Changes in cell adhesion properties are associated with the formation of fluid-filled spaces in an epithelium.

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Abstract (200 words)

The sensory epithelium of the cochlea, the organ of Corti, has complex cytoarchitecture consisting of mechanosensory hair cells intercalated by support cells. The support cells provide important trophic and structural support to the hair cells and must be stiff yet compliant enough to modulate vibrations to the organ of Corti. Once the sensory cells are properly arranged, the support cells undergo significant remodeling to help the sensory epithelium transition from a simple epithelium into a structurally rigid epithelium with fluid-filled spaces. Cadherins are cell adhesion molecules that are necessary for sorting and connecting cells in an intact epithelium. To create the fluid-filled spaces, the cell adhesion properties of adjoining cell membranes between cells must change to allow the formation of spaces within an epithelium. However, the dynamic localization of cadherins has not been properly analyzed as these spaces are formed. There are three cadherins that are reported to be expressed during the first postnatal week of development when the tunnel of Corti forms in the cochlea. We characterized the dynamic localization of cadherins that are associated with the cytoskeletal remodeling at the contacting membranes of the inner and outer pillar cells flanking the tunnel of Corti.

1. Introduction

Epithelial morphogenesis requires complex cytoskeletal remodeling and maturation [1,2] and is essential for proper organ formation and organ function. Cadherin-mediated cell adhesion provides epithelial stability during morphogenesis by inducing cell-cell contact-mediated mechanical coupling with the cellular actin cytoskeleton [2,3]. Cadherins are Ca^{2+}-dependent cell-cell adhesion molecules that lead to the formation of adherens junctions during tissue development [4]. Recruitment of cadherin molecules at the membrane is followed by cadherin clustering and is stabilized by coupling to the actin cytoskeleton [5-7]. Maturation of adherens junctions and strengthening of cell-cell adhesion further induces tension-dependent actin remodeling and recruitment of actin binding proteins to the site of de novo actin synthesis [8,9].

In the cochlea, auditory function is also reliant on proper epithelial morphogenesis of the sensory epithelium, the organ of Corti. The organ of Corti attains a highly specialized organization of mechanosensory hair cells intercalated by support cells [10,11]. Thus far, most studies have focused on the cytoskeleton and cytoskeletal remodelers in the sensory hair cells; but recent studies have shown that the support cells have important electrophysiology, metabolic

58 and sensor properties that are necessary for proper auditory function [12-14]. The support cells also
59 serve as important support structures for the hair cells [15] and help to establish the overall
60 morphology and complex cytoarchitecture of the sensory epithelium. The organ of Corti must be
61 stable enough to withstand vibrational movements of the basilar membrane [14,16] and the
62 movement of fluid through the epithelium [17-19] to facilitate the propagation of sound information.
63 During sensory epithelial morphogenesis, fluid-filled spaces such as the tunnel of Corti, the Nuel
64 space and the outer tunnel are formed within the epithelium [20], which requires stabilization of the
65 cytoskeletal structure. Between late embryonic and early postnatal stages, the support cells
66 undergo significant dynamic morphological changes. However, few studies have uncovered the
67 spatiotemporal characteristics of cytoskeletal modifications on the cochlear sensory epithelium
68 and how the tunnel of Corti is formed during maturation. In this study we focus on the
69 cytoskeletal changes that the support cells undergo and the cell adhesion modification that occur
70 during a specific time in development when fluid-filled spaces form in the sensory epithelium,
71 particularly the pillar cells (PCs) that undergo the most drastic structural cytoskeletal remodeling
72 to form the tunnel of Corti. Since epithelial integrity in the cochlea is dependent on cadherins [21],
73 we also examine whether the cellular spatial localization of cadherins play a role in establishing
74 the integrity of the sensory epithelium with spaces interspersed within the cochlear sensory
75 epithelium.

76 Three cadherin molecules reported to be expressed in the mouse cochlea during the first
77 postnatal week are neural (N-), epithelial (E-), and placental (P-) cadherins [22-24]. The tunnel of
78 Corti forms between the inner pillar cells (IPC) and the outer pillar cells (OPCs), spaces of Nuel
79 and the outer tunnel [24]. A recent study suggested that the abnormal premature enlargement of the
80 tunnel of Corti was caused by loss of P-cadherin [22]; however, from wholemount analysis, P-
81 cadherin localization in the PCs appears to be apical. Cross-sectional views of cadherin
82 localization along the apico-basolateral axis during the narrow temporal window as the tunnel of
83 Corti forms would provide a clearer view of how E-cadherin or N- or P- cadherin localization
84 influence tunnel formation under normal developmental conditions. In this study, we analyze the
85 spatiotemporal localization of these three cadherins in the cochlea.

86

87 **2. Results and Discussion**

88 At neonatal stages of the mouse cochlea, the organ of Corti has already established its
89 classical mosaic pattern of hair cells intercalated by support cells (Figure 1). There are two
90 classes of mechanosensory hair cells: one row of inner hair cells (IHCs) and three rows outer hair
91 cells (OHCs) (Figure 1A) that are innervated by afferents and efferent neurons enabling the
92 transmission of auditory information along the central auditory pathways [25,26]. The inner and
93 outer hair cells are segregated by the IPC and OPC support cells. The outer hair cells are
94 intercalated by a specific subtype of support cells known as Deiters' cells (DCs) (Figure 1A, A').
95 A prior study showed an increase in junctional E-cadherin in the apical junctions of the PCs in
96 wholemount mouse cochleas during the first postnatal week after birth [27]. Thus, E-cadherin
97 signaling is a strong candidate for PC remodeling. Since this study focuses on the support cells,
98 the cross-sectional view of the organ of Corti reveals structural details (Figure 1A, A') that are
99 difficult to appreciate in wholemount cochlear preparations where the stereocilia on hair cells
100 and the overall patterning of the sensory epithelium are the prime focus (Figure 1B).

101 A tunnel of Corti forms between the IPC and the OPC during the neonatal ages of
102 cochlear development; however, a close examination of the spatial changes of the F-actin
103 cytoskeleton and cell adhesion of the cochlea undergoing extensive remodeling and the tunnel of
104 Corti opening, has not been performed. Although the F-actin cytoskeleton establishes the
105 structure and shape of cells, its enrichment can vary in different cell types. In order to identify
106 the correct stage to observe the opening of the tunnel of Corti and characterize the associated
107 subcellular changes in the F-actin cytoskeletal organization, we first analyzed cochlear tissue on
108 E18.5, P6 and P8. On E18.5, although F-actin is present in all cells, Phalloidin labeling shows
109 that F-actin is specifically enriched at membranes contacting both the OHCs and the DCs (Figure
110 2A). Thus, we examined markers for cell adhesion along the OHC-DC membrane contacts.
111 Comparing F-actin with the cell adhesion protein, E-cadherin, we found a striking enrichment of
112 E-cadherin at OHC-DC membrane contacts (Figure 2B). On E18.5, E-cadherin was also
113 localized to the membrane of Hensen's cells (Figure 2B, green arrow). At this stage, there was
114 no marked enrichment of F-actin or E-cadherin in the PCs. Cell adhesion induces bi-directional
115 signaling that promotes the recruitment of phosphorylated actin binding proteins at their tyrosine
116 residues (pTyr), a sign of active actin assembly [28,29]. Immunolabeling for pTyr was undetectable
117 on E18.5, presumably because minimal remodeling of the F-actin cytoskeleton was required at
118 this stage of development (Figure 2C).

119 We next analyzed cochleas on P6. The tunnel of Corti was still not formed at this stage.
120 However, F-actin assembly is enhanced, particularly in the IPC and the IPC-OPC membrane
121 contacts (Figure 2D, arrow). E-cadherin was also localized at the adjoining IPC-OPC
122 membranes, but E-cadherin was concentrated mainly in the apical and mid-regions along the
123 apico-basolateral axis of the IPC-OPC membranes, indicating there was an increase in cell
124 adhesion contacts associated with the increase in F-actin (Figure 2E, arrow). However, there was
125 no apparent increase in the recruitment of pTyr actin binding proteins at the IPC-OPC
126 membranes (Figure 2F).

127 We then performed the same analysis in the P8 cochlea. At this stage, the tunnel of Corti
128 has fully formed (Figure 2G-I). The F-actin bundle that establishes the rigid support structure
129 within the IPC is much more developed (Figure 2G, green arrow) compared to the P6 stage.
130 However, by P8, F-actin, E-cadherin were no longer localized in the mid-regions of the IPC-
131 OPC contact membranes where the tunnel of Corti and Nuel space were formed. F-actin and E-
132 cadherin remain enriched in the adjoining apical IPC-OPC membranes (Figure 2G-H). Despite
133 the formation of the tunnel of Corti, the IPC and the OPC remain connected at their apical
134 junctions where E-cadherin remains localized (Figure 2H). P-Tyr labeling was not present in the
135 PCs at P8, suggesting that a critical window was missed. The time interval between P6 and P8
136 was too large to observe the transient cytoskeletal modifications associated with the formation of
137 the tunnel of Corti.

138 To capture finer temporal resolution of the opening of the tunnel of Corti, we analyzed P7
139 cochleas that were harvested at 3 timepoints throughout the day at 9 am, 1 pm and 5 pm (Figure
140 3). At the first timepoint, the tunnel had not yet opened (closed). We observed an accumulation
141 of F-actin at the mid-region of the IPC-OPC membranes (Figure 3A, arrow) along their apico-
142 basolateral axis, which will go onto to become the axial regions of the PCs that flank the tunnel
143 of Corti. Coincidently, we also saw an increase in E-cadherin cell contacts (Figure 3B, arrow).
144 and pTyr labeling (Figure 3C, arrow). At this stage, F-actin, E-cadherin and pTyr co-localize at
145 the mid-region of the IPC-OPC membranes (Figure 3C' inset, arrow). This is a notable
146 enrichment compared to the P6 when the tunnel was also closed but no pTyr labeling was
147 detectable (Figure 2D-F). P-Tyr labeling was comparatively less pronounced at the apical
148 junction (Figure 3C), which suggests that there comparatively less active F-actin assembly on the

149 apical junctions of the PCs. There was less also reduced F-actin, E-cadherin and pTyr in the
150 basolateral end of the PCs (Figure 3B-C). We also observed a notable increase of pTyr when the
151 Nuel space has a very narrow opening, which suggests a significant recruitment of actin binding
152 proteins during initiation of Nuel space formation (Figure 3C, red arrow). At the mid-timepoint
153 on P7, the tunnel of Corti was open but small (small ToC). F-actin and E-cadherin have already
154 decreased their expression in the mid-axial region of the IPC-OPC membranes but remain
155 enriched at the apical junction (Figure 3D-E). Interestingly, pTyr labeling remained high at the
156 edges of cell membranes (Figure 3F). This suggests that these sites remain enriched for actin
157 binding proteins which help remodel cellular structure of the tunnel. In the final timepoint on P7,
158 the tunnel was significantly larger (large ToC). F-actin and E-cadherin remained at the apical
159 regions of the PCs (Figure 3G-H); while pTyr labeling was relatively minimal at the apical
160 junctions (Figure 3I). This suggests that there is a greater recruitment of pTyr-labeled actin
161 binding proteins where the cochlear spaces are forming and not at stable contacts. The variable
162 localization of E-cadherin on the adjoining IPC-OPC membranes along the apico-basolateral axis
163 suggests that a reduction in cell adhesion precedes the opening of the tunnel of Corti at the mid-
164 region of the PC membranes and is accompanied by a large recruitment of pTyr-labeled proteins.
165 This data also show that P7 is critical timepoint when the support cells in the sensory epithelium
166 undergo significant cellular remodeling to attain its complex cytoarchitecture. Conversely,
167 stabilized F-actin will require less recruitment of actin binding proteins as there are no sites for
168 de novo F-actin synthesis e.g., in the apical junctions of the PCs.

169 N-cadherin is another cadherin known to be expressed in the developing cochlea [23]. We
170 examined whether N-cadherin is spatially positioned to influence the opening of the spaces in the
171 cochlear sensory epithelium. On P6, N-cadherin is visible in the inner hair cell (IHC), inner
172 phalangeal and inner border cell, but is clearly absent in the PCs and the DCs (Figure 4A). This
173 expression pattern remains consistent in P7 cochleas at the same three timepoints when the
174 tunnel of Corti was still closed (Figure 4B), has a small opening (Figure 4C) and when the tunnel
175 was fully open (Figure 4D). Thus, the stable expression of N-cadherin and its consistent absence
176 of expression in the PCs suggests that N-cadherins do not influence the opening of the tunnel of
177 Corti.

178 A recent study showed that P-cadherin is expressed in the PCs of the cochlea during early
179 postnatal stages. Upon Thyroxine administration, P-cadherin was translocated from the PC
180 membranes to the cytoplasm, thereby reducing cell-cell adhesion [22]. The study analyzed
181 wholemount cochleas; thus, the expression of P-cadherin along the entire apico-basolateral axis
182 of PCs was unclear. To determine its spatial localization, we analyzed P-cadherin expression. On
183 P6, P-cadherin was localized in the IPCs, but enriched at the top apical end of the IPC (Figure
184 5A, white arrow). In addition to the PCs, we found P-cadherin was also expressed in the
185 membranes of the Hensen's cells and the cells in the outer sulcus region (Figure 5A, green
186 arrow). The expression of P-cadherin was much more substantial in these lateral non-sensory
187 cells compared with E-cadherin. On P7, while the tunnel of Corti was still closed, P-cadherin
188 remained enriched in the apical portion of the PCs, the Hensen's cells and the outer sulcus cells
189 (Figure 5B). The apical spatial localization remained unchanged while the tunnel of Corti opened
190 (Figure 5C-D). The expression of P-cadherin remained stable in the Hensen's and outer sulcus
191 cells as well.

192 We found that there was significant recruitment of pTyr labeled proteins co-localized
193 along with F-actin and E-cadherin at IPC-OPC membrane prior to the opening of the tunnel of
194 Corti (Figure 3). We postulated that in preparation for the tunnel opening, actin binding proteins
195 are recruited to facilitate the remodeling of F-actin in the PCs. Thus, we examined the expression
196 of Cofilin, an F-actin severing protein, on P6 and the three stages of tunnel of Corti formation on
197 P7 (Figure 6). Previous studies showed cofilin labeling in the HCs [30,31], but Cofilin localization
198 in the SCs has not been reported. Thus, we were surprised to find a much higher punctate
199 labeling of Cofilin in the SCs compared to the HCs. Relative to P6 (Figure 6A), immunolabeling
200 shows an increase in Cofilin localization in the mid-region of the IPC-OPC junction of the closed
201 P7 closed tunnel of Corti (Figure 6B, arrow). This suggests that during this critical period,
202 Cofilin recruitment is associated with actin remodeling and will aid in disrupting cadherin
203 localization and consequently, disrupting cell adhesion at the mid-region of the mid-region of the
204 IPC-OPC walls.

205 Although P-cadherin was the first cell adhesion protein to be expressed in the PCs and
206 continued to be expressed in the apical portion on P7, the stable P-cadherin expression is
207 unlikely to influence the opening of the tunnel of Corti under normal developmental conditions.

208 On the other hand, the dynamic and transient localization of E-cadherin in the mid- regions of
209 the IPC-OPC membranes, and its subsequent downregulation preceding the opening of the tunnel
210 of Corti makes the dynamic downregulation E-cadherin a much more likely mechanism for
211 opening the tunnel under normal developmental conditions. However, it is possible that in a
212 disease context, the loss of P-cadherin can exacerbate the tunnel opening and final size of the
213 tunnel, disrupting the overall stability of the sensory epithelium. Perhaps both E-cadherin and P-
214 cadherin trafficking are impacted when the cytoarchitecture of the sensory epithelium is
215 disrupted under disease conditions. Thus, follow up studies are needed to validate this. In this
216 study, we show evidence that significant actin remodeling takes place in the SCs during P6- P8
217 and paves way for future investigations on actin assembly in the SCs on which little focus has
218 been placed thus far. Here we closely analyze the cell adhesion characteristics associated with
219 the opening of the tunnel of Corti because they are expressed at much higher levels. We predict
220 that the same decrease in cadherin trafficking will promote the formation of the Nuel space and
221 the outer tunnel.

222

223 **3. Experimental Procedures**

224 ***3.1 Mice***

225 Time-mated Swiss Webster dams (Envigo, Indianapolis, IN, USA) were denoted as embryonic
226 day (E)0.5 on the first day a plug was observed. E18.5 embryos, and postnatal day (P)6-P8 pups
227 were euthanized by decapitation, in accordance with the Institutional Animal Care and Use
228 Committee (IACUC) guidelines at The Jackson Laboratory, Bar Harbor, and heads or ears were
229 harvested and fixed in 4% paraformaldehyde (PFA) (Electron Microscopy Sciences, Hatfield,
230 PA, USA).

231 ***3.2 Histology***

232 After fixation, E18.5 heads were bisected and processed through a 10%, 20%, and 30% sucrose
233 gradient series, embedded with TFM and cryofrozen. For postnatal studies, cochleas were
234 extracted on P6, P7 and P8, and fixed in 4% PFA for three hours at 4°C, immersed in a 30%
235 sucrose and transferred to a 1:1 solution of 30% sucrose: Tissue Freezing Medium (TFM)
236 (General Data Healthcare, Cincinnati, OH, USA) before final embedding in 100% TFM and

237 cryofrozen. Tissues were cryosectioned at 12 μm for histology at all ages. To capture the
238 opening of the tunnel of Corti (ToC) on P7, inner ears were harvested and fixed at 9 am, 1 pm
239 and 5 pm.

240 ***3.3 Immunofluorescence***

241 Tissues were washed in PBS/0.5% Triton and blocked at for at room temperature (RT) 1 hour
242 with 2% Donkey serum (Jackson ImmunoResearch, West Grove, PA, USA) in PBS/0.5% Triton,
243 followed by overnight primary antibody incubation at 4°C. Primary antibodies included: rat anti-
244 E-cadherin (1:250, AB11512, Abcam, Waltham, MA, USA), sheep anti- N-cadherin (1:250,
245 AF6426, R&D Systems, Minneapolis, MN, USA), goat anti- P-cadherin (1:250, AF761, R&D
246 Systems), mouse anti-phosphoTyrosine (1:250, zms1b282, Sigma-Aldrich), anti-Cofilin (1:250,
247 5175, Cell Signaling Technology, Danvers, MA, USA). For N-cadherin, P-cadherin and Cofilin
248 immunolabeling, antigen retrieval was performed prior to primary antibody incubation with a 10
249 mM sodium citrate/0.05% Tween20 buffer at 6.0 pH for 15 min at 99°C. Since phalloidin
250 labeling of F-actin is incompatible with antigen retrieval, we co-labeled with an actin antibody
251 (1:250, MA5-11869, Invitrogen). Alexa-conjugated secondary antibodies (Invitrogen) were
252 incubated for 2 hours at RT. Nuclei were counter stained with DAPI (Abcam, Waltham, MA,
253 USA) and tissues were mounted with Fluoromount G mounting medium (Life Technologies
254 Corporation, Carlsbad, CA, USA).

255 ***3.4 Data Acquisition and Image processing***

256 60X magnification images were acquired on a Zeiss LSM 800 confocal microscope (ZEISS,
257 Oberkochen, BW, Germany) in The Jackson Laboratory microscopy core. Z-stacks were
258 analyzed using the FIJI software (NIH). Data were collected on N=6 cochleas for each time
259 point. TIFF images were processed in Adobe Photoshop and assembled in Adobe Illustrator
260 (Adobe, San Jose, CA, USA).

261

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5. Conflict of Interest

The authors declare no conflicts of interest or competing financial interests.

6. Author Contributions

H.J.B., study conceptualization and design, development of methodology, acquisition, data analysis, writing and revision of the manuscript. A.M.S., study design, acquisition, data analysis, and revision of the manuscript. V.M., funding, material support, study conceptualization and design, development of methodology, acquisition, analysis, and interpretation of data; writing and revision of the manuscript. All authors read and approved the final manuscript.

7. Ethics Approval

All experiments were performed under IACUC protocol 18013: PI Vidhya Munnamalai, in compliance with the U.S. Department of Health and Human Services and reviewed by The Jackson Laboratory Institutional Animal Care and Use Committee.

8. Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

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10. Figure Legends

Figure 1: Cross-sectional and top-down view of organ of Corti reveal cytoarchitecture of the support cells and hair cells.

(A) Phalloidin labeling of F-actin (green) and Myo6 labeling of HCs (magenta) in a P1 sectioned cochlea shows structural details of the F-actin cytoskeleton in the sensory epithelium. (A') Phalloidin labeling alone. White Arrow indicates the location of the PCs.

(B) Top-down view of a Phalloidin-labeled P1 wholemount cochlea shows the overall organization and location of the IHC, PC (black arrow) and the three rows of OHCs.

Figure 2: Significant remodeling of F-actin and E-cadherin occurs in the cochlear sensory epithelium between late embryonic and early postnatal ages.

(A-C) Phalloidin labeling of F-actin, and E-cadherin and pTyr immunolabeled proteins in E18.5 cochleas. Dotted lines outline the PCs, asterisks denote the HCs.

(D-E) Phalloidin labeling of F-actin, and E-cadherin and pTyr immunolabeled proteins in P6 cochleas. Arrows indicate the mid-regions of IPC-OPC membranes that will become the axial regions of the mature PCs.

(G-I) Phalloidin labeling of F-actin, and E-cadherin and pTyr immunolabeled proteins in P6 cochleas. Arrows indicate the apical regions of IPC-OPC membrane contacts. Dotted lines outline the tunnel of Corti and the space of Nuel.

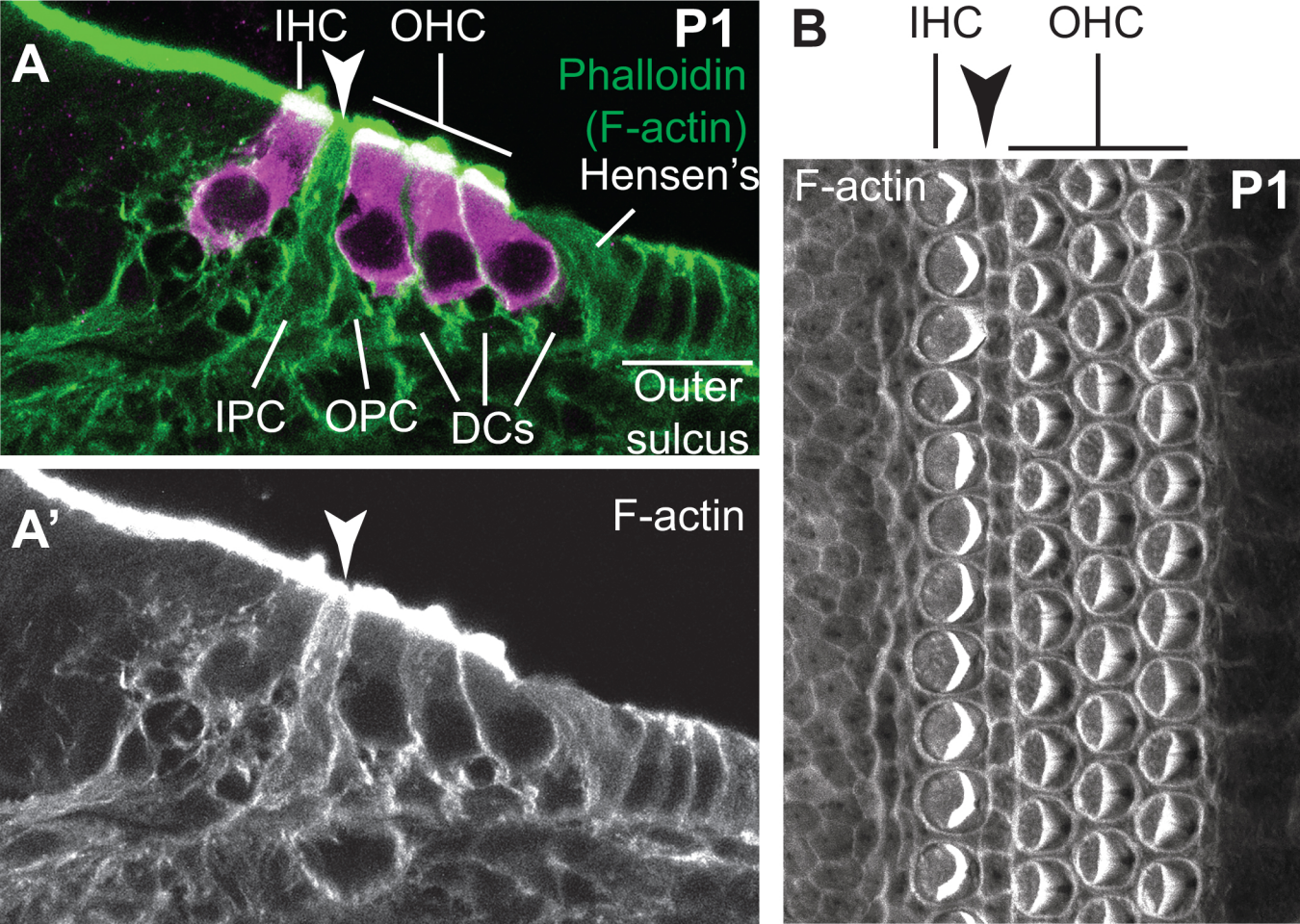
Nuclei are counter stained with DAPI (magenta).

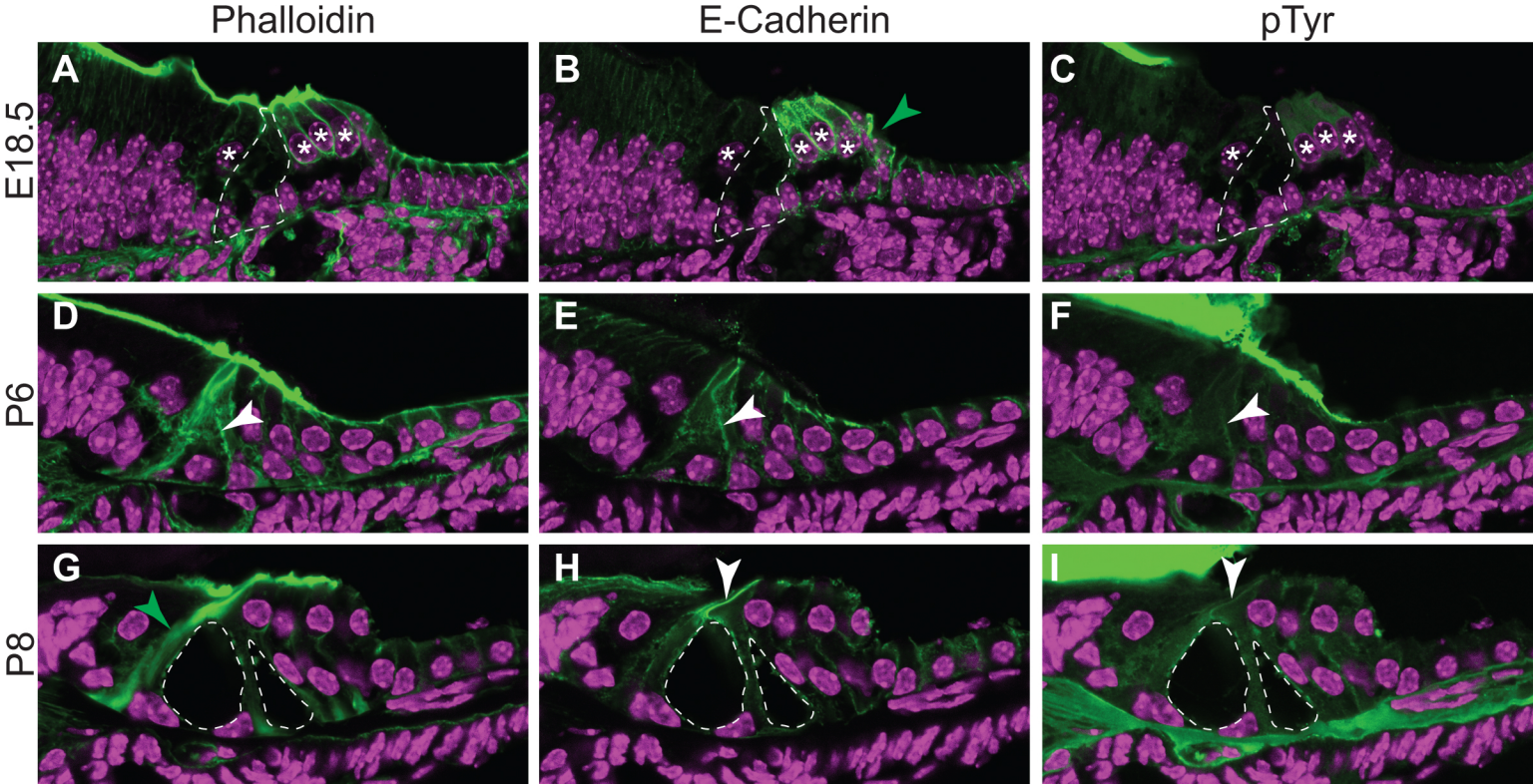
Figure 3: Fine temporal resolution of the opening of the tunnel of Corti reveals novel cytoskeletal details.

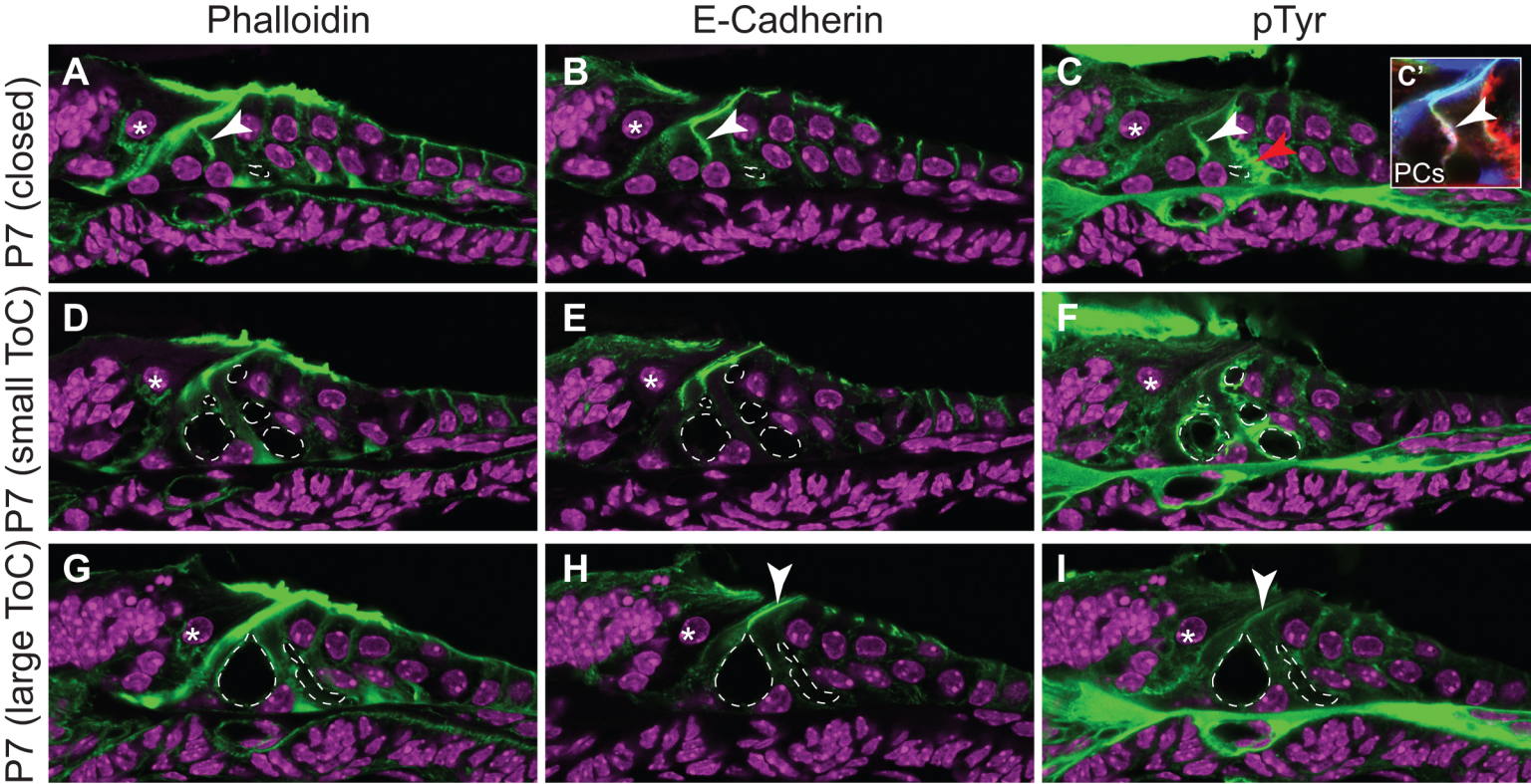
(A-C) Phalloidin labeling of F-actin, and E-cadherin and pTyr immunolabeled proteins in P7 cochleas harvested at 9 am. Arrows indicate the Arrows indicate the mid-regions of IPC-OPC membrane contacts. C' Inset- overlay of mid-region of IPC-OPC junction. E-cadherin (green), pTyr (red) and Phalloidin (blue). White arrow indicates co-localization of the three markers at the mid-region of the IPC-OPC junction. Red arrow indicates pTyr accumulation at OPC-DC contact flanking Nuel space. Dotted lines denote the locations of spaces that will form the tunnel of Corti and the space of Nuel

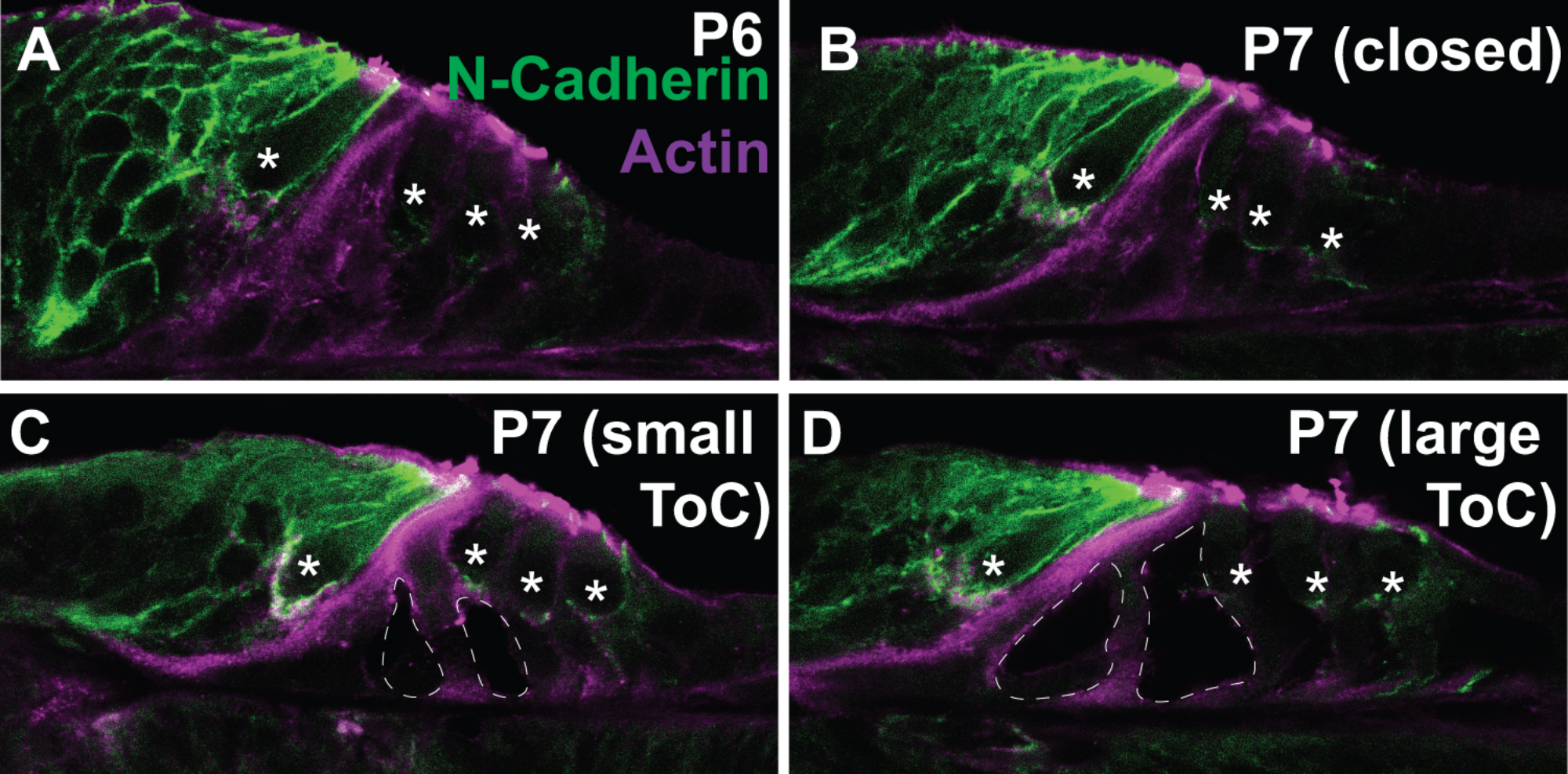
406 (D-F) Phalloidin labeling of F-actin, and E-cadherin and pTyr immunolabeled proteins in P7
407 cochleas harvested at 1 pm. Dotted lines denote the locations of spaces that will form the tunnel
408 of Corti and the space of Nuel.
409 (G-I) Phalloidin labeling of F-actin, and E-cadherin and pTyr immunolabeled proteins in P7
410 cochleas harvested at 5 pm. Arrows indicate the apical junctional contacts between the IPCs and
411 the OPCs.
412 Asterisks denote the IHCs, and nuclei are counter stained with DAPI (magenta).
413
414 Figure 4: N-cadherin localization remains stable from embryonic to postnatal ages.
415 (A) On P6, N-cadherin is localized to the membranes of the inner border cell, inner phalangeal
416 cell and the IHC.
417 (B-C) On P7, N-cadherin is localized to membranes of the inner border, IHC and inner
418 phalangeal cell membranes remains stable as the tunnel of Corti is formed.
419 Asterisks denote the hair cells and actin is immunolabeled in magenta.
420
421 Figure 5: P-cadherin localization remains stable from embryonic to postnatal ages.
422 (A) On P6, P-cadherin is localized to the membranes of the IPC (white arrow), and Hensen's and
423 outer sulcus cells (green arrow).
424 (B-C) On P7, P-cadherin is localized to the membranes of the IPCs and the OPCs, Hensen's cells
425 and outer sulcus cells. Arrows indicate the apical portion of the IPC-OPC adherens junctions.
426 Asterisks denote the hair cells, and actin is immunolabeled in magenta.
427
428 Figure 6: Cofilin is enriched in the support cells of the sensory epithelium in the postnatal
429 cochlea.
430 (A) On P6, Cofilin labeling is punctate in the cytoplasm and slightly enriched at the PC
431 membranes. Cofilin is expressed in the SCs, but not the HCs.
432 (B) On P7, before the tunnel of Corti forms, Cofilin is selectively enriched in the mid-region of
433 the IPC-OPC junctions.
434 (C-D) As the tunnel begins to open, Cofilin remains enriched at the edge of the cell membrane
435 that retracts to form the tunnel of Corti.

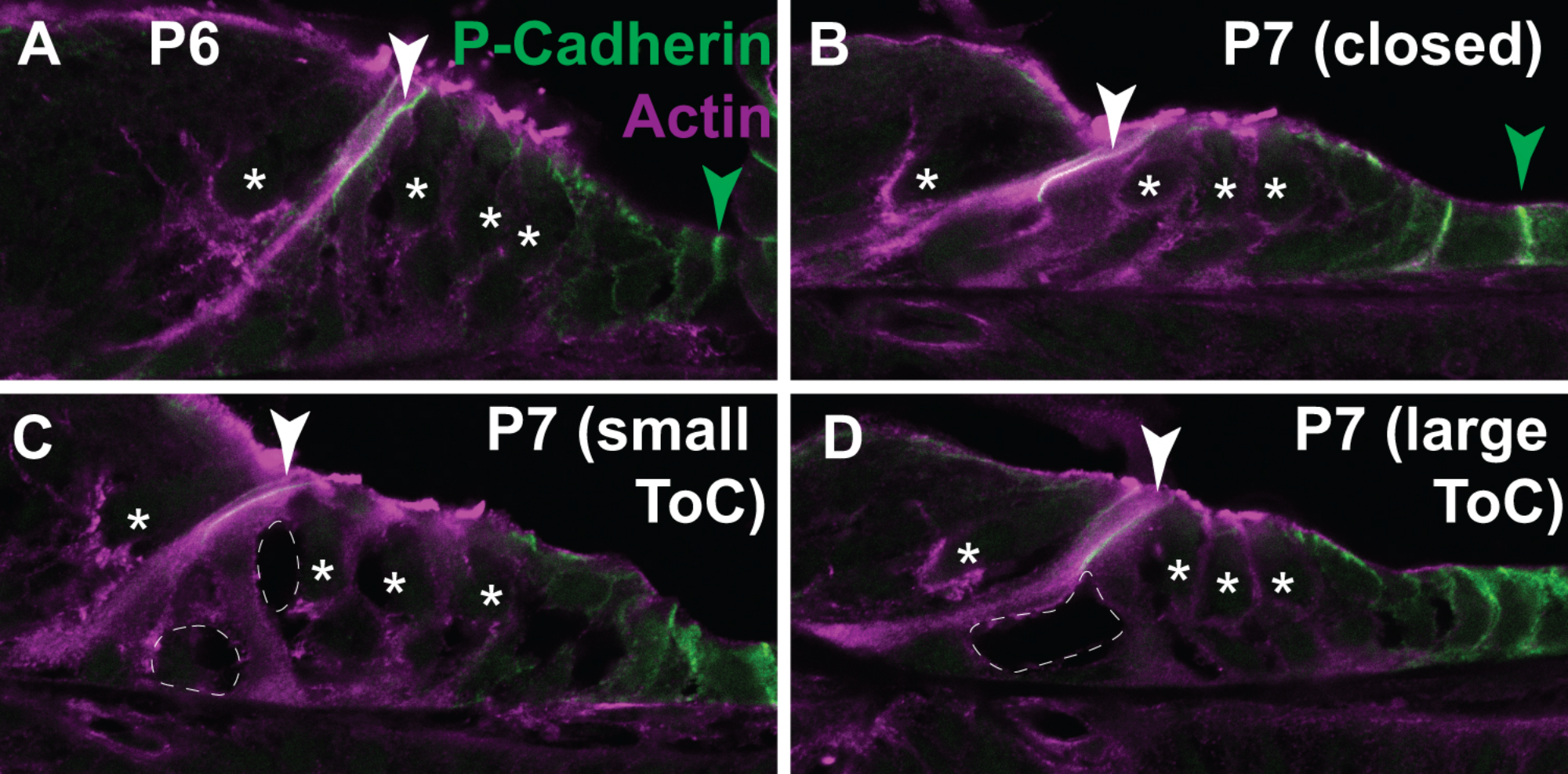
436 Arrows indicate the apical regions of the pillar cells. Asterisks denote the hair cells, and actin in
437 immunolabeled in magenta. Yellow arrow indicates cofilin enrichment.
438

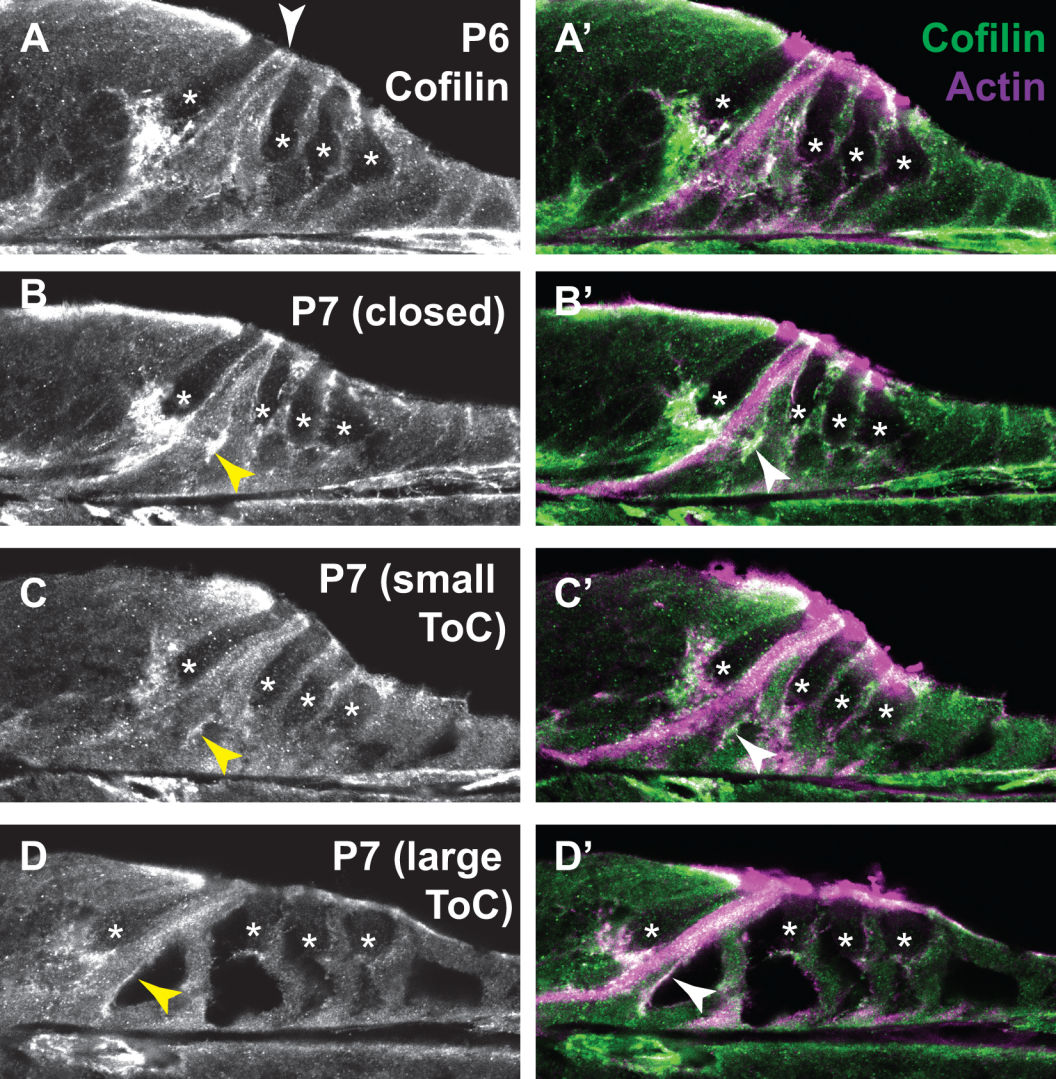

A
IHC
OHC
P1
Phalloidin
(F-actin)
Hensen's
IPC
OPC
DCs
Outer
sulcus
A'
F-actin
B
IHC
OHC
F-actin
P1


Phalloidin
E-Cadherin
pTyr
E18.5
P6
P8
A
B
C
D
E
F
G
H
I


Phalloidin
E-Cadherin
pTyr
P7 (closed)
P7 (small ToC)
P7 (large ToC)
A
B
C
C'
PCs
D
E
F
G
H
I


A
P6
N-Cadherin
Actin
B
P7 (closed)
C
P7 (small ToC)
D
P7 (large ToC)


A
P6
P-Cadherin
Actin
B
P7 (closed)
C
P7 (small ToC)
D
P7 (large ToC)


A
P6
Cofilin
A'
Cofilin
Actin
B
P7 (closed)
B'
C
P7 (small
ToC)
C'
D
P7 (large
ToC)
D'