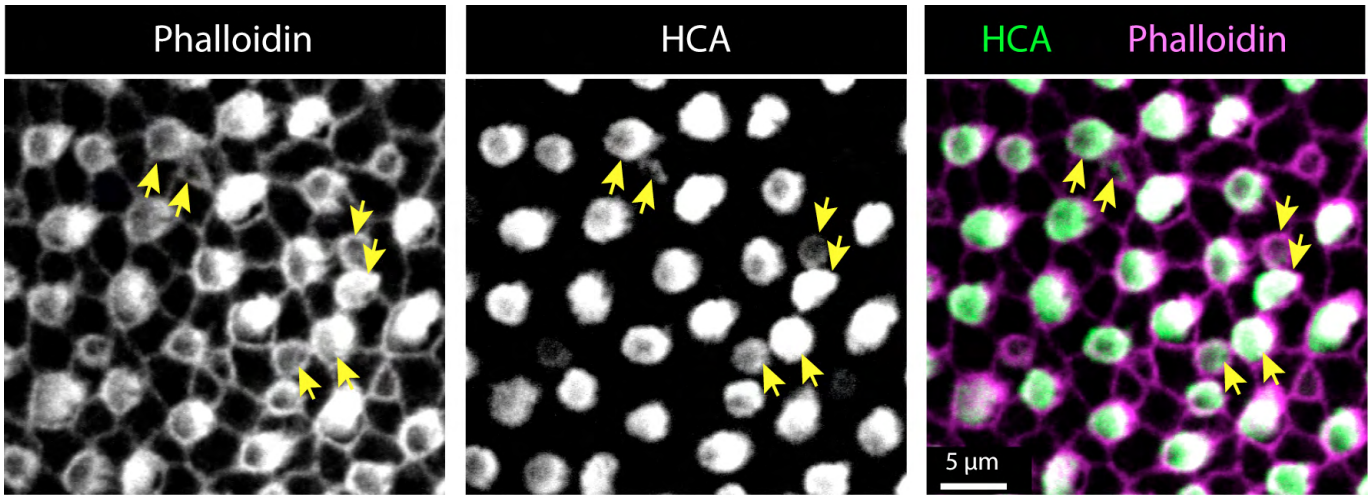


**Fig. S1. Surface view of an E10 basilar papilla (distal region) immunostained for HCA and labelled with fluorescent phalloidin.** Note the presence of contacts between hair cells with strong HCA staining and some hair cells with very faint or no expression of HCA (arrows), but a characteristic enrichment of actin at their periphery.

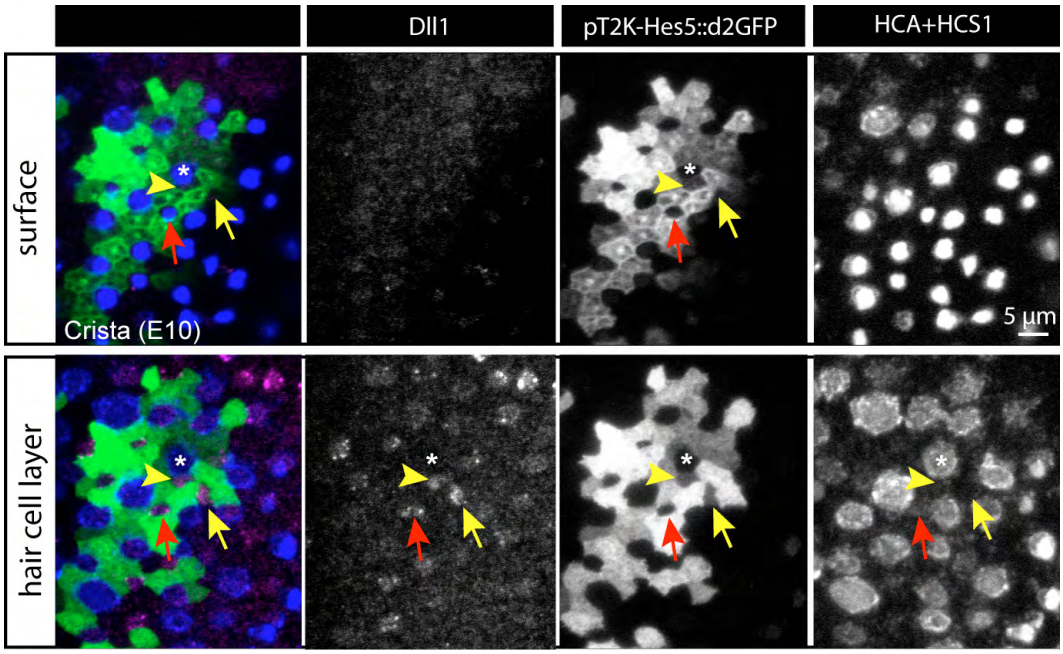


**Fig. S2. Immunostaining for Dll1, HCA and otoferlin (HCS1) in relation to Hes5::d2EGFP activity in an E10 sensory crista.** Two different optical planes from the same region, and parallel to the lumenal surface, are shown. The expression of Dll1 is reduced in differentiated hair cells (asterisk) expressing HCA and otoferlin. The Dll1-positive cells (yellow arrow and arrowhead and white arrow) have low levels of Hes5::d2EGFP, can be HCA positive (red arrow), but do not express otoferlin. Note that one of these cells (yellow arrowhead) is in direct contact with a more differentiated hair cell (asterisk).

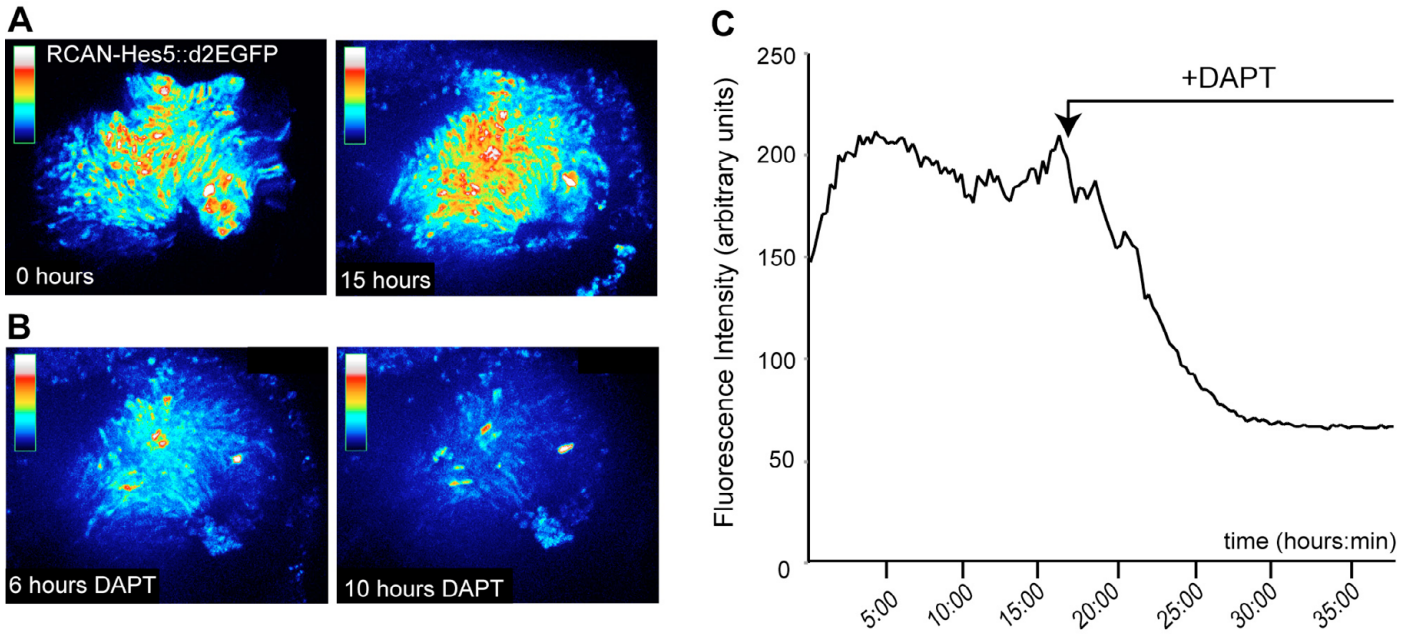


**Fig. S3. Treatment with the γ-secretase inhibitor DAPT (20 μM) reduces EGFP fluorescence levels in organotypic cultures of E10 crista stably transfected with Hes5::d2EGFP.** (**A**) Low-magnification surface view of a crista transfected with RCAN-Hes5::d2EGFP and imaged at 15-minute intervals on a Nipkow spinning-disc confocal microscope (see Movie 1). In control medium, levels of fluorescence show slight fluctuations but remain relatively stable over 15 hours. (**B**) The same crista following DAPT addition to the medium. After a delay of ~2 hours, fluorescence levels start to decrease, reaching a minimum level in ~10 hours (*n*=3). Similar results and timecourse for reduction in fluorescence were obtained in samples transfected with pT2K-Hes5::d2EGFP and with pT2K-Hes5::nd2EGFP (*n*=5; not shown). (**C**) Quantification of mean fluorescence intensities (arbitrary units) before and after DAPT addition in the crista shown in A,B.