

Fig. S1. Normal junctional E-cadherin localization in *Lis1*^{cKO-early} hair cells. E-cadherin immunostaining (red) in E18.5 control (A) and *Lis1*^{cKO-early} (B) organ of Corti at the level of the adherens junctions. Arrowheads mark the pillar cell row and brackets indicate outer hair cell rows. Scale bars: 6 μ m.

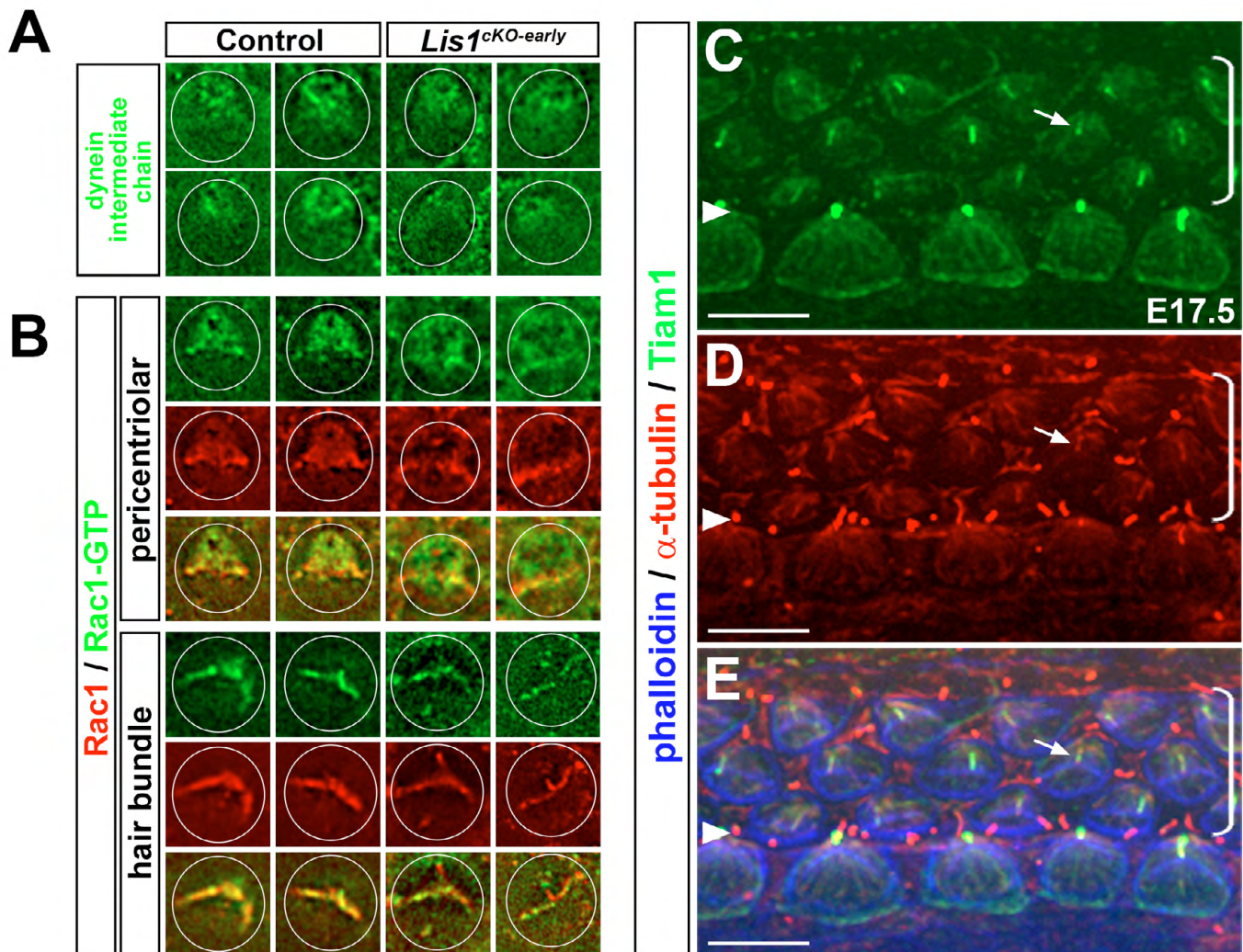


Fig. S2. Subcellular localization of dynein, Rac1-GTP and Tiam1 in hair cells at E17.5. (A) Dynein intermediate chain immunostaining (green) in individual control (left) and *Lis1*^{cKO-early} (right) hair cells. Dynein in control hair cells is enriched in the pericentriolar region. In *Lis1*^{cKO-early} hair cells, dynein staining in the pericentriolar region is more diffuse. (B) Rac1-GTP (green) and Rac1 (red) immunostaining in individual control (left) and *Lis1*^{cKO-early} (right) hair cells. Each panel is an optical section taken at the level of the hair cell centrosome or the hair bundle as indicated. White circles mark individual hair cell boundaries. (C-E) Tiam1 (green) and α -tubulin (red) immunostaining in a wild-type organ of Corti. Tiam1 is enriched on the hair cell kinocilium (arrows). Arrowheads mark the pillar cell row and brackets indicate outer hair cell rows. Scale bars: 6 μ m.

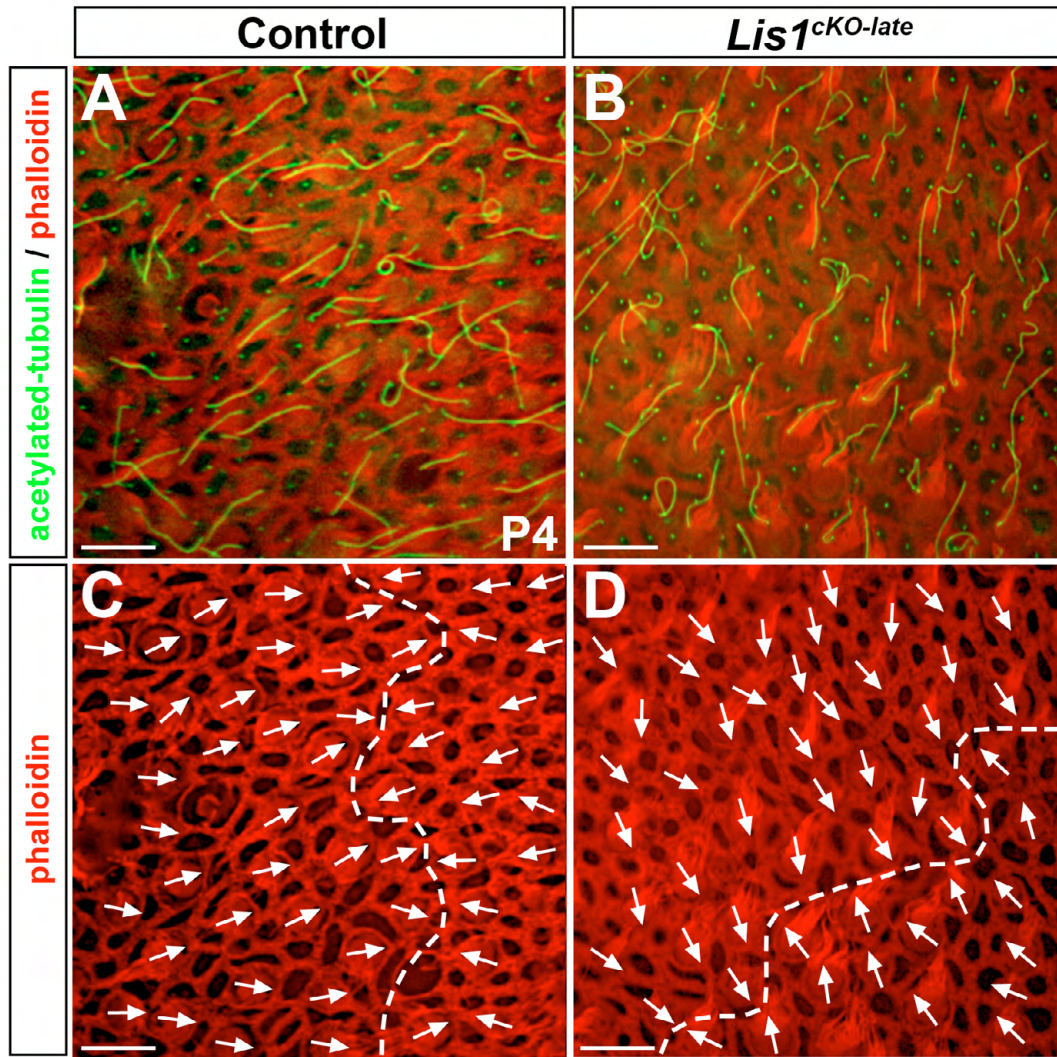


Fig. S3. Normal hair bundle morphology and polarity in the *Lis1*^{cKO-late} utricle. Acetylated-tubulin (green) and phalloidin (red) staining in P4 control (A,C) and *Lis1*^{cKO-late} (B,D) utricles. (A,B) Hair bundle morphology and kinocilium position are normal in *Lis1*^{cKO-late} utricular hair cells (B) compared with controls (A). (C,D) Planar polarity of hair cells appears normal in *Lis1*^{cKO-late} utricles (D) compared with controls (C). Panels show z-sections taken at the level of the cuticular plate. Arrows indicate planar polarity as revealed by actin staining of the cuticular plate. Dashed lines indicate the line of reversal. Scale bars: 6 μ m.

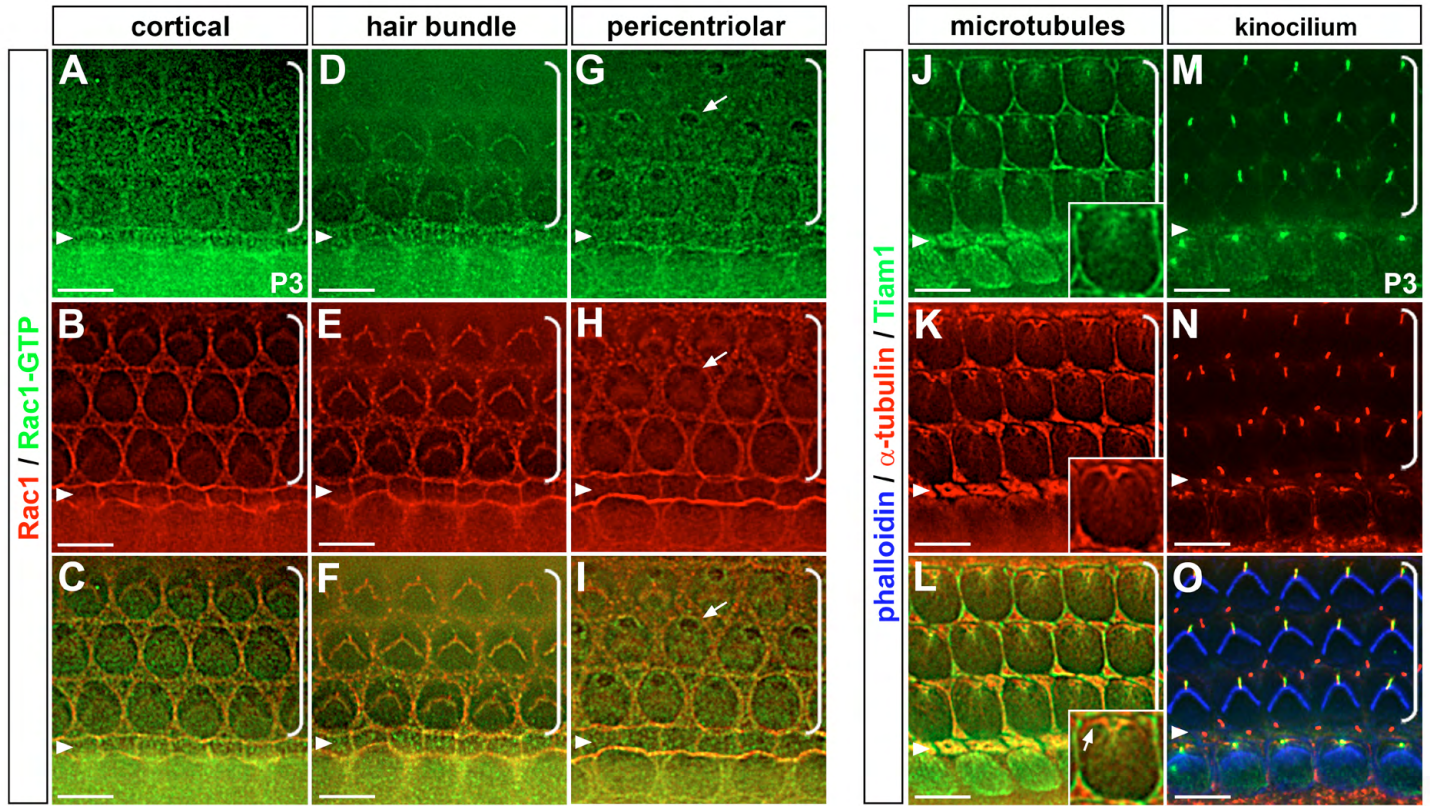


Fig. S4. Rac1-GTP and Tiam1 localization in wild-type organ of Corti at P3. (A-I) Rac1-GTP (green) and total Rac1 (red) immunostaining at the level of Dvl2 cortical crescents (A-C), the hair bundle (D-F) and the pericentriolar region (G-I). (J-O) Tiam1 (green) and α -tubulin (red) immunostaining at the level of centriolar microtubules (J-L) and the kinocilium (M-O). Tiam1 localization on microtubules is restricted to the pericentriolar region (J-L); bundled microtubules along the cell cortex lack significant Tiam1 staining (insets, arrow). Arrowheads mark the pillar cell row and brackets indicate outer hair cell rows. Scale bars: 6 μ m.