**Supplementary Figure 1: Expression analysis of Ca\(\alpha\)\(\beta\) subunits in IHCs**

Detection of Ca\(\alpha\)\(\beta\)1, Ca\(\alpha\)\(\beta\)2, Ca\(\alpha\)\(\beta\)3, Ca\(\alpha\)\(\beta\)4 and otoferlin (positive control) mRNAs (from top to bottom) by RT-PCR in 8 single WT (left panels) and Ca\(\alpha\)\(\beta\)2\(^{-/-}\) (right panel) IHCs. Expected sizes of the PCR products are 552 bp, 703 bp, 621 bp, 872 bp and 217 bp for
CaVβ1, CaVβ2, CaVβ3, CaVβ4, and otoferlin, respectively. The RT-PCR reaction was also tested by applying brain cDNA (positive control) and by aspirating bath solution next to the IHCs (negative control), as well as reactions without reverse transcriptase (no RT control).

**Supplementary Methods**

**Single IHC RT-PCR**

IHCs from the apical coils of freshly dissected organs of Corti were harvested after cleaning off supporting cells at a high bath perfusion rate (3 ml/min). Each individual IHC was aspirated and the pipette content was transferred into first strand cDNA synthesis mix containing after the dilution: 50mM Tris-HCl, pH 8.3, 75mM KCl, 5mM MgCl2, 5mM DTT, 100 units of SuperScript™ II Reverse Transcriptase (Invitrogen) and 40 units RNaseOUT™ Ribonuclease inhibitor (Invitrogen). Reverse transcription was performed with oligo(dT)primers according to manufacturer’s instructions. Aspirated bath solution was used as a negative control. Each cDNA mix was used as a template for two subsequent PCR reactions with nested primers specific for CaVβ1-4 as used previously (Knirsch, 2007) and otoferlin cDNA. Primer sequences are listed in Table 2.
Table I: Primer sequences used for single cell RT-PCR

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<th>Forward primer 5’-3’</th>
<th>Reverse primer 5’-3’</th>
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<td>CaVβ1</td>
<td>GCCCAAGGACTTCCTACACATCAAGG</td>
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References

Supplementary Figure 2: Experiments at 2 mM extracellular Ca\(^{2+}\) concentration and effects of the dihydropyridine agonist BayK8644.

A, Average $\Delta C_m$ and $Q_{Ca}$ for various depolarization durations in WT (black, n = 11) and Ca\(\gamma\)β\(_2\)\(^{-}/\) (grey, n = 13) IHCs obtained in the perforated patch configuration in 2 mM extracellular Ca\(^{2+}\).

B, I-V relationship for WT (black, n = 11) and Ca\(\gamma\)β\(_2\)\(^{-}/\) (grey, n = 13) IHCs obtained from the last 2 ms of 10 ms depolarizations in 2 mM extracellular Ca\(^{2+}\).

C, I-V relationship in 10 mM Ca\(^{2+}\) for WT IHCs in the presence (grey, n = 8) and absence (black, n=8) of 5 μM BayK8644. The peak Ca\(^{2+}\) current is increased by ~ 60%.
D, I-V relationship as in C for Cavβ2+/− IHCs in the presence (grey, n = 8) and absence (black, n=8) of 5 μM BayK8644. The peak Ca2+ current is increased by ~70%.
Supplementary Figure 3: Colocalization of Ca$$^{2+}$$ hotspots and ribbon synapses

A and B, Representative projections of confocal sections of IHCs following immunolabelling of ribbons (anti-CtBP2, red) and GluR2/3 receptors (anti-GluR2/3, green) obtained from WT (A) and Ca$$\gamma$$β$$\beta$$-/- (B) mice. Scalebar is 10 µm.

C and F: Exemplary Ca2+ microdomains evoked by 390 ms step depolarizations to -14 mV (average of 6 recordings) of a WT (C) and a Ca$$\gamma$$β$$^+$$-/- IHC (F). The cells were patched with 0.4 mM Fluo-5N, 40 µM CtBP2/RIBEYE-staining peptide (Zenisek et al., 2004) and 2 mM EGTA in the patch pipette. In red is displayed the signal from a fluorescently
labeled peptide interacting with the RIBEYE protein, a component of the synaptic ribbon. In green is displayed the change in fluorescence of the Fluo-5N Ca\textsuperscript{2+} indicator caused by depolarization of the cell. Scalebar is 2 \(\mu\)m.

D and E: Separated channels showing the ribbon label (B) and the Ca\textsuperscript{2+} microdomain (C) from the WT cell (A).

G and H: Separated channels showing the ribbon label (E) and the Ca\textsuperscript{2+} microdomain (F) from the Ca\textsubscript{V}\beta\textsubscript{2} \textsuperscript{-/-} cell in (B).

**References**

Supplementary Figure 4: Classification of sound-responsive neurons

A-D, Examples of different types of PSTHs from sound-responsive units of WT (black) and CaVβ2−/− (grey) mice. For WT, representative examples were chosen, whereas for CaVβ2−/− mice, we selected examples most closely matching to the WT data.

E and F, Distribution of spontaneous rates and positions of sound-responsive units as taken from the depth of the recording electrode (starting position approximately 200µm above the surface of the cochlear nucleus) in WT (E) and CaVβ2−/− mice (F).

Supplementary Methods

Classification of unit types

Classification of sound-responsive units in the region of the cochlear nucleus was not as straightforward in CaVβ2−/− mice as in the descriptions of Rhode et al. (1983a, b) and Taberner and Liberman (2005), as spike rates were low, onset responses were often lacking, and distinctive characteristics such as chopping patterns or notches were not well defined. Effectively, we sorted PSTH patterns according to the following criteria:

Primary-Like: Onset response, exponential adaptation to sustained rate. As discussed in Taberner and Liberman (2005), we assume that most of these units (when located in the auditory nerve/deep posteroventral cochlear nucleus area rather than the anteroventral cochlear nucleus) represent auditory nerve fibers, though some contribution from bushy cells cannot be entirely excluded.

Chopper: Onset response, irregular adaptation of sustained response with several peaks. Most other CaVβ2−/− examples showed a less pronounced chopping pattern.

Primary-Like with Notch: Onset response, followed by a notch-like decrease in spike rate and subsequent exponential adaptation similar to Primary-Like units.
Onset: Onset response, sharp decrease to a very low sustained rate. Maximal rates in Ca\(\nu\)\(\beta\)_2\(^{-/-}\) mice were never as high as in WT mice and delayed, sustained rates were relatively high.

In addition, we encountered a relatively large number of units in the cochlear nucleus of Ca\(\nu\)\(\beta\)_2\(^{-/-}\) mice that did not qualify for the above criteria, mostly because they lacked onset responses. These were not further considered (‘atypical’).

References

