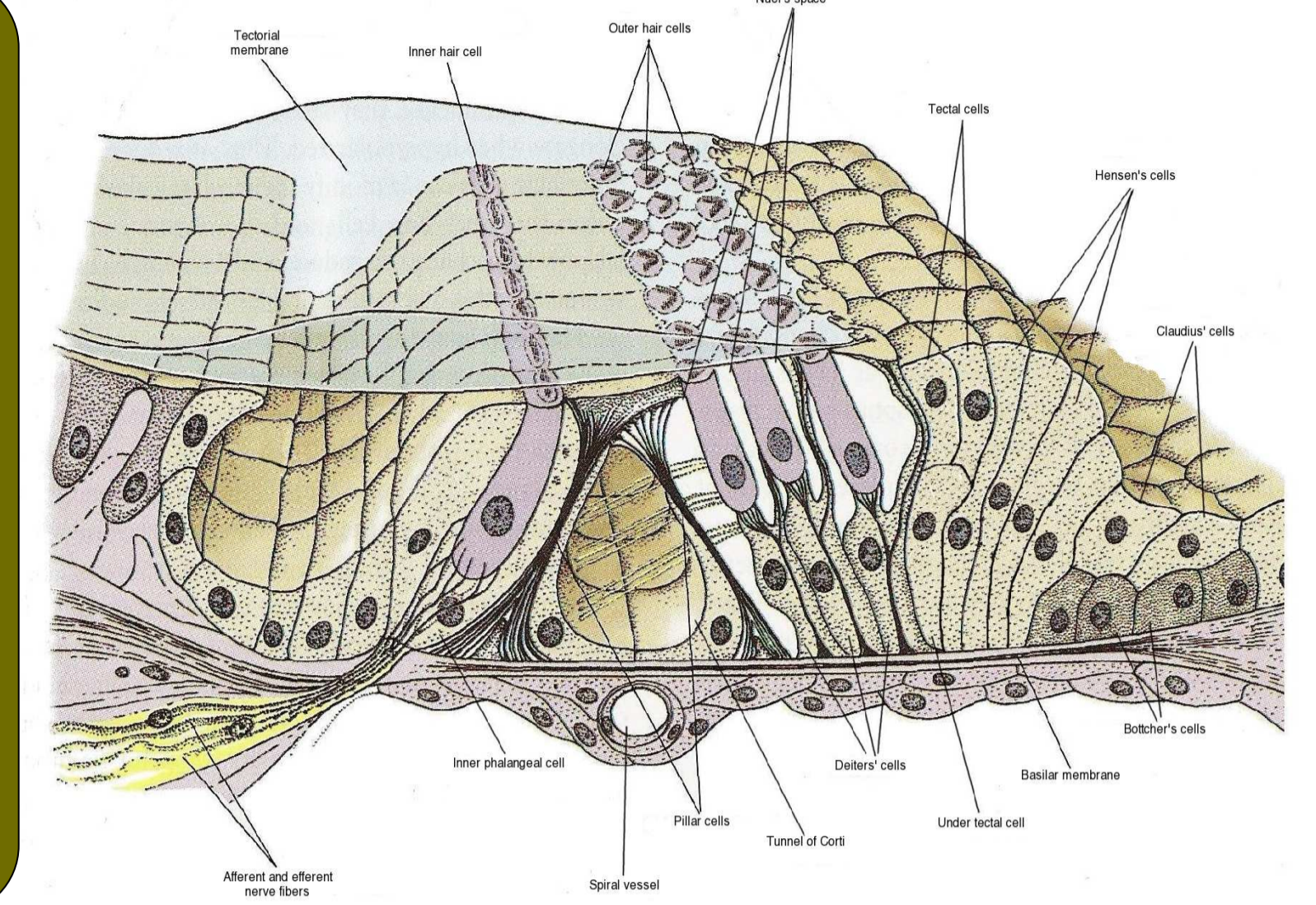


SPATIO-TEMPORAL LOCALIZATION OF INTERMEDIATE FILAMENTS IN THE ORGAN OF CORTI BETWEEN THE EMBRYONIC DAY 18 (E18) AND THE POST-NATAL DAY 15 (P15) IN RAT

Introduction

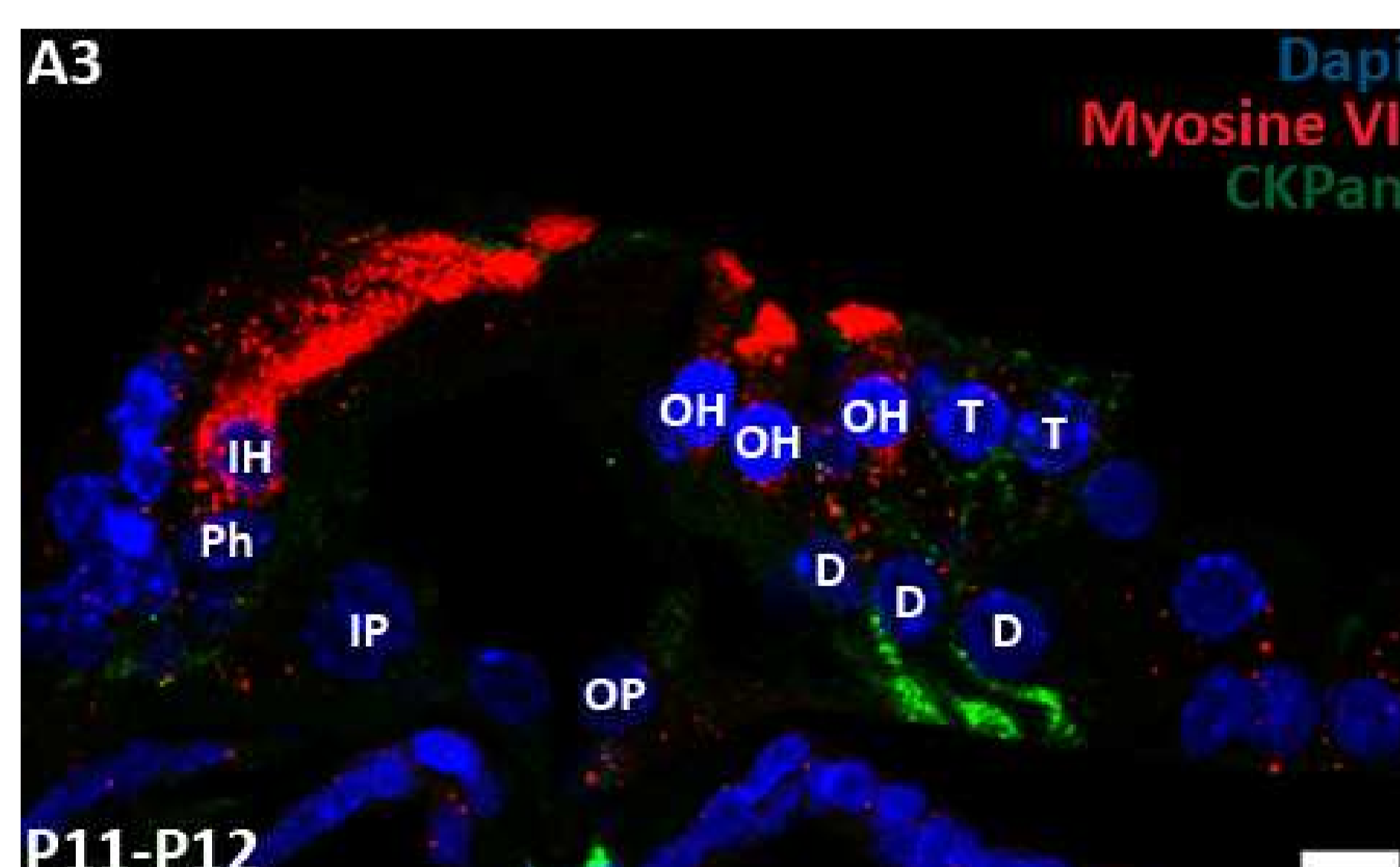
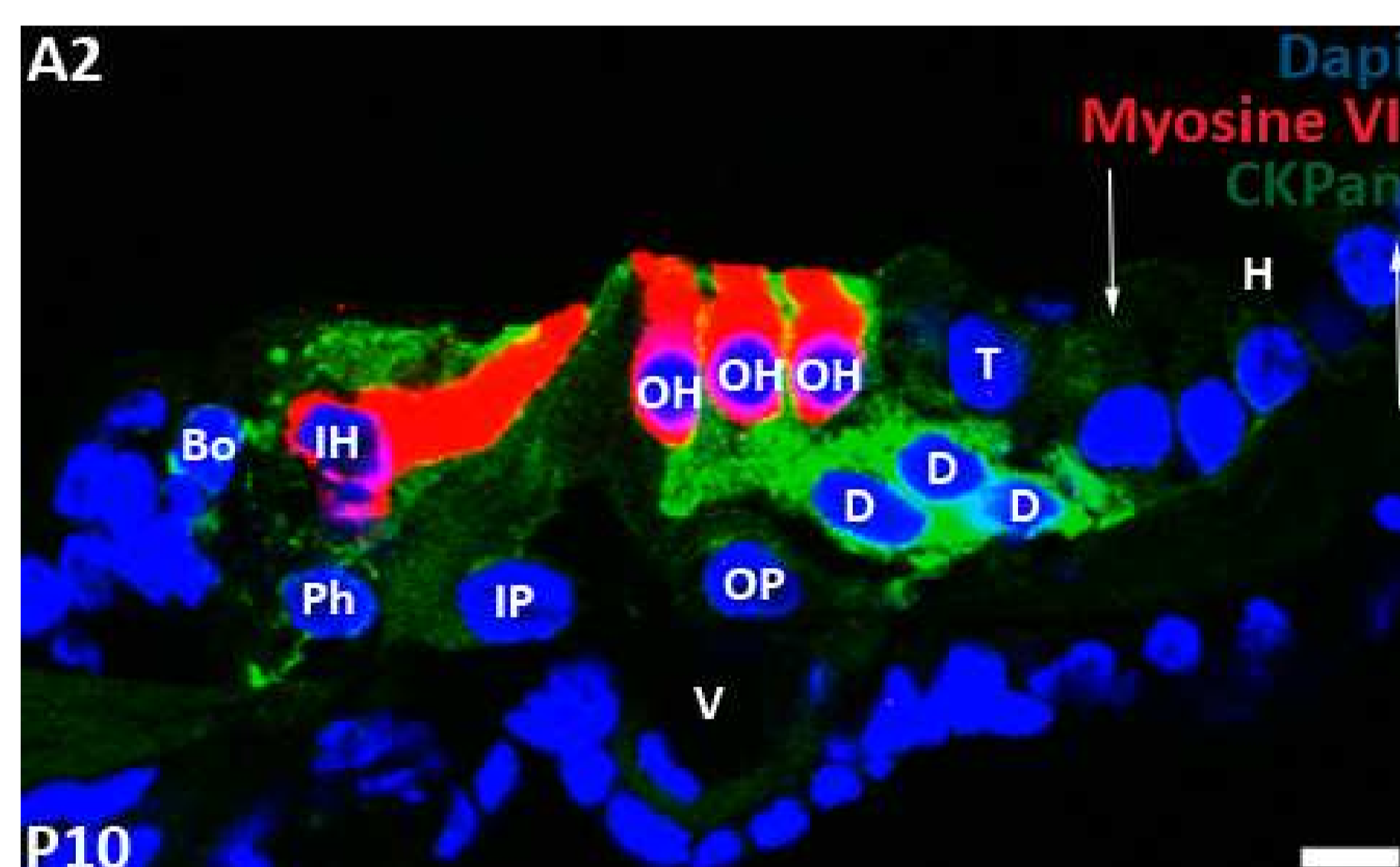
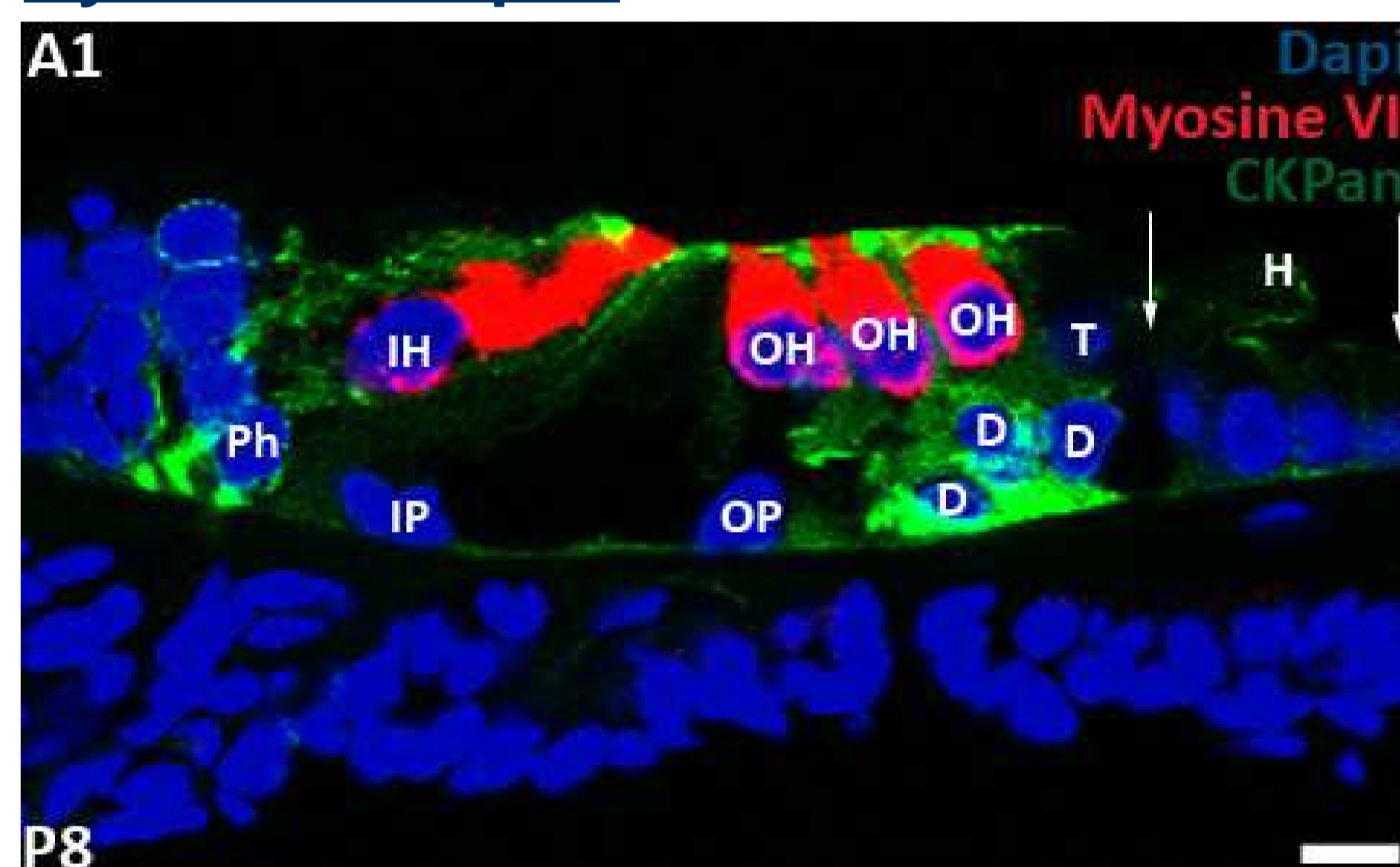
In mammals, the perception of sound is mediated by an epithelial sensory patch located in the cochlear region of the inner ear named the organ of Corti (OC) [Kelley and Bianchi, 2002 ; Kelley, 2006]. The latter is composed of mechanosensory hair cells and nonsensory supporting cell types. The hair cells are modified epithelial cells that utilise a group of derived microvilli, referred to as stereocilia, to perceive pressure waves induced through sound. Based on their morphology and physiology, two types of hair cells can be distinguished: inner and outer hair cells. Likely, at least four types of supporting cells can be identified in the OC: inner pillar cell, outer pillar cell, inner phalangeal cell and Deiters' cells. These cells are arranged in a regular mosaic pattern running along the length of the snail-like cochlea from base to apex. One of the most striking aspects of this mosaic is that specific cell types are arranged in discreet rows. The edge of the OC located closest to the modiolus is composed of a single row of alternating inner hair cells and inner phalangeal cells. The edge of the Corti's organ located closest to the stria vascularis is composed of three rows of outer hair cells and Deiters' cells that are also arranged into a regular alternating mosaic. Finally, the single row of inner hair cells and the three rows of outer hair cells are separated by the tunnel of Corti that is a space delimited by a single row of inner pillar cells and a single row of outer pillar cells. The formation of Corti's tunnel appears after birth in rat [Roth and Bruns, 1992].



Although the structure of the auditory organ in mature mammals, the organ of Corti, is clearly established, its development is far to be elucidated. Using antibodies directed against different proteins of intermediate filaments cytoskeleton, we studied the spatial-temporal localization of cytokeratins (Cytokeratin pan (CK1, 4, 5, 6, 8, 10, 13, 18 and 19) and CK8, typical of epithelial cells) and vimentin (V9, typical of mesenchymal cells), we investigated by confocal microscopy the setting up of supporting cells' cytoskeleton during the differentiation of the OC in rat from the embryonic day 18 (E18) to postnatal day 15 (P15).

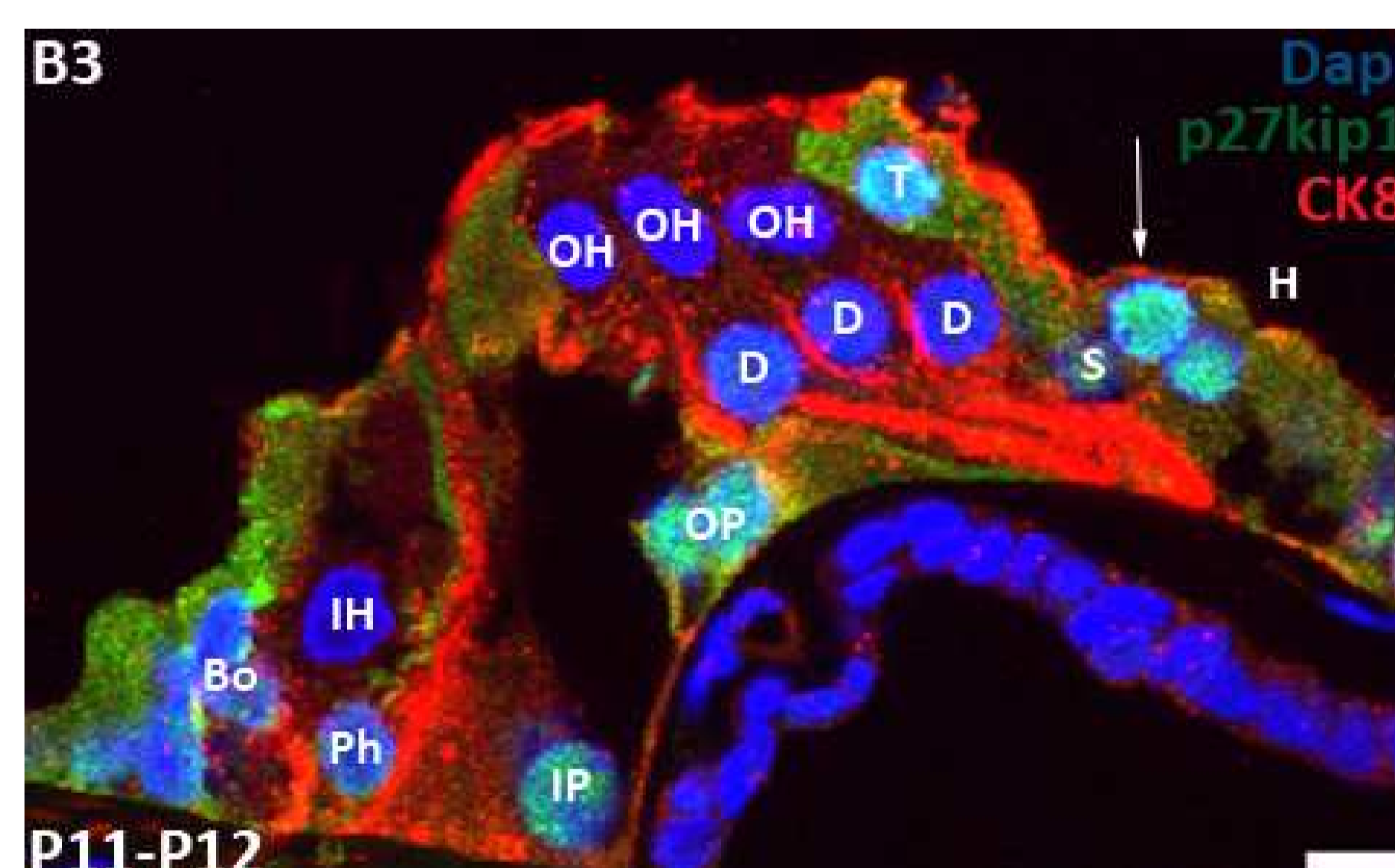
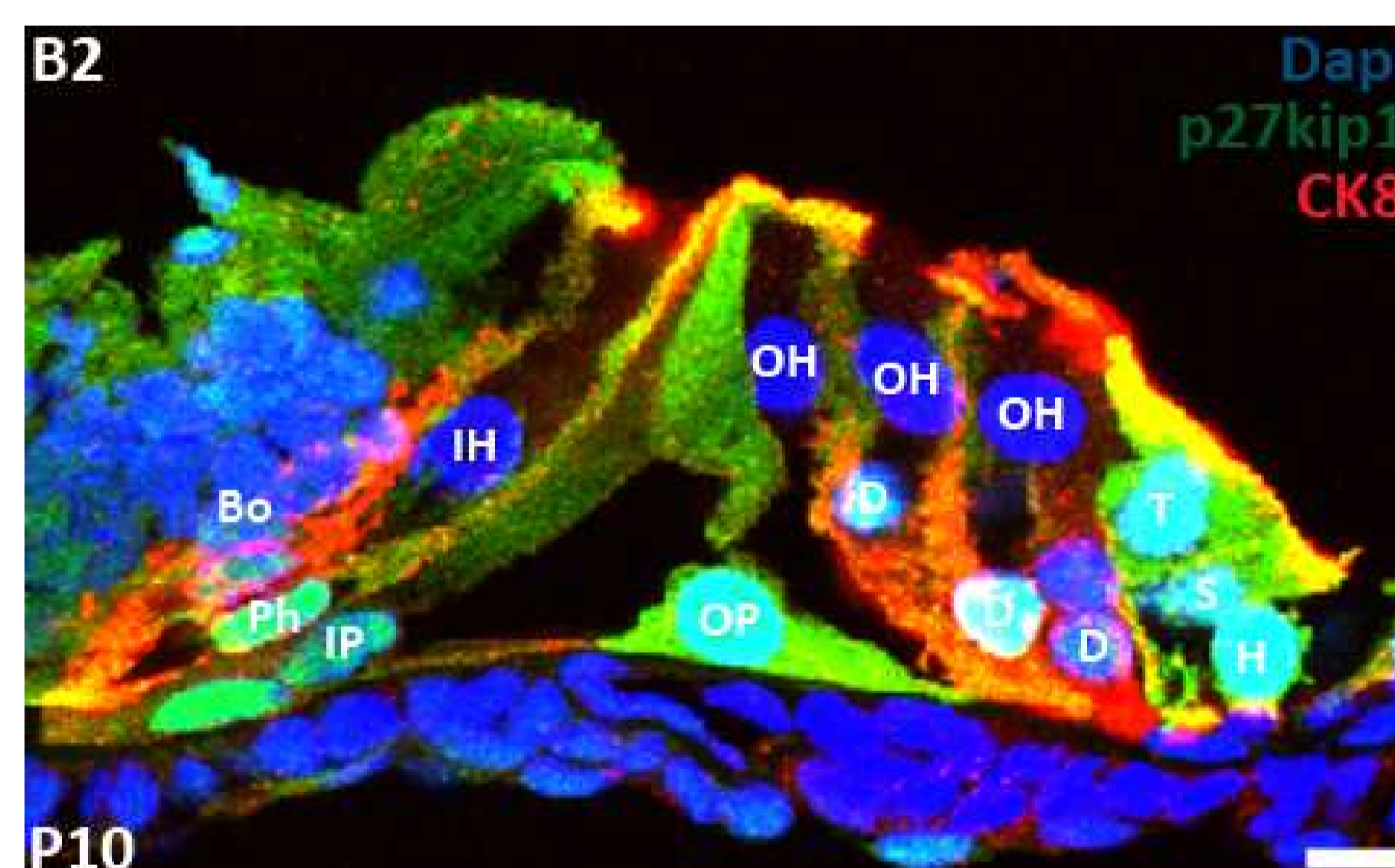
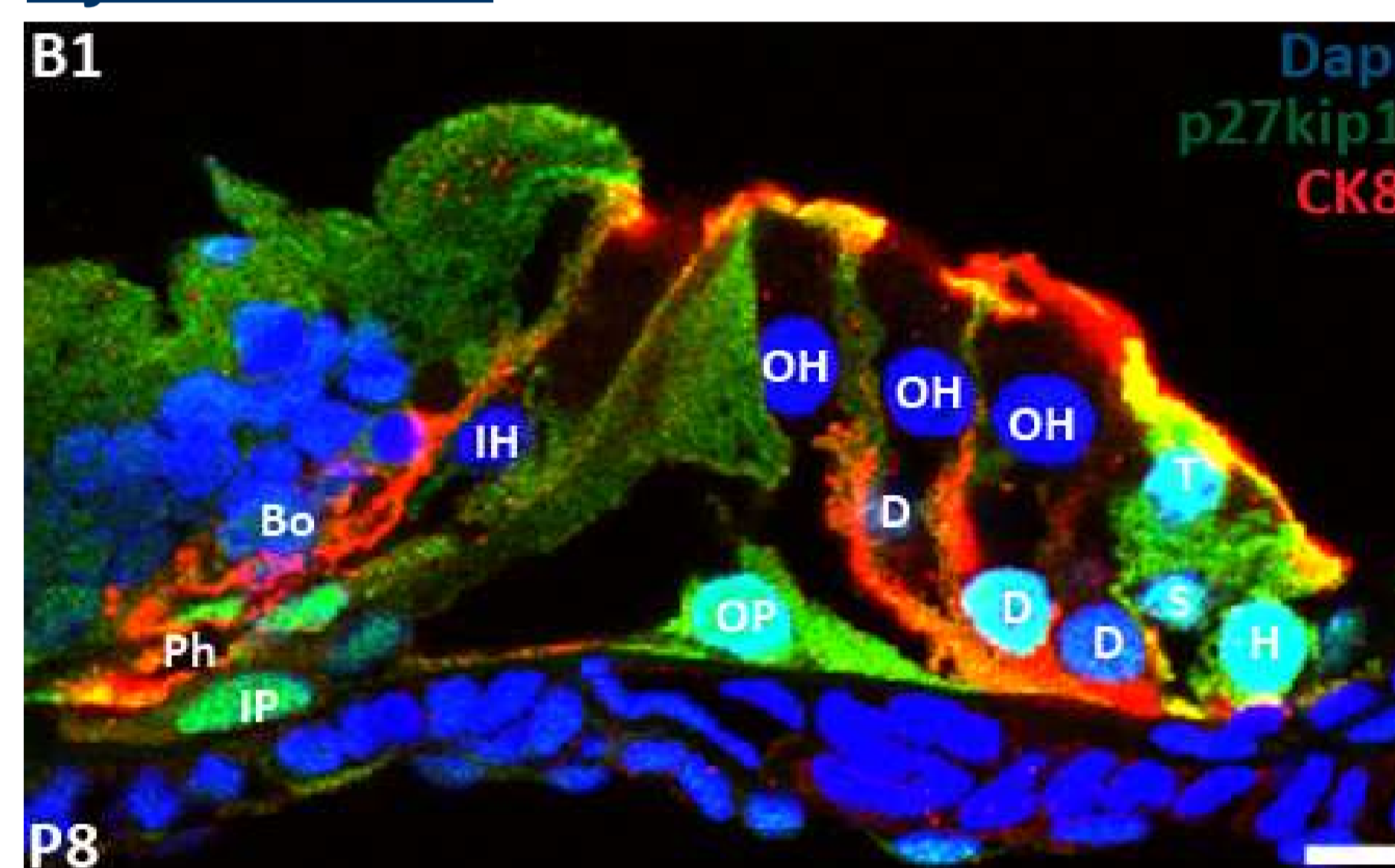
Results

Cytokeratins pan



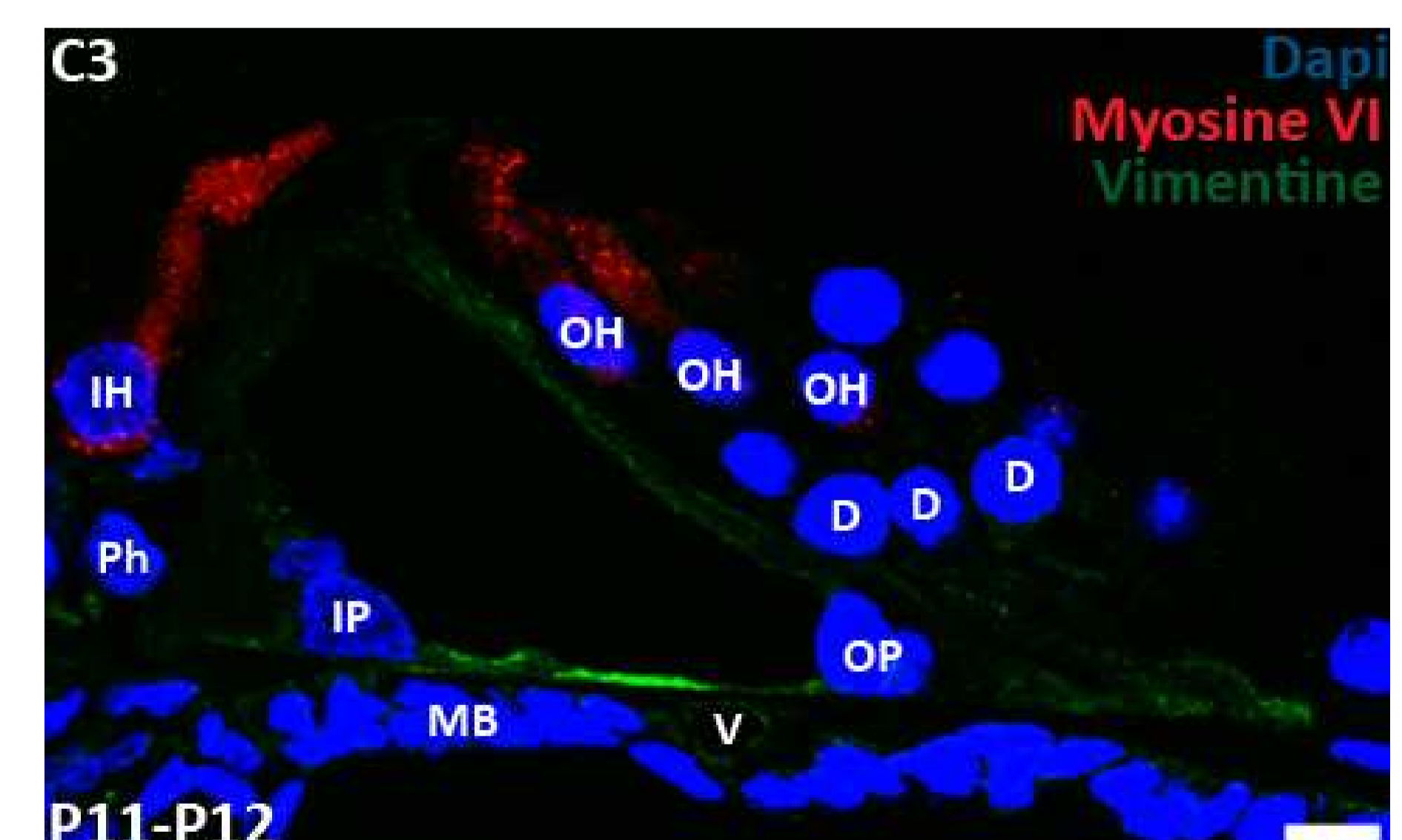
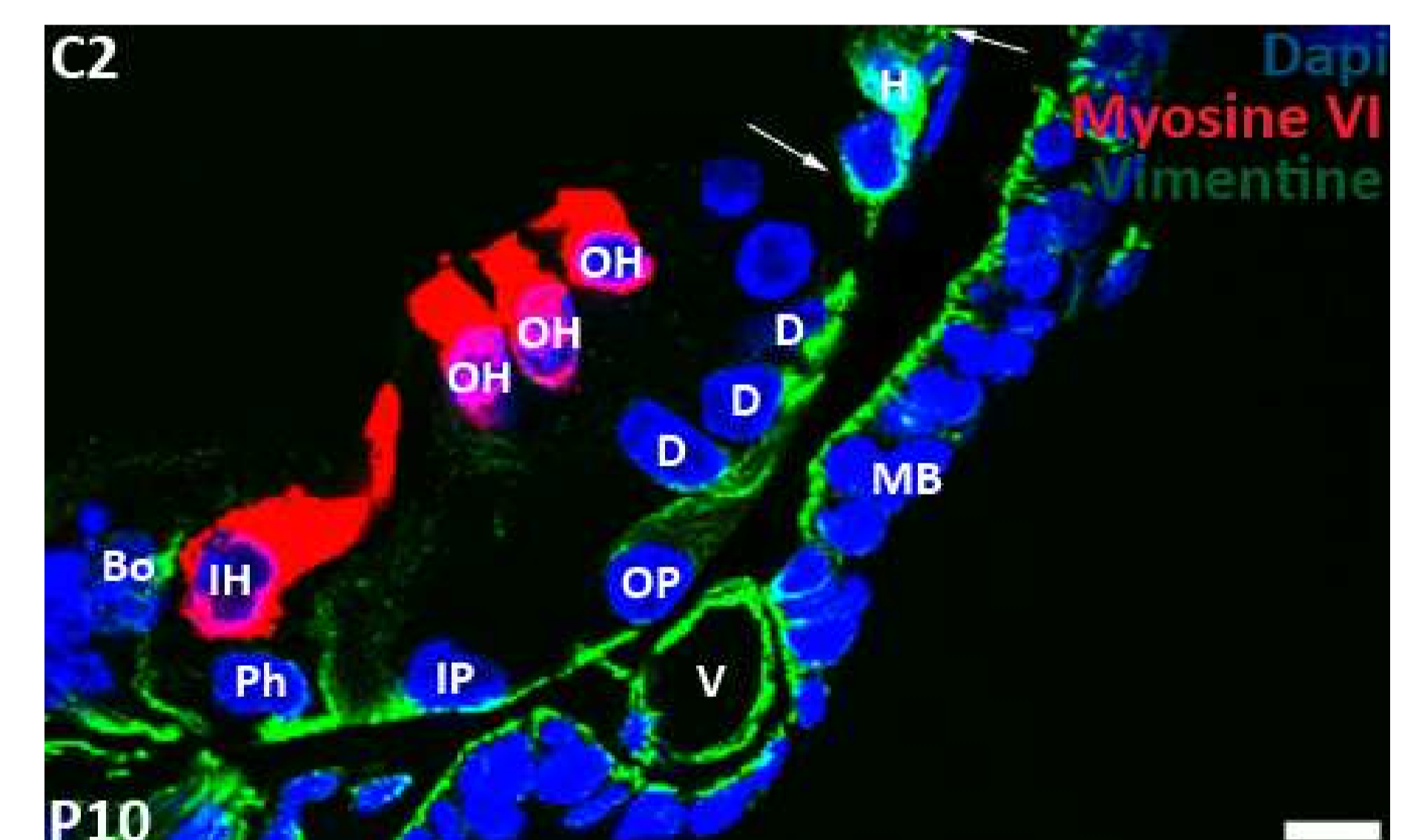
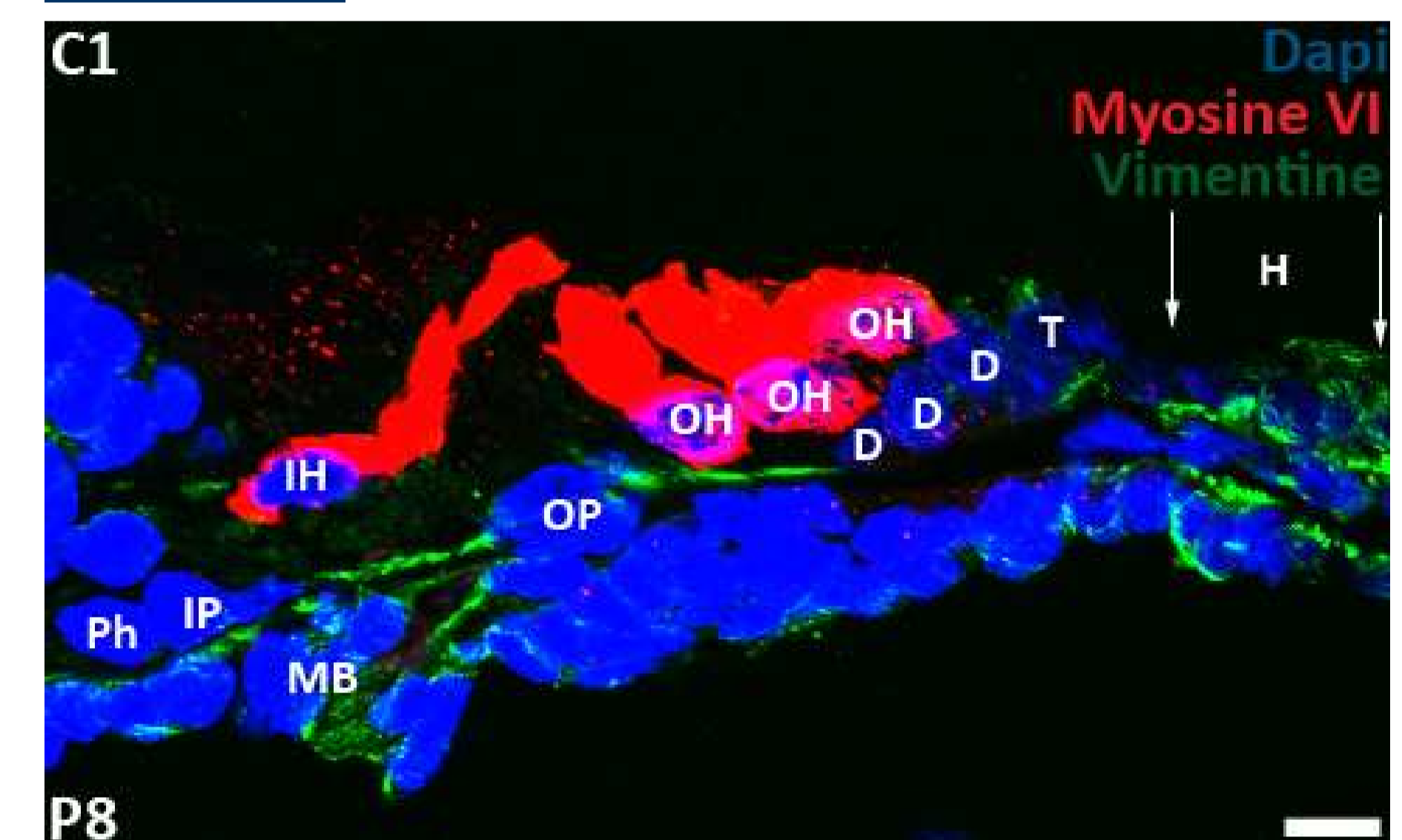
Figures A1-A3: Spatiotemporal distribution of Cytokeratin pan during the mammalian auditory organ development from P8 to P11-P12 in the basal part of the cochlea. Between P8 (A1) and P10 (A2), there is the cytokeratin in the pillar cells, the Deiters' cells and near the phalangeal and border cells. At P11-P12 (A3), the basal parts of the pillar cells and of the Deiters' cells are labelled. Bo: Border cell; D: Deiters' cell; H: Hensen's cell; IH: Inner Hair cell; IP: Inner Pillar cell; OH: Outer Hair cell; OP: Outer Pillar cell; Ph: Phalangeal cell; T: Tectal cell. μm . Red: Myosin VI; Green: Cytokeratins pan; Blue: Dapi. Bars A1 and A3: 7,5 μm and A2: 5 μm .

Cytokeratin 8



Figures B1-B3: Spatiotemporal distribution of Cytokeratin 8 (CK8) during the mammalian auditory organ development from P8 to P11-P12 in the basal part of the cochlea. Between P8 (B1) and P10 (B2), there is the CK8 in the pillar cells, the Deiters' cells and near the phalangeal cell, border cells, tectal cells and the Hensen's cells. At P11-P12 (B3), the pillar cell, the Deiters' cells are labelled. Bo: Border cell; D: Deiters' cell; H: Hensen's cell; IH: Inner Hair cell; IP: Inner Pillar cell; OH: Outer Hair cell; OP: Outer Pillar cell; Ph: Phalangeal cell; T: Tectal cell. μm . Red: CK8; Green: p27^{KIP1}; Blue: Dapi. Bars B1 and B3: 7,5 μm and B2: 5 μm .

Vimentin



Figures C1-C3: Spatiotemporal distribution of vimentin V9 during the mammalian auditory organ development from P8 to P11-12 in the basal part of the cochlea. Between P8 (C1) and P10 (C2), the vimentin is present in the pillar cells, the Deiters' cells and near the phalangeal and bordering cells. At P11-12, the pillar cells and Deiter's cell are labelled. Bo: Border cell; D: Deiters' cell; H: Hensen's cell; IH: Inner Hair cell; IP: Inner Pillar cell; OH: Outer Hair cell; OP: Outer Pillar cell; Ph: Phalangeal cell; T: Tectal cell. μm . Red: Myosin VI; Green: Vimentin; Blue: Dapi. Bars B1 and B2: 5 μm and B3: 7,5 μm .

Conclusions

These results suggest that the epithelial-mesenchymal transition (EMT) might be implicated in the opening of Corti's tunnel between the pillar cells and the formation of the Nuel's spaces between the Deiters' cell and their outer hair cells, at P8 and at P10 respectively. Indeed, the molecular hallmarks of EMT include de novo expression of mesenchymal proteins such as vimentin; the loss of cytokeratins and other epithelial-associated markers but a certain number of carcinoma, like breast, prostate, lung, colon and ovary, express CK8 and CK18. Furthermore, the EMT includes the E-Cadherin down-regulation responsible for the loss of cell-cell adhesion and the subsequent detachment from the parent epithelium. The study of this proteins is necessary to understand the differentiation's process proceeding in the auditory organ.