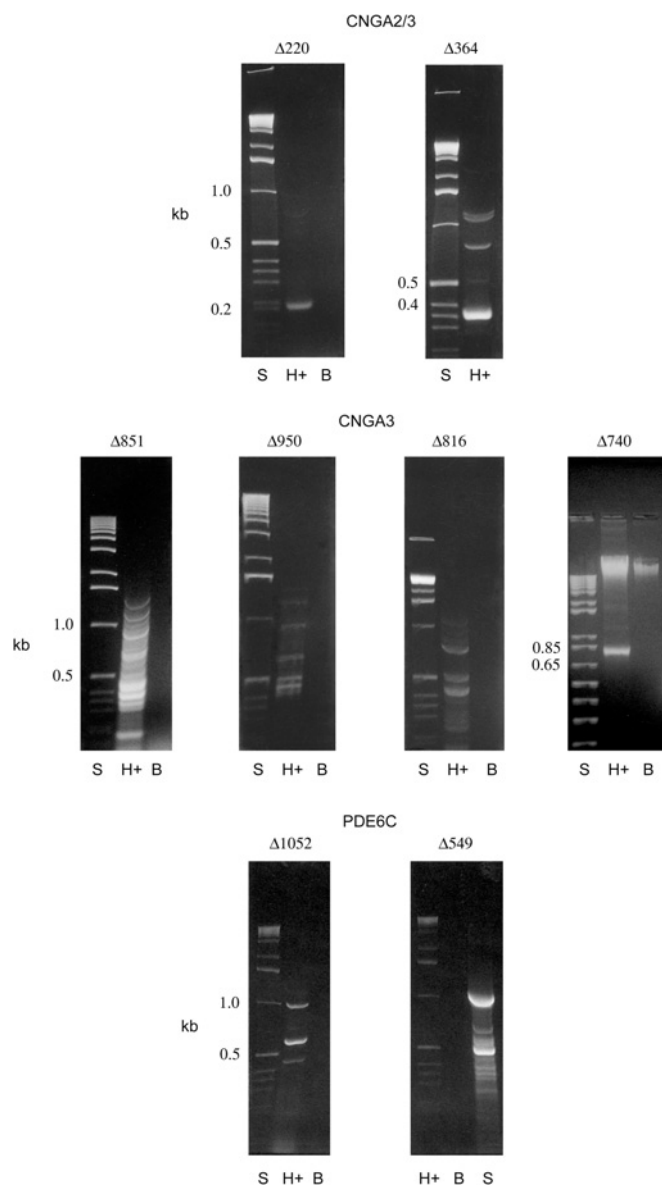


Figure S2 Degenerate primer analysis of expression of CNGA2/CNGA3 and PDE6C in the trout saccular hair cell layer

Degenerate primers CNGA2/3-1 and CNGA2/3-2 (Table S1) directed amplification of products 220 and 364 bp respectively. CNGA3 amplification products from the use of CNGA3-A, CNGA3-B, CNGA3-C and CNGA3-D (Table S1) were 851 bp, 950 bp, 816 bp and 740 bp in length, corresponding in position to A, B, C and D of Figure S1. PDE6C-1 and PDE6C-2 degenerate primers (Table S1) directed amplification of products of 1052 and 549 bp. In all cases, there was no amplification of PCR products in the sample where reverse transcriptase had been omitted or sterile water substituted for cDNA in the PCR reaction (negative controls). Lanes: S, 1 kb or 1 kb plus DNA ladder (Invitrogen) H+, hair cell cDNA; B, water blank as negative control.

Table S1 CNGA3 degenerate PCR primers for trout saccular hair cell layer cDNA

Degenerate primers that are cross-reactive for the nucleotide sequence of olfactory CNG subunits were applied in PCR to trout saccular hair cell cDNA and the products were cloned. Predicted Δ denotes predicted length of PCR product in number of base pairs. The first set of primers (CNGA2/3-1) targeted both CNGA2 and CNGA3 sequences (corresponding to CNGA2 nucleotide-position numbering for catfish, GenBank® accession number M-83111). The second set of primers (CNGA2/3-2) corresponded to the CNGA3 sequence of zebrafish (GenBank® accession number XM_001337717). The primer pairs used to direct PCR amplification of the CNGA3 isoforms (A–D) are shown, with nucleotide position numbering according to GenBank® accession number AF393839 for trout pineal CNGA3 (nucleotide 288 is the first nucleotide in the start codon). Degeneracy in CNGA3 primers was introduced with the use of additional sequence for CNGA3 in zebrafish (GenBank® accession number XM-001337717) and CNGA2 in catfish (GenBank® accession number M83111). Primer positions for PDE6C are numbered according to *Danio rerio* PDE6C, cGMP-specific, cone, alpha prime coding sequence (GenBank® accession number NM_200871). DS, downstream; US, upstream.

| Target | Primers | Nucleotide position | Predicted Δ |
|-----------|------------------------------------|---------------------|--------------------|
| CNGA2/3-1 | 5'-GHKCHATGATYKCYAACATGA-3' | 1094–1114 | 220 |
| | 5'-GSACRTRTRATRGCRATYTC-3' | 1294–1312 | |
| CNGA2/3-2 | 5'-GHKCHATGATYKCYAACATGA-3' | 1222–1242 | 364 |
| | 5'-TRTACATYTCYYKNCCDATRTC-3' | 1563–1584 | |
| CNGA3-A | US: 5'-ATGGCRAAGATYTYGACAGAMC-3' | 0288–0309 | 851 |
| | DS: 5'-CAGCCCCTGTTC AATAACC-3' | 1119–1139 | |
| CNGA3-B | US: 5'-CTSTGGVTDKKBVTGGAYTAY-3' | 1050–1071 | 950 |
| | DS: 5'-TRTACATYTCYYKNCCDATRTC-3' | 1986–2007 | |
| CNGA3-C | US: 5'-GCCATGATYKCYAACRTGAAC-3' | 1647–1667 | 816 |
| | DS: 5'-SCGWYTTCACTKTWKWCTC-3' | 2442–2460 | |
| CNGA3-D | US: 5'-GATGAGGAAATCGCTAATGCAG-3' | 2283–2304 | 740 |
| | DS: 5'-AAGCAGTGGTATCAACGCAGAGT-3' | RACE primer | |
| PDE6C-1 | US: 5'-GARMGWCAAGTTCACAAARGCTY-3' | 759–777 | 1052 |
| | DS: 5'-GTRCCHVKGTGGTCRATATCRGTG-3' | 1787–1810 | |
| PDE6C-2 | US: 5'-GCTSACDGAYATYGARMGWC-3' | 746–765 | 549 |
| | DS: 5'-SWRTTSAGHRCBGACCAKCC-3' | 1275–1294 | |

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