vestibule

A. harmonin b
B. F-actin
C. E17
D. harmonin b
E. harmonin b
F. P4

HB

harmonin b
Supl-Fig.5

A. HeLa cells transfected with harmonin b

B. HeLa co-transfected with harmonin b and hEcad-cad23 chimera
**Supplementary data**

**Figure 1, supplementary: harmonin b at the apex of the hair bundle**

(A-C) From E16 onwards, harmonin b labelling (green) becomes progressively restricted at the tips of the F-actin-labeled (red) stereocilia in differentiated vestibular hair cells (arrowheads), as shown at E17.

(D,E) A hair bundle from a vestibular hair cell at P4. Harmonin b is essentially enriched at the apex of the hair bundle (HB). (F) schematic diagram illustrating the stereociliar composition of the hair bundle, and the green dots (arrows) represents the harmonin b labelling at the tips of the stereocilia.

Bars: 5 μm.

**Figure 3, supplementary: F-actin bundling of the CC2-Cter harmonin b fragment.**

(A) In the presence of the untagged harmonin CC2-Cter fragment, F-actin assembles into large bundles. The absence of GST was confirmed by immunoblotting.

(B) F-actin cosedimentation assay. Lanes 1 and 2 are soluble and pellet fractions of GST-CC2-Cter harmonin b obtained upon low speed centrifugation, respectively. No GST-CC2-Cter harmonin fragment is recovered in the pellet fraction (lane 2). The same amount of GST-tagged harmonin CC2-Cter fragment is incubated with F-actin for 30 min at 37 °C (lanes 3 and 4). Upon centrifugation, almost all GST-CC2-Cter harmonin is recovered with F-actin in the pellet fraction.
To further confirm the direct binding of harmonin b to F-actin, glutathione-sepharose beads (GB) coupled with GST or GST-harmonin CC2-Cter fragment were mixed with 10 µM bundled F-actin. After three washes in high salt buffer, F-actin was then visualised using rhodamine-phallodin (Molecular Probes). F-actin (red) binds to glutathione-Sepharose beads coated with the GST-CC2-Cter harmonin fragment (C) but not to beads coated with GST alone (D). Right panels are higher magnification views of the areas boxed in C, and D, respectively.

Bars: 5 µm in A, 20 µm in (C,D).

**Figure 5, supplementary: Harmonin b and hEcad-cad23 in HeLa cells.**

(A) In HeLa cells producing harmonin b, almost all harmonin b labelling (red) associates with long F-actin labelled filaments (green) that spreads throughout the cell cytoplasm. No harmonin b enrichment is seen at the cell-cell contacts (arrowheads).

(B) In contrast, in HeLa cells producing hEcad-cad23 and harmonin b, the harmonin b labelling is enriched at the cell-cell contacts, where it entirely colocalises with the hEcad-cad23 chimeric protein (arrowheads). The presence of hEcad-cad23 clearly modifies the harmonin b-actin pattern, *i.e.* long harmonin b-bound curvy filaments (seen in A) changed into punctuated labelled structures (in B), thus suggesting an association between harmonin b and cadherin 23.

Bars: 10 µm
Figure 6, supplementary: Harmonin interacts with cadherin 23 and with myosin VIIa.

(A) Schematic representation of the yeast two-hybrid harmonin preys isolated using the cadherin 23 cytodomain (aa 3086-3354) as the bait. The inner ear two-hybrid cDNA library was repeatedly screened to saturate the library. In each screen, performed as described (Fromont-Racine et al., 1997), a theoretical 95% coverage of the inner ear two-hybrid cDNA library was reached to minimise the rate of false negatives. Then, obtention of more than two independent clones for a given prey is a good argument for the specificity of the interaction. Eighteen independent harmonin clones (out of 25) were obtained for the cadherin 23 cytodomain. This indicates that harmonin is likely one of the major partners of cadherin 23.

(B) Harmonin b colocalises with myosin VIIa and F-actin in co-transfected HeLa cells.

Harmonin b (revealed by H1b) colocalises perfectly with the myosin VIIa tail (revealed by a monoclonal anti-myosin VIIa). Similarly to the hEcad-cad23 chimera, the presence of the myosin VIIa tail prevents the formation of long, curvy, harmonin b-bound actin filaments.

(C) Yeast two-hybrid harmonin preys obtained using the SH3-MyTH4-FERM domains of myosin VIIa (aa 1562-2215) as the bait. Six independent clones of harmonin (out of 7) were obtained for the myosin VIIa C-terminal tail.

Grey boxes indicate the interacting domain deduced from the prey clones.

Bar: 20 µm.
Reference: