

# Supplementary Information

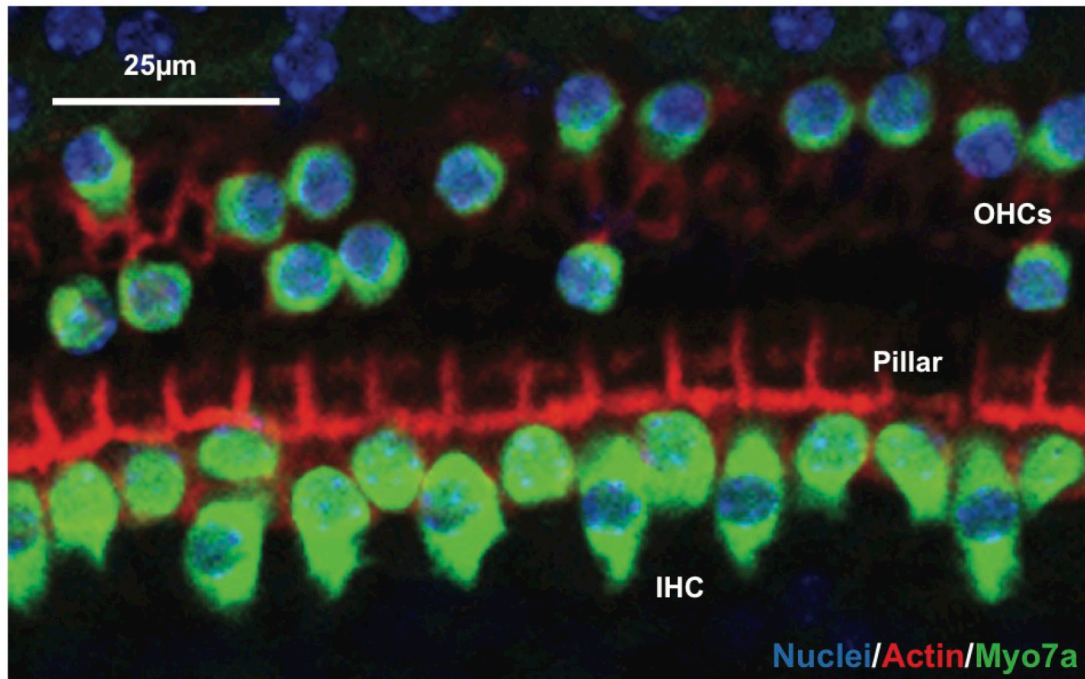
## Susceptibility of outer hair cells to cholesterol chelator 2-hydroxypropyl- $\beta$ -cyclodextrine is prestin-dependent

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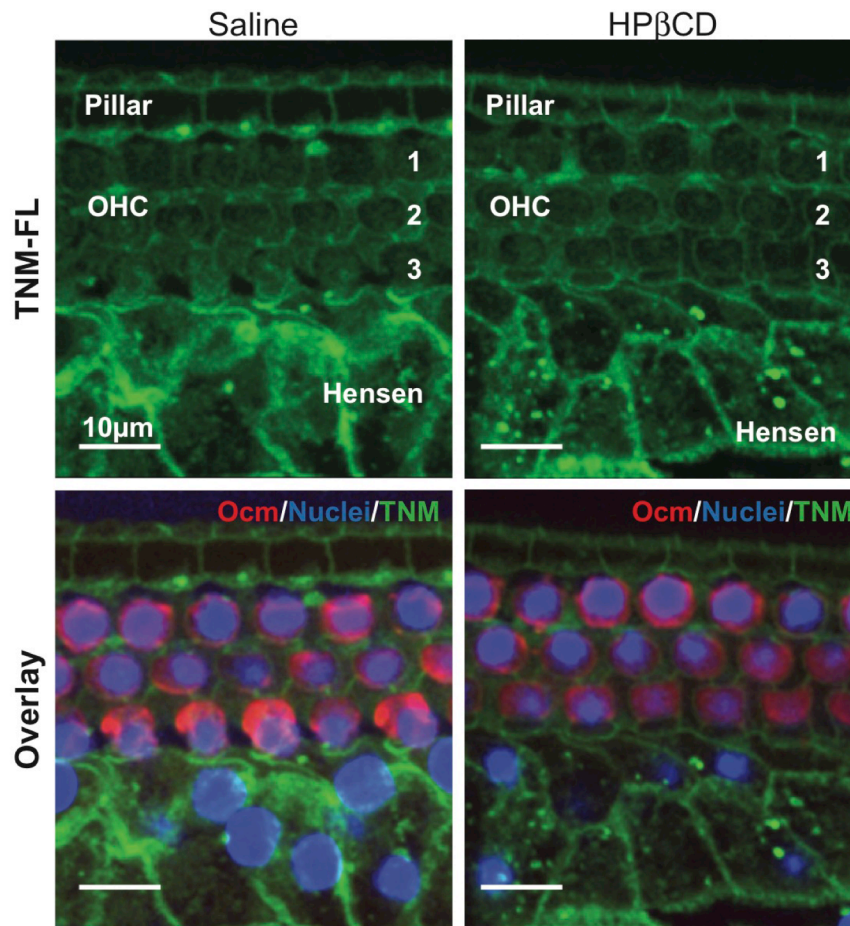
Prestin-KO (Mid-Base)



**Supplemental Fig. 1. Only OHCs are damaged by HP $\beta$ CD treatment.**

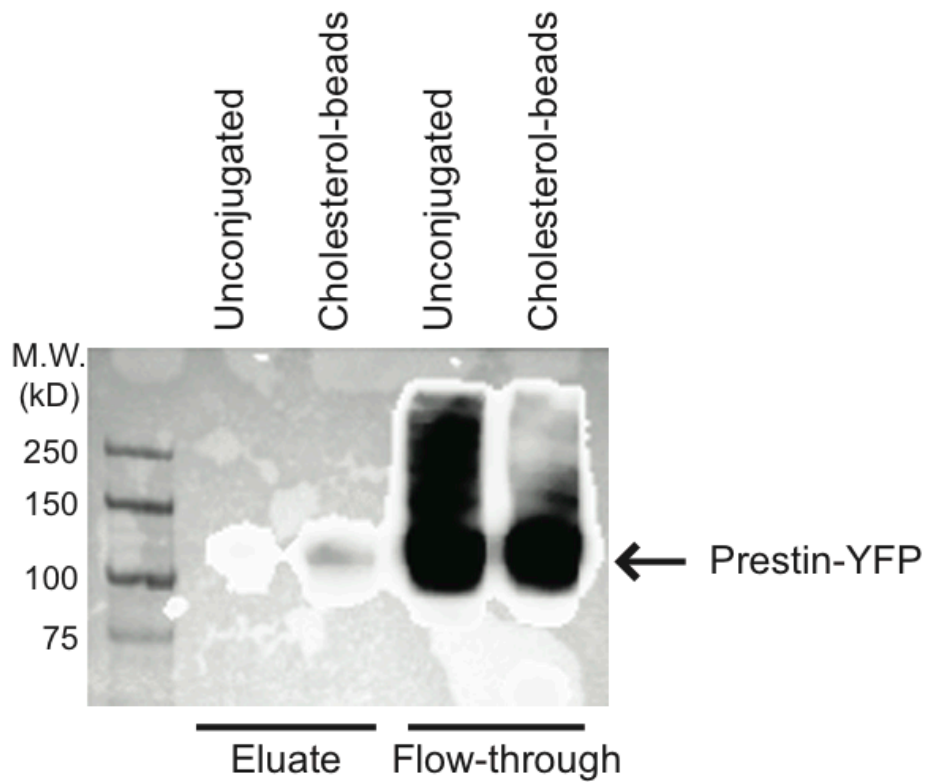
Representative image of HP $\beta$ CD-treated cochlea from prestin-KO mice, stained with anti-Myosin 7a for IHCs and OHCs, Phalloidin-Alexa 546 for actin, and Hoechst 33342 for nuclei. All the IHCs and pillar cells remained intact while some OHC loss occurred in the mid-to-basal region. Scale bar, 25 $\mu$ m.

Prestin-KO (Mid-Base)



**Supplemental Fig. 2. Cholesterol staining of prestin-KO cochleae.**

Comparison of saline- and HPβCD-treated prestin-KO cochleae at the mid-to-basal region. An HPβCD-treated OC exhibits similar TNM-FL staining intensities as in the saline control. Red, anti-oncomodulin; Green, TNM-FL; Blue, Hoechst 33342 for nuclei. Scale bars, 5μm.



**Supplemental Fig. 3. Pull-down assays using stable HEK293-prestin-YFP cells.** Cell lysates containing membrane fractions were prepared from HEK293 cells stably expressing prestin-YFP, and incubated with unconjugated control beads or cholesterol-conjugated resins, separated on SDS-PAGE and probed with anti-GFP antibody. Prestin-YFP was pulled-down with cholesterol-beads (Lane 2) but not with unconjugated control beads (Lane 1). The flow-through data (Lanes 3 and 4) showed that the unbound prestin-YFP in both unconjugated control and cholesterol-beads conditions.

**Supplemental Movie 1. Time-lapse images of HP $\beta$ CD-treated WT OHCs.**

OHCs from prestin-WT were isolated and placed on a glass-bottom 35mm dish. HP $\beta$ CD was added to the final concentration of 1 mM at t=0 (0:00), and imaged at 4 sec intervals for over 5 min. Scale bar, 5 $\mu$ m.

**Supplemental Movie 2. Time-lapse images of HP $\beta$ CD-treated WT OC. Sense**

organs from WT mice were dissected and placed on a glass-bottom 35mm dish. HP $\beta$ CD was added to the final concentration of 10 mM at t=0 (0:00), and imaged at 4 sec intervals for over 12 min. Scale bar, 25 $\mu$ m.

**Supplemental Movie 3. Time-lapse images of HP $\beta$ CD-treated prestin-KO**

**OC.** Sense organs from prestin-KOs were dissected and placed on a glass-bottom 35mm dish. HP $\beta$ CD was added to the final concentration of 10 mM at t=0 (0:00), and imaged at 4 sec intervals for over 12 min. Scale bar, 25 $\mu$ m.