



Additional file 1

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Phenotypic analyses in *ha* mutant. (A-F) Whole-mount *in situ* hybridization of molecular markers expressed in the OVs. (A)(B) Differentiation markers, *eya-1* (across the vesicles) and *pax-2* (medial region) are normally expressed in *ha* embryos. All OVs are dorsal views. (C)(D) Normal expression patterns of marker genes at stage st. 30. *dlx-3b* is restricted to the dorsal wall of the OV and *bmp-4* marks the neural cristae regions, showing that regionalization and neural differentiation patterns are not disturbed in *ha* mutant. Mutant OVs are enlarged. ac, anterior cristae; lc, lateral cristae; pc, posterior cristae. All OVs are lateral views. Left side shows anterior. (E)(F) The transcripts of otolith matrix proteins, *starmaker-like* (st. 22) and *sparc-1* (st. 24) are comparably detected in the mutant OV. All OVs are dorsal views. Mutant OVs are enlarged at st. 24. (G) TEM images from a *wt* specimen and a mutant one (representative images are shown in G to G'''). Asterisks: growing otolith precursors; Arrows: fine substances; 's': seeding particles; 'g': globules. (H) Immunohistochemistry for OMP-1 revealed crystalized 'otolith' of *ha* in hatching stage (st. 40) that looks similar to *wt* otolith and contains at least some organic materials. (I) double colored-Immunofluorescence of acetylated  $\alpha$ -tubulin and  $\gamma$ -tubulin (root of cilia). Many short cilia ( $<1\mu\text{m}$ ) are visible in the OV of *ha* as well as *wt*. (J) Scatter plot showing total number of cilia in the OV of *wt* and *ha* at various stages. Total number of cilia in the OV is obtained by counting the signal of  $\alpha$ -tubulin (cilia) and  $\gamma$ -butulin (root of cilia). In each stage, there was no significant difference in total number of cilia between *wt* and *ha* ( $>0.05$  Student's t-test). (K) Otolith development in the OV of *ktu* mutant. In *ktu*, seeding particles are coalesced at the prospective macula region (*Upper*; dorsal views of left OV), and otoliths appear normal at later stage just like *wt* (*Middle*; dorsal views of left OV and *Lower*; lateral views of left OV). Asterisks: otoliths, Arrow: paste-like substance