SUPPLEMENTAL DATA

Supplemental Figure 1

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Exon 48   NRPDTAFVWFLNPLKSIKYLICTRYKWLIIKIVLALLGLLMLALFLYSLPGYMVKKLLGA 60
          NRPDT+F+WFLNPLKS +Y +   Y+WL++K +L  L LL+ ALFLYSLPGY+ KK+LGA
Exon 47   NRPDTSFIWFLNPLKSARYFLWHTYRWLLLKFLLLFLLLLLFALFLYSLPGYLAKKILGA 60
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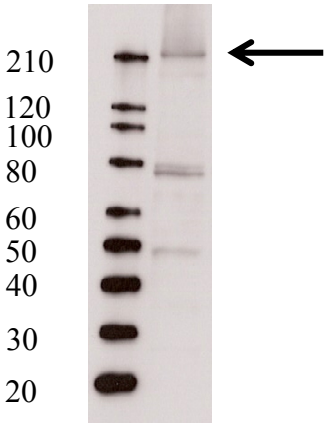
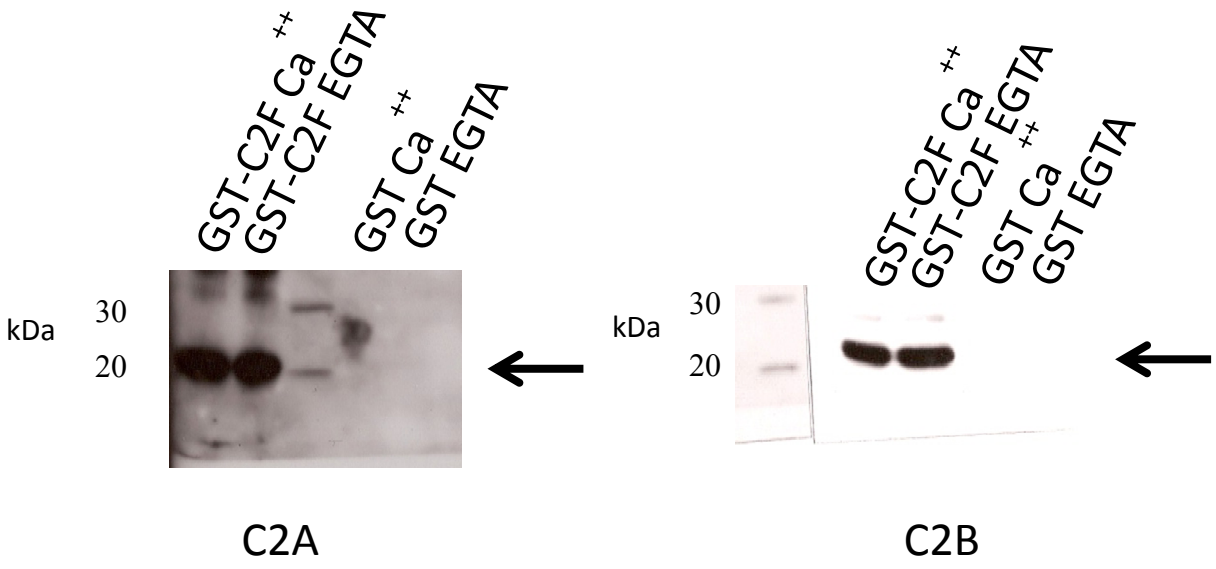
Otoferlin transmembrane domain splice variants share limited identity. The transmembrane domain is coded by exon 48 in cochlea and exon 47 in brain. A 19 amino acid stretch, predicted to form a transmembrane helix (underlined letters), shares only 53% identity between the splice variants, whereas the whole exons 47 and 48, composed of 60 amino acids each, share 63% identity.

Supplemental Figure 2



Western detection of otoferlin protein in rat brain lysate. 10 μg of total protein from rat brain lysate was immunoblotted and reacted with a custom anti-otoferlin primary antibody (1:1,000; see text) and anti-rabbit secondary antibody (Santa Cruz, 1:6,000). Bands were detected by chemiluminescence (Promega). A band above 210 is visible (arrow). The predicted molecular mass for otoferlin is 227 kDa .

Supplemental Figure 3



Otoferlin C2F-C2A and C2F-C2B interactions are independent of calcium. In pull-down assays, GST-C2F was used as a probe to detect otoferlin C2A and C2B interactions. Left (C2F-C2A): Lane 1: 100 µM calcium. Lane 2: 1 mM EGTA. Lane 3: molecular mass standards. Lane 4: GST with calcium, as control. Lane 5: GST without calcium: negative control. The molecular mass for C2A fusion protein is predicted to be 19 kDa (arrow). Bands corresponding to C2A are visible in calcium and EGTA samples with almost equal intensity. Right (C2F-C2B): Lane 1: molecular mass standard. Lane 2: C2F with 100 µM calcium. Lane 3: C2F with 1 mM EGTA. Lane 4: GST with 100 µM calcium. Lane 5: GST with 1 mM EGTA. C2B fusion protein is estimated to be 23 kDa in size (arrow). C2A and C2B fusion products were reacted with anti-Xpress antibody (Invitrogen, 1:5,000) and donkey anti-mouse HRP conjugate secondary antibody (Santa Cruz, 1:6,000) and detected by chemiluminescence (GE Lifesciences).

Supplemental Table 1

Primers	Forward	Reverse	Product size (bp)
Otoferlin C2A	ATGGCCCTGATCGTCCACCTCAAGAC	AGGTAGCAGACCATCTGTCTC	461
C2B	GATCCTGACTCAGTGTCTCTAG	CACGGCGACATCACACTTTACATAG	488
C2C	ACACCCCACAAGGCCAACGAG	GCAACTACACGCTGCTGGACGAG	491
C2D	GCCAAGCTGGAACTCTACC	CCTACTGGCTGCCTTTGAGCTG	560
C2E	CGCATAGTAGGCCGATTCAA	CACTTTGGTCCCCATGGGAGA	563
C2F	CCGTTGCTCAACCCTGACAAG	GTGTCAAAGGCTGGTGGCCCCTC	608
C2F *pachanga*	A GCAGGAGGGCAAACAGGACA	T TGTGTCCTGTTTGCCCTCCTGCTG	608
Syntaxin-1A SNARE	CTATGTTCATGGACATGGCCATGCTG	CTATCCAAAGATGCCCCCGAT	225
Syntaxin-1B	ATGACAAAGCAAGCCCTGAAT	GATATTTCACAGCTTTCTTG	210

Primers for otoferlin C2 domains and syntaxins. Otoferlin C2 domains were identified based on amino acid sequences using the SMART program (smart.embl.heidelberg.de) and primers were designed from upstream and downstream regions of the predicted C2 domains that included at least 40-50 amino acids at the N and C terminal regions to allow proper configuration of the fusion products. C2F *pachanga* primers were designed to create a mutation from aspartate to glycine (Schwander et al. (2007) *J. Neurosci.* **27,** 2163-2175). The upstream primer of C2F and downstream primer of C2F *pachanga*, and upstream primer of C2F *pachanga* and downstream primer of C2F, were paired in a PCR reaction to amplify fragments, which were then used as templates in another PCR reaction using C2F upstream and downstream primers to obtain full C2F sequence with the intended mutation. The resulting C2F *pachanga* product was sequence-verified before cloning and expression. Specific primers were designed based on rat sequences for otoferlin (NM_001276720), syntaxin-1 SNARE domain (AF217191), and syntaxin-1B (NM_012700).