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# Quantitative Analysis of Supporting Cell Subtype Labeling Among CreER Lines in the Neonatal Mouse Cochlea

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5 1 **Quantitative Analysis of Supporting Cell Subtype**  
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8 2 **Labeling among CreER Lines in the Neonatal Mouse**  
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11 3 **Cochlea**  
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## Abstract

Four CreER lines that are commonly used in the auditory field to label cochlear supporting cells (SCs) are expressed in multiple SC subtypes, with some lines also showing reporter expression in hair cells (HCs). We hypothesized that altering the tamoxifen dose would modify CreER expression and target subsets of SCs. We also used two different reporter lines, *ROSA26<sup>tdTomato</sup>* and *CAG-eGFP*, to achieve the same goal. Our results confirm previous reports that *Sox2<sup>CreERT2</sup>* and *Fgfr3-iCreERT2* are not only expressed in neonatal SCs, but also in HCs. Decreasing the tamoxifen dose did not reduce HC expression for *Sox2<sup>CreERT2</sup>*, but changing to the *CAG-eGFP* reporter decreased reporter-positive HCs 7-fold. However, there was also a significant decrease in the number of reporter-positive SCs. In contrast, there was a large reduction in reporter-positive HCs in *Fgfr3-iCreERT2* mice with the lowest tamoxifen dose tested, yet only limited reduction in SC labeling. The targeting of reporter expression to inner phalangeal and border cells was increased when *Plp-CreERT2* was paired with the *CAG-eGFP* reporter, however the total number of labeled cells decreased. Changes to the tamoxifen dose or reporter line with *Prox1<sup>CreERT2</sup>* caused minimal changes. Our data demonstrate that modifications to the tamoxifen dose or the use of different reporter lines may be successful in narrowing the numbers and/or types of cells labeled, but each CreER line responded differently. When the *ROSA26<sup>tdTomato</sup>* reporter was combined with any of the four CreER lines, there was no difference in the number of tdTomato-positive cells after one or two injections of tamoxifen given at birth. Thus tamoxifen-mediated toxicity could be reduced by only giving one injection. While the

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4 44 *CAG-eGFP* reporter consistently labeled fewer cells, both reporter lines are valuable  
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7 45 depending on the goal of the study.  
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12 47 **Keywords:** Plp-CreER, Sox2-CreER, Fgfr3-iCreER, Prox1-CreER, mouse genetics,  
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15 48 Cre/loxP  
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## 19 50 **Introduction**

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22 51 The mammalian cochlea is a highly organized structure containing sensory hair cells  
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24 52 (HCs) surrounded by supporting cells (SCs), which are divided into subtypes based on  
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27 53 location (Figure 1A). Cells of the greater epithelial ridge (GER), inner phalangeal cells  
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29 54 (IPhCs), and border cells (BCs) are located medial to inner HCs (IHCs). Inner pillar cells  
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32 55 (IPCs) and outer pillar cells (OPCs) separate IHCs and outer HCs (OHCs). Deiters' cells  
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34 56 (DCs) surround OHCs with Hensen cells (HeCs) and Claudius cells (CCs) located  
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37 57 lateral to the last row of OHCs (Raphael and Altschuler, 2003). While HCs and SCs are  
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39 58 distinct cell types, they are derived from the same pool of progenitor cells (Yang et al.,  
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42 59 2010; Cai et al., 2013; Driver et al., 2013).  
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44 60  
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46 61 Much research has focused on HCs including studies of planar cell polarity (Denman-  
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48 62 Johnson and Forge, 1999; Lewis and Davies, 2002), mechanotransduction (Hudspeth  
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51 63 and Corey, 1977; LeMasurier and Gillespie, 2005), otoprotection (Huang et al., 2000;  
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53 64 Lefebvre et al., 2002), and regeneration (Corwin and Warchol, 1991; Rubel et al., 1995).  
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56 65 While less is known about SCs, several functions have been described including  
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58 66 protection of HCs from excitotoxicity (Spicer and Schulte, 1996; Kikuchi et al., 2000;  
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4 67 Boettger et al., 2002; Furness et al., 2002), phagocytosis of dying HCs (Abrashkin et al.,  
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6 68 2006; Taylor et al., 2008; Anttonen et al., 2014), and sealing the epithelial surface after  
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9 69 HC death (McDowell et al., 1989; Raphael and Altschuler, 1991a, b). Studies have also  
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11 shown that SCs release factors which promote the formation of synapses and survival  
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14 71 of HCs and auditory nerves (Pirvola et al., 1992; Flores-Otero et al., 2007; Sugawara et  
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16 72 al., 2007; Tritsch et al., 2007; Gomez-Casati et al., 2010; Tritsch and Bergles, 2010;  
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19 73 Zuccotti et al., 2012). In the neonatal mouse cochlea, SCs are the source of  
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21 74 regenerated HCs (Bramhall et al., 2014; Cox et al., 2014). These functions have been  
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24 75 attributed to SCs as a group, with little known about the function of individual SC  
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26 76 subtypes, primarily because tools are limited.  
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31 78 Genetically-modified mouse models that target cochlear cells have provided critical  
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33 79 tools to increase our understanding of inner ear physiology and response to damage.  
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35 80 The CreER/loxP system, which allows cell type-specific and temporal control of gene  
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38 81 expression, has been used to delete or over-express genes, to label cell populations for  
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41 82 fate-mapping, and to ablate specific cell types. Here, we used four CreER alleles known  
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43 83 to target broad populations of SCs. The CreER enzyme driven by both the *Fgfr3*-  
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45 84 *iCreERT2* and *Sox2<sup>CreERT2</sup>* lines is also expressed in neonatal HCs (Cox et al., 2012;  
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48 85 Bramhall et al., 2014; Walters et al., 2015). We hypothesized that modifying the  
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51 86 tamoxifen induction paradigm and/or changing the paired reporter line would label  
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53 87 subpopulations of SCs as well as decrease HC labeling in *Fgfr3-iCreERT2* and  
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55 88 *Sox2<sup>CreERT2</sup>* lines. This hypothesis is based, in part, on previous studies showing that the  
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58 89 *ROSA26<sup>tdTomato</sup>* reporter produces a brighter fluorescent protein that is more stable than  
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60 90 the fluorophores produced by other reporter lines such as *CAG-eGFP* or *Rosa26<sup>LacZ</sup>*  
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91 (Madisen et al., 2010). With the *Fgfr3-iCreERT<sup>2</sup>* line, a 10-fold reduction in tamoxifen or  
92 changing to the *CAG-eGFP* reporter produced a large reduction of labeled OHCs.  
93 However, the number of reporter-positive SCs also decreased. Reducing the tamoxifen  
94 dose in *Sox2<sup>CreERT2+/-::ROSA26<sup>tdTomato/+</sup></sup>* mice produced a minimal effect. However,  
95 changing to the *CAG-eGFP* reporter reduced reporter-positive HCs and SCs. *Plp-*  
96 *CreERT<sup>2</sup>* is largely expressed in IPhCs/BCs of the neonatal cochlea with some PCs/DCs  
97 also labeled. Reduction in tamoxifen dose had a minimal effect when the  
98 *ROSA26<sup>tdTomato</sup>* reporter was used. However, using the *CAG-eGFP* reporter with a  
99 reduced tamoxifen dose, fewer PCs/DCs were labeled, but this also decreased the  
100 number of reporter-positive IPhCs/BCs. As previously described, *Prox1<sup>CreERT2</sup>* is limited  
101 to PCs/DCs in the neonatal cochlea, with relatively fewer IPCs labeled. Alterations in  
102 tamoxifen dose caused few changes in labeled cells.

## 103 104 **Materials and Methods**

### 105 **Mice**

106 *Sox2<sup>CreERT2</sup>* (stock #17593; Arnold et al., 2011), *Plp-CreERT<sup>2</sup>* (stock # 5975; Doerflinger  
107 et al., 2003), and *ROSA26<sup>CAG-loxP-stop-loxP-tdTomato</sup>* (*ROSA26<sup>tdTomato</sup>*) mice, also referred to  
108 as Ai14, (stock #7914; Madisen et al., 2010) were obtained from The Jackson  
109 Laboratory (Bar Harbor, ME). *Fgfr3-iCreERT<sup>2</sup>* mice (Rivers et al., 2008; Young et al.,  
110 2010) were provided by Dr. William Richardson (University College London, UK),  
111 *Prox1<sup>CreERT2</sup>* mice (Srinivasan et al., 2007) were provided by Dr. Guillermo Oliver (St.  
112 Jude Children’s Research Hospital, Memphis, TN) and *CAG-loxP-stop-loxP-eGFP*  
113 (*CAG-eGFP*) mice (Nakamura et al., 2006) were provided by Dr. Jeffery Robbins

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114 (Cincinnati Children’s Hospital, Cincinnati, OH). Genotyping for all mouse lines was  
115 performed by Transnetyx, Inc. (Cordova, TN). Mice of both genders were used and all  
116 animal work was performed in accordance with approved animal protocols from the  
117 Institutional Animal Care and Use Committee at Southern Illinois University School of  
118 Medicine.

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**Tamoxifen Injections**

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121 CreER recombination was induced by intraperitoneal (IP) injections of tamoxifen  
122 (Sigma-Aldrich – St. Louis, MO) dissolved in 100% corn oil. Mice received one injection  
123 per day on either postnatal day (P) 0, P1, or on both P0 and P1 with the dose per  
124 injection ranging from 0.3 mg/40 g to 5 mg/40 g. Stock solutions were diluted so that  
125 the total volume of each injection remained the same. To measure CreER leakiness,  
126 samples that were CreER-positive and reporter-positive but did not receive tamoxifen  
127 were analyzed as controls. Samples were evaluated between P5-P7.

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**Immunostaining**

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130 Samples were post-fixed in 4% paraformaldehyde overnight (Polysciences, Inc –  
131 Warrington, PA) and subsequently stored in 10 mM Phosphate Buffered Saline (Sigma-  
132 Aldrich – St. Louis, MO) at 4° C. Cochleae were dissected into apical, middle, and  
133 basal sections using a whole-mount or surface preparation method. Routine  
134 immunostaining was performed on free-floating cochlear turns as previously described  
135 (Montgomery and Cox, 2016) with the following primary antibodies: anti-myosin VIIa  
136 (1:200, cat #25-6790, Proteus BioSciences – Ramona, CA), anti-Sox2 (1:500, cat #sc-  
137 17320, Santa Cruz – Dallas, TX), anti-GFP (1:1000, cat #ab13970, Abcam –

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4 138 Cambridge, MA), and anti-GFP conjugated to Alexa 488 (1:50 cat#A21311, Invitrogen –  
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7 139 Grand Island, NY). All secondary antibodies were Alexa-conjugated from Invitrogen  
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9 140 (Waltham, MA) and were used at a 1:1000 dilution. Images were taken using a Leica  
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11 141 SP5 confocal microscope and image analysis was performed using Leica LAS AF LITE  
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14 142 software.

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### 19 144 **Cell counts**

22 145 HCs and SCs were identified by immunostaining for myosin VIIa and Sox2, respectively.  
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24 146 Myosin VIIa is expressed in the cytoplasm of HCs and Sox2 is expressed in SC nuclei,  
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26  
27 147 while both tdTomato and eGFP are expressed throughout the cell in both cytoplasm and  
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29 148 nuclei. SC subtypes that expressed either the tdTomato or eGFP reporter were  
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32 149 quantified in two randomly chosen 200  $\mu$ m regions per cochlear turn, averaged, and  
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34 150 expressed as a percentage of labeled cells compared to total cells. For the *Fgfr3*-  
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36 151 *iCreERT2* and *Sox2<sup>CreERT2</sup>* lines, all labeled SC subtypes were pooled into one value. For  
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39 152 the *Plp-CreERT2* and *Prox1<sup>CreERT2</sup>* lines, SC subtypes were counted individually for  
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41 153 IPCs, OPCs, DCs, and IPhC/BCs.

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46 155 Reporter labeling in HCs was detected in *Fgfr3-iCreERT2* and *Sox2<sup>CreERT2</sup>* lines. For the  
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49 156 *ROSA26<sup>tdTomato</sup>* reporter, the entire organ of Corti was imaged, measured, and divided  
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51 157 into six equal sections for quantification of tdTomato-positive HCs to determine if a  
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54 158 gradient of tdTomato expression was present. For the *CAG-eGFP* reporter, eGFP-  
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56 159 positive HCs were quantified in 2 sections: a 250  $\mu$ m region at the most apical tip  
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59 160 (where the majority of eGFP-positive cells were found) and the rest of the organ of



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4 161 Corti. Data for both reporter lines are expressed as a percentage of the reporter-positive  
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6 162 HCs compared to the total HCs within each region.  
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## 11 164 **Statistical analysis**

13  
14 165 All data are presented as mean  $\pm$  SEM. One-way or two-way ANOVA followed by  
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16 166 Tukey's post-hoc tests, Student's *t*-test, and Pearson's correlations were performed  
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19 167 using Graphpad Prism 6.0 2 (Graphpad Software Inc – La Jolla, CA).  
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## 26 170 **Results**

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30 171 The CreER/loxP system allows cell-type specific gene expression through the use of  
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32 172 cell-type specific promoters that drive expression of the Cre enzyme. Temporal control  
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35 173 is achieved by fusing a modified estrogen receptor (ER) to Cre, which restricts the  
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38 174 CreER protein to the cytoplasm. Only in the presence of tamoxifen will CreER  
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40 175 translocate to the nucleus for excision of loxP sites (Feil et al., 1996; Hayashi and  
41  
42 176 McMahon, 2002). Using four independent CreER mouse lines (*Fgfr3-iCreERT2*,  
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44 177 *Sox2<sup>CreERT2</sup>*, *Plp-CreERT2*, and *Prox1<sup>CreERT2</sup>*) that have previously been shown to label  
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47 178 broad populations of SCs in the neonatal cochlea, we sought to label subpopulations of  
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50 179 SCs by altering the tamoxifen induction paradigm and/or reporter line.  
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56 181 The *ROSA26<sup>tdTomato</sup>* reporter line was developed to improve the fluorescent labeling of  
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59 182 cells in a Cre-dependent manner over standard reporter lines such as *CAG-eGFP*. It  
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183 uses two ubiquitously expressed promoters (*ROSA26* and *CAG*) to drive expression of  
184 tdTomato, as well as the woodchuck hepatitis virus post-transcriptional regulatory  
185 element (*WPRE*) to enhance mRNA stability. Endogenous fluorescence of tdTomato is  
186 ~2.8 times brighter than eGFP and is readily detected, whereas as detection of eGFP  
187 often requires amplification with immunostaining prior to imaging (Madisen et al., 2010;  
188 Madisen et al., 2015). Therefore different patterns of reporter labeling are seen when  
189 *ROSA26<sup>tdTomato</sup>* and *CAG-eGFP* reporter lines are used with the same CreER allele and  
190 tamoxifen induction paradigm. We also tested for Cre leakage, which can occur when  
191 some of the CreER enzyme enters the nucleus in the absence of tamoxifen. To  
192 determine the leakiness of these four CreER lines, we performed control experiments  
193 using CreER-positive::reporter-positive mice that did not receive tamoxifen.

194

195 ***Fgfr3-iCreER<sup>T2</sup>***

196 Fibroblast growth factor receptor 3 (*Fgfr3*) is necessary for development of PCs in the  
197 organ of Corti (Colvin et al., 1996). At P0, *Fgfr3* expression is thought to be confined to  
198 PCs and DCs in the lateral compartment of the cochlea (Peters et al., 1993; Mueller et  
199 al., 2002; Pirvola et al., 2002; Hayashi et al., 2007). However recent single-cell RNA-  
200 seq analyses have detected a low level of *Fgfr3* expression in some HCs at P1-2 (Burns  
201 et al., 2015; Waldhaus et al., 2015). The *Fgfr3-iCreER<sup>T2</sup>* mouse line is a transgenic  
202 allele, where CreER is driven by the *Fgfr3* promoter (Young et al., 2010). Previous  
203 characterization of the *Fgfr3-iCreER<sup>T2</sup>* allele showed that after tamoxifen injection at  
204 neonatal ages, reporter expression was detected in the vast majority of PCs and DCs

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4 205 within the organ of Corti (Cox et al., 2012). Specifically when *Fgfr3-*  
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6 206 *iCreERT2/+::ROSA26<sup>tdTomato/+</sup>* mice were injected with tamoxifen (3 mg/40 g, IP) at both  
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9 207 P0 and P1, 100% of PCs and DCs expressed tdTomato. However, there were also 25-  
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11 208 75% of OHCs labeled (Cox et al., 2012). To verify this expression pattern and to  
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14 209 establish a basis for comparison, we repeated this induction protocol in *Fgfr3-*  
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16 210 *iCreERT2/+::ROSA26<sup>tdTomato/+</sup>* mice and found that 94.4 %  $\pm$  5.5% of PCs and DCs were  
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19 211 labeled throughout the organ of Corti (Figure 1B, Table 1). Similar to previously  
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21 212 published results, this dosing paradigm also showed robust labeling of OHCs (43.8%  $\pm$   
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23 213 2.9%; Figure 2B, Table 1). To investigate whether there was a gradient of HC labeling  
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25  
26 214 across cochlear turns, the organ of Corti was divided into six sections of equal length  
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29 215 (Figure 2A) and the percentage of labeled OHCs was counted within each section. The  
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31 216 percentage of tdTomato+ OHCs declined in an apical to basal gradient (98.5%  $\pm$  0.7%  
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33 217 in the first segment of apex and 13.2%  $\pm$  3.1% in the last segment of the base; Pearson  
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36 218 correlation coefficient  $r^2 = 0.81$ ,  $P = 0.049$ ; Figure 2B, Table 1). Three tdTomato-positive  
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38 219 IHCs were detected in the apical tip of one sample (data not shown). In addition, no  
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41 220 tdTomato-positive SCs or HCs were detected in controls that were not treated with  
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43 221 tamoxifen (Figure 1B-E, 2B-E).

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50 223 To attenuate HC labeling and target only PCs and DCs, Cre-mediated recombination  
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52 224 was induced with a 10-fold lower dose of tamoxifen (0.3 mg/40 g, IP) at P1 only. The  
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55 225 percentage of tdTomato-positive HCs was reduced to 3.9%  $\pm$  2.2% of total OHCs  
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57 226 across the entire cochlea (Figure 2B, F-M, Table 1). Yet the apical to basal gradient  
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60 227 remained with 14.6%  $\pm$  10.6% OHCs labeled in the first apical section and 0.2%  $\pm$  0.2%

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228 in the most basal section (Pearson correlation coefficient  $r^2 = 0.81$ ,  $P = 0.049$ ; Figure  
229 2B, F-M, Table 1). However, this dosing paradigm also reduced total PC/DC labeling to  
230 77.7%  $\pm$  4.9% (Figure 1B, F-H", Table 1).

231

232 Because HC labeling was still present with the reduced tamoxifen dose, *Fgfr3-iCreERT2*  
233 mice were next paired with the *CAG-eGFP* reporter. Using the previously published  
234 tamoxifen induction paradigm (3 mg/40 g, IP at P0/P1) in *Fgfr3-iCreERT2/+::CAG-*  
235 *eGFP+/loxP* mice, we observed 37.8%  $\pm$  3.4% eGFP-positive PCs and DCs throughout  
236 the cochlea (Figure 1B, L-N", Table 1). However, similar to the 0.3 mg/40 g tamoxifen  
237 paradigm in *Fgfr3-iCreERT2/+::ROSA26<sup>tdTomato/+</sup>* mice, 5.8%  $\pm$  2.6% eGFP-positive OHCs  
238 were still detected (Figure 3B, F-M, Table 1). Because the apical tip of the cochlea  
239 contained a large number of eGFP-positive HCs which quickly declined, the cochlea  
240 was divided into two sections for quantification: the first 250  $\mu$ m of the apex and the  
241 remainder of the cochlea (Figure 3A). Upon quantification, there was a significant  
242 difference between the two regions with the largest amount of OHC labeling in the most  
243 apical section (40.5%  $\pm$  6.1% compared to 4.4%  $\pm$  2.5%; paired Student's *t* test  $t(2) =$   
244 9.822,  $P = 0.0102$ ); Figure 3B, Table 1). No eGFP-positive SCs or HCs were observed in  
245 control animals that were not treated with tamoxifen (Figure 1B, I-K, 3B-E).

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247 **Sox2<sup>CreERT2</sup>**

248 *Sox2* is a transcription factor expressed in all SCs throughout the organ of Corti as well  
249 as in immature HCs (Hume et al., 2007; Dabdoub et al., 2008). Low levels of *Sox2*

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4 250 expression have been detected in some HCs at P1-2 using single-cell RNA-seq  
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7 251 analyses (Burns et al., 2015; Waldhaus et al., 2015). The *Sox2<sup>CreERT2</sup>* mouse line is a  
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9 252 knock-in allele, where CreER was inserted into the endogenous *Sox2* locus (Arnold et  
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12 253 al., 2011). *Sox2<sup>CreERT2</sup>* expression has previously been reported in both HCs and SCs in  
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14 254 the neonatal organ of Corti (Bramhall et al., 2014; Walters et al., 2015). When paired  
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16 255 with the *ROSA26<sup>tdTomato</sup>* reporter line and tamoxifen (3 mg/40 g, IP) given at P0/P1,  
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19 256 more than 85% of SCs and more than 50% of HCs throughout the cochlea were labeled  
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21 257 (Walters et al., 2015). To determine whether HC labeling could be reduced, we  
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24 258 performed a tamoxifen dose response analysis. For comparison, we repeated the  
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26 259 dosing paradigm from Walters et al., (2015). In *Sox2<sup>CreERT2+/-::ROSA26<sup>tdTomato/+</sup></sup>* mice  
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29 260 with tamoxifen (3 mg/40 g, IP) injected at P0/P1, 100%  $\pm$  0.0% of SCs from the GER to  
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31 261 HeCs throughout the cochlea expressed tdTomato (Figure 4A, Table 1). 35.3%  $\pm$  4.8%  
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33 262 tdTomato-positive HCs were also observed which appeared to decrease in a gradient  
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36 263 from apical to basal turn of the cochlea (Figure 5A, Table 1). To quantify this gradient,  
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38 264 we divided the organ of Corti into six segments of equal length as before (Figure 2A).  
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41 265 No difference was observed between the percentage of IHCs and OHCs labeled in any  
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43 266 of the six segments (Figure 5A). However, the percentage of tdTomato-positive HCs  
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46 267 was 97.2%  $\pm$  1.7% in the most apical segment which declined to 2.7%  $\pm$  1.2% in the  
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48 268 most basal segment (Figure 5A, Table 1; Pearson correlation coefficient  $r^2 = 0.996$ ,  $P =$   
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50 269 0.00002). In addition, *Sox2<sup>CreERT2+/-::ROSA26<sup>tdTomato/+</sup></sup>* mice showed some Cre leakiness  
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53 270 with approximately 20 tdTomato-positive SCs detected in the entire cochlea in controls  
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55 271 that did not receive tamoxifen (Figure 4A-D, 5A-D).  
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273 To determine if a reduced tamoxifen dose leads to a reduced number of labeled HCs,  
274 *Sox2<sup>CreERT2+/-</sup>::ROSA26<sup>tdTomato/+</sup>* mice were injected with tamoxifen (3 mg/40 g, IP) at P0  
275 only. Again, 99.9% ± 0% of SCs throughout the cochlea were labeled (Figure 4A, E-G”,  
276 Table 1). There was minimal change in the percentage of tdTomato-labeled HCs  
277 (34.5% ± 4.7%) with no significant reduction seen compared to the dual injection of  
278 tamoxifen (Figure 5A, E-L, Table 1).

279  
280 We next used *CAG-eGFP<sup>+/-loxP</sup>* mice to investigate whether a different reporter would  
281 minimize HC labeling. *Sox2<sup>CreERT2+/-</sup>::CAG-eGFP<sup>+/-loxP</sup>* mice were injected with tamoxifen  
282 (3 mg/40 g, IP) at P1 only. Unlike the *ROSA26<sup>tdTomato</sup>* reporter, no eGFP-positive cells  
283 were observed in controls that did not receive tamoxifen (Figure 4A, H-J, 6A-D). As  
284 predicted, there was a large reduction in the total number of eGFP-positive HCs with the  
285 majority of the labeled cells located in the apex (Figure 6A, E-L). Therefore, we  
286 quantified eGFP-positive HCs using the same parameters used for *FGFR3-*  
287 *iCreERT2+::CAG-eGFP<sup>+/-loxP</sup>* samples (Figure 3A). Throughout the entire organ of Corti,  
288 only 3.2%± 0.4% HCs were labeled with eGFP (Figure 6A, Table 1), which is far less  
289 than the number of tdTomato-positive HCs observed with tamoxifen induction at either  
290 P0 only (34.5%± 4.7%) or P0/P1 (35.3%± 4.8% Figure 5A, Table 1). Within the first 250  
291 µm of the apical tip in *Sox2<sup>CreERT2+/-</sup>::CAG-eGFP<sup>+/-loxP</sup>* mice, 44.8% ± 10.8% of HCs were  
292 eGFP-positive, however, only 1.6% ± 0.5% eGFP-positive HCs were observed  
293 throughout the remainder of the cochlea (paired Student’s *t* test  $t(3.967)= 2, P=0.0581,$   
294 Figure 6A, Table 1). Parallel to the reduction in eGFP-positive HCs, fewer SCs were  
295 labeled with eGFP throughout the cochlea (66.0% ± 5.9% compared to 100%± 0.0%

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296 tdTomato-positive SCs with tamoxifen given at P0/P1 and 99.9% ± 0.0% tdTomato-  
297 positive SCs with tamoxifen given at P0 only; Figure 4A, K-M", Table 1).

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299 ***Plp-CreER<sup>T2</sup>***

300 Proteolipid protein (*Plp*) is expressed in oligodendrocytes and Schwann cells of the  
301 central and peripheral nervous systems (Fuss et al., 2000; Mallon et al., 2002). In the  
302 mammalian cochlea, *Plp* is expressed in PCs and DCs in the late embryonic stage  
303 (Morris et al., 2006). *Plp-CreER<sup>T2</sup>* is a transgene that has previously been reported to  
304 label IPhCs and BCs, as well as some PCs and DCs in the neonatal organ of Corti  
305 (Doerflinger et al., 2003; Gomez-Casati et al., 2010; Cox et al., 2012; Mellado Lagarde  
306 et al., 2014). Specifically, *Plp-CreER<sup>T2/+</sup>::ROSA26<sup>eYFP/+</sup>* mice injected with tamoxifen (3  
307 mg/40 g, IP) at P0/P1 had 47% eYFP-positive IPhs/BCs, 3.4% eYFP-positive PCs, and  
308 5.2% eYFP-positive DCs with no statistical differences detected among cochlear turns  
309 (Liu et al., 2014). When paired with the *ROSA26<sup>LacZ</sup>* reporter and tamoxifen (33 mg/kg,  
310 IP) given from P0-P7, *Plp-CreER<sup>T2</sup>* was expressed only in SCs with the majority of  
311 labeled cells being IPhCs/BCs, but no quantification was given (Gomez-Casati et al.,  
312 2010). In *Plp-CreER<sup>T2/+</sup>::Rosa26<sup>tdTomato/+</sup>* mice given tamoxifen (3 mg/40 g, IP) at P0/P1,  
313 tdTomato expression was observed in 50% of IPhCs/BCs in the apex, 80% of  
314 IPhCs/BCs in the middle and base, and 5-10% of PCs and DCs throughout the whole  
315 cochlea (Cox et al., 2012). Similar to Cox et al. (2012), Mellado Lagarde et al., (2014)  
316 found fewer tdTomato-positive IPhCs/BCs in the apex (25.9%) compared to middle and  
317 basal turns (71.9% and 86.1%, respectively) with the same tamoxifen induction

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318 paradigm. In order to set a standard with which to compare alternate tamoxifen dosing  
319 paradigms, we repeated the tamoxifen dose published in Cox et al., (2012) and Mellado  
320 Lagarde et al., (2014). Similar to the previous reports, there was an apical to basal  
321 gradient of increasing numbers of tdTomato-positive SCs (Figure 7A, Table 1).  
322 Additionally, fewer IPhCs/BCs expressed tdTomato in the apex ( $48.2\% \pm 4.7\%$ )  
323 compared to the middle ( $79.9\% \pm 2.5\%$ ), and base ( $89.2\% \pm 3.7\%$ ; one-way ANOVA  
324  $F(1.313, 3.940)=71.03$ ,  $P=0.0010$  with Tukey's multiple comparisons test: Figure 8A,  
325 Table 1). Interestingly, this paradigm also showed SC labeling lateral to IHCs in PCs  
326 and DCs. Specifically,  $13.5\% \pm 4.6\%$  of IPCs and  $10.6\% \pm 2.0\%$  OPCs were labeled  
327 throughout the organ of Corti (Figure 8A-D, Table 1). There also appeared to be a  
328 gradient for DC labeling with fewer tdTomato-positive DCs in the apex ( $5.8\% \pm 1.9\%$ )  
329 and middle ( $9.8\% \pm 2.8\%$ ) than in the base ( $29.3\% \pm 4.7\%$ ; one-way ANOVA  $F(1.193,$   
330  $3.579)=39.29$ ,  $P=0.0044$  with Tukey's multiple comparisons test; Figure 8D, Table 1).

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332 We attempted to label only IPhCs/BCs by reducing the tamoxifen dose to one injection  
333 given at either P0 only or P1 only, while maintaining the same concentration (3 mg/40  
334 g). Expression of tdTomato in IPhCs/BCs of *Plp-CreERT2/+::Rosa26<sup>tdTomato/+</sup>* mice that  
335 received tamoxifen at P0 only did not differ from tamoxifen injections given at P1 only or  
336 at both P0/P1 (Figure 7E-J, 8A, Table 1). There was also no difference in the number of  
337 tdTomato-positive IPCs, OPCs, and DCs with a P0 only or P1 only injection compared  
338 to the P0/P1 injection, (Figure 7E-J, 8A-D, Table 1).

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340 Because reduction in tamoxifen dose did not reduce labeling of PCs/DCs, we next  
341 generated *Plp-CreER<sup>T2/+</sup>::CAG-eGFP<sup>+loxP</sup>* mice. When Cre recombination was induced  
342 with tamoxifen (3 mg/40 g, IP) at P0/P1, fewer IPhC/BC were labeled by eGFP than  
343 tdTomato in all turns of the cochlea (apex = 21.8% ± 10.9% eGFP-positive IPhCs/BCs  
344 compared to 48.2% ± 4.7% tdTomato-positive IPhCs/BCs; middle = 28.2% ± 2.1%  
345 eGFP-positive IPhCs/BCs compared to 79.9% ± 2.5% tdTomato-positive IPhCs/BCs;  
346 base = 38.1% ± 3.9% eGFP-positive IPhCs/BCs compared to 89.2% ± 3.7% tdTomato-  
347 positive IPhCs/BCs; two-way ANOVA  $F(2, 45)=30.39$ ,  $P<0.0001$  with Tukey's multiple  
348 comparisons test; Figure 7Q-S, 8A, Table 1). There was no difference in IPC, OPC, or  
349 DCs labeling between eGFP and tdTomato reporter lines with tamoxifen induction (3  
350 mg/40 g, IP) at P0/P1 (Figure 7Q-S, 8B-D, Table 1).

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352 Since minimal changes in the *Plp-CreER<sup>T2</sup>* expression pattern occurred with the *CAG-*  
353 *eGFP<sup>+loxP</sup>* reporter, we also investigated a lower dose of tamoxifen to reduce PC/DC  
354 labeling. Reducing the amount of tamoxifen given to *Plp-CreER<sup>T2/+</sup>::CAG-eGFP<sup>+loxP</sup>*  
355 mice to a single injection (3 mg/40 g, IP) at P0 only reduced the number of eGFP-  
356 positive cells only in specific turns of the cochlea. The eGFP-positive IPhCs/BCs in the  
357 middle turn were reduced (24.3% ± 5.9%) compared to *Plp-CreER<sup>T2/+</sup>::Rosa26<sup>tdTomato/+</sup>*  
358 mice (79.9% ± 2.5%; two-way ANOVA  $F(6, 45)=97.06$ ,  $P<0.0001$  with Tukey's multiple  
359 comparisons test; Figure 7N-P, 8A, Table 1). OPCs were also reduced in the middle  
360 turn (0.0% ± 0.0% eGFP-positive OPCs compared to 15.7% ± 3.4% tdTomato-positive  
361 OPCs; two-way ANOVA  $F(6, 60)=8.524$ ,  $P<0.0001$  with Tukey's multiple comparisons  
362 test) and DCs were reduced in the base (5.9% ± 4.8% eGFP-positive DCs compared to

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363 29.3% ± 4.7% tdTomato-positive DCs; two-way ANOVA  $F(6, 45)=6.313$ ,  $P<0.0001$  with  
364 Tukey's multiple comparisons test; Figure 7N-P, 8D, Table 1). Unlike *Fgfr3-iCreERT2*  
365 and *Sox2<sup>CreERT2</sup>*, neither *Plp-CreERT2/+::Rosa26<sup>tdTomato/+</sup>* nor *Plp-CreERT2+/-::CAG-*  
366 *eGFP<sup>+/-loxP</sup>* mice showed reporter expression in HCs throughout the cochlea. Finally, no  
367 tdTomato-positive or eGFP-positive cells were detected in *Plp-CreERT2* control mice  
368 which did not receive tamoxifen (Figure 7A-D, K-M, 8A-D).

370 ***Prox1<sup>CreERT2</sup>***

371 *Prox1* is a transcription factor that is expressed in IPCs, OPCs, and DCs throughout the  
372 neonatal organ of Corti, but is downregulated by ~P14 (Bermingham-McDonogh et al.,  
373 2006). The *Prox1<sup>CreERT2</sup>* mouse line is a knock-in allele, where CreER was inserted into  
374 the endogenous *Prox1* locus (Srinivasan et al., 2007). Previously, Yu et al. (2010)  
375 characterized *Prox1<sup>CreERT2</sup>* mice using *ROSA26<sup>LacZ</sup>* and *ROSA26<sup>eYFP</sup>* reporters and  
376 induced Cre recombination with tamoxifen injections (3 mg/40 g, IP) at P0 and P1. LacZ  
377 and eYFP expression were found to only label PCs and DCs, however a small number  
378 of cells were labeled (~13% in the apex, ~10% in the middle, and ~7% in the base, Yu  
379 et al., 2010). When the same tamoxifen induction paradigm was used with the  
380 *Rosa26<sup>tdTomato</sup>* reporter, tdTomato also labeled only PCs and DCs, but many more cells  
381 were labeled (Mellado Lagarde et al., 2013). As a basis for comparison, we replicated  
382 this paradigm and quantified tdTomato expression at P6. As previously reported,  
383 *Prox1<sup>CreERT2</sup>* expression was limited to PCs and DCs and no HC labeling was observed.  
384 The tdTomato labeling of PCs and DCs combined was higher in the apex (71.1% ±

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4 385 1.2%) and middle (57.2% ± 1.5%) compared to basal turns (44.3% ± 3.3%; one-way  
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7 386 ANOVA  $F(1.018, 2.036)=37.08$ ,  $P=0.0248$  with Tukey's multiple comparisons test;  
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9 387 Figure 9A). Similar to the previous report, there was differential labeling among SC  
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11 388 subtypes. Specifically, tdTomato was expressed in fewer IPCs (25.1%± 2.9%)  
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14 389 compared to OPCs (70.1%± 2.6%) and DCs (76.1% ± 0.1%; one-way ANOVA  $F(1.122,$   
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16 390  $2.245)=141.1$ ,  $P=0.0045$  with Tukey's multiple comparisons test; Figure 9N, Table 1). In  
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19 391 addition, the number of tdTomato-positive IPCs was higher in the apex (45.9% ± 1.6%)  
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21 392 compared to the base (7.4% ± 1.2%; one-way ANOVA  $F(1.021, 2.042)=35.11$ ,  
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24 393  $P=0.0260$  with Tukey's multiple comparisons test; Figure 9N, Table 1). There was no  
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26 394 difference in tdTomato-positive OPCs across turns; however, significantly fewer DCs  
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29 395 expressed tdTomato in the base (61.7% ± 4.0%) compared to the apex (89.6% ± 3.0%;)  
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31 396 and middle (80.7% ± 0.6%; one-way ANOVA  $F(1.302, 2.603)=18.53$ ,  $P=0.0299$  with  
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34 397 Tukey's multiple comparisons test; Figure 9P, Table 1). Our results are quite similar to  
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36 398 Mellado-Lagarde et al. (2013). No tdTomato-positive cells were detected in  
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38 399 *Prox1<sup>CreERT2+/-</sup>::Rosa26<sup>tdTomato/+</sup>* control samples that did not receive tamoxifen (Figure  
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41 400 9A-D, N-P).  
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47 402 Because so few IPCs were labeled with this induction paradigm, we attempted to  
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50 403 increase the number of tdTomato-positive IPCs by using a higher dose of tamoxifen. In  
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52 404 *Prox1<sup>CreERT2+/-</sup>::Rosa26<sup>tdTomato/+</sup>* mice injected (IP) with 5 mg/40 g tamoxifen at P0/P1, we  
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55 405 again observed fewer tdTomato-positive IPCs (24.5% ± 0.9%) than OPCs (71.4% ±  
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57 406 1.3%) or DCs (79.5%± 0.4%; one-way ANOVA  $F(1.038, 2.077)=927.6$ ,  $P=0.0009$  with  
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60 407 Tukey's multiple comparisons test; Figure 9K-P, Table 1) across the whole cochlea. We  
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408 did not observe an increase in tdTomato labeling in IPCs at this dose (24.5% ± 0.9%)  
409 compared to the 3 mg/40 g dose (25.1%± 2.9%), nor did increasing the dose increase  
410 tdTomato expression in OPCs or DCs (Figure 9K-M, O-P, Table 1). Interestingly,  
411 however, 17 OHCs and 1 IHC in the apical tip of one sample expressed tdTomato with  
412 the 5 mg/40 g dose (Figure 9Q-S).

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414 We next attempted to eliminate IPC labeling to make *Prox1<sup>CreERT2</sup>* label just OPCs and  
415 DCs using two different adjustments to the tamoxifen regimen. In *Prox1<sup>CreERT2+/+</sup>*  
416 *::Rosa26<sup>tdTomato/+</sup>* mice injected with tamoxifen (3 mg/40 g, IP) at P0 only, there were no  
417 differences in the number of tdTomato-positive IPCs, OPCs, or DCs compared to the  
418 P0/P1 injection with the same dose (Figure 9H-J, N-P, Table 1). Similarly, when the  
419 tamoxifen dose was reduced to 0.75 mg/40 g (IP) at P0 only, there was no significant  
420 difference in the number of tdTomato-positive IPCs (16.8% ± 4.0%) compared to the 3  
421 mg/40 g dose given at P0/P1 (25.1% ± 2.9%), however this number was significantly  
422 lower compared to the 3 mg/40 g dose given at P0 only (33.4% ± 2.4%; one-way  
423 ANOVA  $F(4, 10)=25.88$ ,  $P<0.0001$  with Tukey's multiple comparisons test, Figure 9E-G,  
424 N, Table 1). Additionally, tdTomato expression was reduced in OPCs (37.9% ± 2.5%;  
425 one-way ANOVA  $F(4, 10)= 201.3$ ,  $P<0.0001$  with Tukey's multiple comparisons test)  
426 and DCs (41.5% ± 2.4%; one-way ANOVA  $F(4, 10)= 519.5$ ,  $P<0.0001$  with Tukey's  
427 multiple comparisons test; Figure 9E-G, O-P, Table 1) compared to 3 mg/40 g given at  
428 either P0 (77.7% ± 3.4% OPCs and 77.9 ± 2.4% DCs) or P0/P1 (70.1% ± 2.6% OPCs  
429 and 76.1% ± 0.1% DCs).

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431 **Discussion**

432 Advances in the science of genetic modification have produced an arsenal of tools that  
433 can be used to study the expression or deletion of specific genes in specific cell types.  
434 This has allowed the development of more targeted therapeutics, as well as broadened  
435 our understanding of living organisms on a cellular and genetic level. To better employ  
436 these tools, we should understand their capabilities and limitations. To that end, we  
437 have endeavored to provide a guide for the use of four CreER mouse lines that are  
438 commonly used in the investigation of the neonatal organ of Corti. We were able to  
439 reduce HC labeling and increase labeling of SC subpopulations by reducing the  
440 tamoxifen dose when *Fgfr3-iCreER<sup>T2</sup>* mice were paired with the tdTomato reporter. In  
441 addition, changing to the *CAG-eGFP* reporter achieved this goal for both *Fgfr3-iCreER<sup>T2</sup>*  
442 and *Sox2<sup>CreERT2</sup>* mice; however this also reduced the total amount of SC labeling. Both  
443 the remaining two CreER lines only labeled SCs with the previously used tamoxifen  
444 doses and no changes in tamoxifen dose altered the expression pattern in *Prox1<sup>CreERT2</sup>*  
445 mice. However, a handful of HCs in the apical tip of the cochlea were labeled when we  
446 increased the tamoxifen dose. Altering tamoxifen dose had little impact on *Plp-CreER<sup>T2</sup>*  
447 activity. Although when paired with the *CAG-eGFP* reporter, the number of labeled  
448 PCs/DCs decreased, fewer IPhCs/BCs labeled as well. Importantly, we learned that for  
449 all CreER lines a single dose of tamoxifen at P0 is equivalent to doses given at both P0  
450 and P1 using the tdTomato reporter. This is an important finding because reduced  
451 tamoxifen exposure may reduce toxicity and mortality.

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4 453 The four CreER lines we chose to study use promoters expressed at high levels in  
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7 454 postnatal SCs to drive expression of CreER and therefore their expression patterns  
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9 455 were previously expected to label only those cell types. However, low levels of *Fgfr3*  
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12 456 and *Sox2* expression have been detected in some HCs at P1-2 using single-cell RNA-  
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14 457 seq analyses (Burns et al., 2015; Waldhaus et al., 2015), which correspond with our  
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16 458 results using *Sox2<sup>CreERT2</sup>* and *Fgfr3-iCreERT2*. Interestingly, *Fgfr3-iCreERT2* HC  
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19 459 expression occurred primarily in OHCs, with only a handful of labeled IHC in the apical  
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21 460 tip. *Fgfr3* expression begins at approximately embryonic day (E) 15.5 in the region  
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24 461 where PCs, DCs, and OHCs will form (Peters et al., 1993; Pirvola et al., 1995; Mueller  
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26 462 et al., 2002; Hayashi et al., 2007). Therefore it is possible that *Fgfr3* is not expressed in  
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29 463 the progenitor cells that give rise to IHCs. Alternatively, since *Fgfr3-iCreERT2* is a  
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31 464 transgenic allele, expression could be affected by the location of the transgene insertion  
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34 465 site in the genome. Importantly, reducing the tamoxifen dose 10-fold (from 3 mg/40 g  
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36 466 given at both P0/P1 to 0.3 mg/40 g given at P1 only) caused a significant reduction in  
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38 467 the amount of labeled HCs (from ~44% to ~ 4%), while there was a minimal reduction in  
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41 468 PC and DC labeling. In contrast, *Sox2<sup>CreERT2</sup>* activity was detected in both IHCs and  
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43 469 OHCs. This was expected since *Sox2* is expressed throughout the developing cochlea  
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46 470 (Kiernan et al., 2005; Hume et al., 2007; Dabdoub et al., 2008). In addition, immature  
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48 471 HCs are known to express *Sox2* in the first postnatal week (Kiernan et al., 2005; Hume  
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51 472 et al., 2007; Walters et al., 2015) which overlaps with the ages when tamoxifen was  
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53 473 injected. When the *Rosa26<sup>tdTomato</sup>* reporter was paired with *Sox2<sup>CreERT2</sup>*, the number of  
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55 474 tdTomato-positive HCs did not change with varying doses of tamoxifen. Switching to the  
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475 CAG-eGFP reporter was able to reduce HC expression (from ~ 35% to ~3%), however  
476 it also reduced SC labeling (~100% to ~66%).

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478 Two of the four CreER lines investigated (*Sox2<sup>CreERT2</sup>* and *Fgfr3-iCreERT2*) expressed  
479 an apical to basal decreasing gradient of reporter expression in HCs with both  
480 *ROSA26<sup>tdTomato</sup>* and CAG-eGFP reporter lines. *Prox1<sup>CreERT2</sup>* also showed an apical to  
481 basal decreasing gradient of reporter expression in SC subtypes. Cochlear HCs and  
482 SCs are derived from the same pool of progenitor cells (Yang et al., 2010; Cai et al.,  
483 2013; Driver et al., 2013) and the organ of Corti is still maturing during the first postnatal  
484 week. Therefore genes necessary for progenitor cells are being downregulated, while  
485 those required for the transition from a progenitor cell fate to a HC or SC fate are being  
486 upregulated during the first few days after birth. *Atoh1* is one of the first genes to be  
487 expressed prior to HC commitment and is upregulated at ~E13.5 in the basal turn of the  
488 cochlea (Woods et al., 2004). Between ~E13.5 and E15.5, *Atoh1* expression extends  
489 throughout the rest of the cochlea allowing IHCs to differentiate first, followed by OHCs  
490 and then SCs (Kelley, 2007). Therefore, cells in the basal turn of the cochlea are  
491 approximately two days more mature than cells in the apical turn. Genes that are  
492 expressed in early cochlear differentiation like *Sox2*, *Prox1*, and *Fgfr3* would have  
493 higher expression levels in the less mature cells of the apical turn at P0, matching the  
494 gradient of CreER activity we observed. However, the lack of gradient seen for SCs in  
495 *Sox2<sup>CreERT2</sup>* mice likely occurred because expression of *Sox2* persists in SCs throughout  
496 the life of the animal (Oesterle et al., 2008). Interestingly, *Plp-CreERT2* showed the  
497 opposite gradient with increased numbers of labeled IPhCs/BCs and DCs in middle and

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4 498 basal turns compared to the apex. Maturation of the organ of Corti also occurs in a  
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7 499 wave from the base to the apex of the cochlea and therefore genes required for  
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9 500 differentiation are first expressed in the base. Little is known about how *Plp* expression  
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11 501 patterns change in the developing cochlea. However our data may suggest that *Plp* is  
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14 502 expressed later than *Sox2*, *Prox1*, and *Fgfr3* and only in cells that have committed to a  
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16 503 SC fate as part of the maturation process.  
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21 505 Of note, *Prox1<sup>CreERT2</sup>* labeled more OPCs and DCs compared to IPCs. This was  
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23 506 somewhat surprising since PROX1 is detected in all IPCs using immunostaining  
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26 507 (Bermingham-McDonogh et al., 2006; Cox et al., 2014), however there are known  
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28 508 differences between IPCs located in the medial compartment of the cochlea and  
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31 509 OPCs/DCs located in the lateral compartment. For example, *Hes5* is detected in OPCs,  
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33 510 DCs, and IPhCs/BCs, but not IPCs (Cox et al., 2014) and *CD44* is expressed only in  
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36 511 OPCs (Hertzano et al., 2010). Similarly, *Lgr5* is expressed in IPCs, IPhCs/BCs, and the  
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38 512 third row of DCs, but not in the rest of the DC pool or in OPCs (Chai et al., 2011; Shi et  
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41 513 al., 2012). Taken together, even though IPCs and OPCs are both classified as PCs and  
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43 514 have similar morphologies, they are very distinct cell types. Another interesting result  
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46 515 obtained with *Prox1<sup>CreERT2</sup>* was the expression of the tdTomato reporter in a handful of  
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48 516 HCs in the apical tip when the highest tamoxifen dose (5 mg/40 g, P0/P1) was given. In  
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51 517 the embryonic cochlea, *Prox1* is expressed in progenitor cells beginning at ~E14.5 and  
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53 518 is downregulated in cells as they take on a HC fate. By ~E18.5 *Prox1* is not detectable  
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56 519 in HCs using immunostaining (Bermingham-McDonogh et al., 2006); however, the  
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58 520 sensitivity of antibodies is known to be limited and low levels of *Prox1* may persist in  
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521 neonatal HCs of the apical turn that is revealed when a high dose of tamoxifen is  
522 combined with the *ROSA26<sup>tdTomato</sup>* reporter.

523  
524 Interestingly, *Sox2<sup>CreERT2</sup>* showed differential Cre leakiness when paired with the two  
525 different reporter lines. Cre leakiness refers to the phenomenon where some CreER  
526 molecules are able to enter the nucleus and excise loxP sites in the absence of  
527 tamoxifen. With the *Rosa26<sup>tdTomato</sup>* reporter, there were several tdTomato-positive SCs  
528 in the organ of Corti, but no eGFP-positive SCs were detected when the *CAG-*  
529 *eGFP<sup>+/-loxP</sup>* reporter was used. Because *Rosa26<sup>tdTomato</sup>* produces a more stable and  
530 brighter fluorescent protein than *CAG-eGFP<sup>+/-loxP</sup>* (Madisen et al., 2010), it is possible  
531 that smaller quantities of CreER enzyme that translocated into the nucleus in the  
532 absence of tamoxifen were sufficient to induce tdTomato expression. However, no  
533 tdTomato-positive cells were detected in the controls for *Fgfr3-iCreERT2*, *Plp-CreERT2*,  
534 or *Prox1<sup>CreERT2</sup>*.

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537 For this study, we used two reporter lines to assess the activity of four CreER lines in  
538 the neonatal organ of Corti. However, each floxed allele is different because the location  
539 of the loxP sites within the gene and the distance between loxP sites affects the  
540 efficiency of the Cre enzyme to bind and excise DNA located between loxP sites (Sauer  
541 and Henderson, 1989; Kuhn et al., 1995; Feltri et al., 1999; Kellendonk et al., 1999).  
542 While our study is primarily useful for fate-mapping purposes, it is difficult to translate  
543 the reporter expression patterns we measured to other floxed alleles. Some alleles may  
544 be similar to the *ROSA26<sup>tdTomato</sup>* reporter, while others may closely resemble the *CAG-*

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4 545 *eGFP* reporter line. This illustrates the importance of using more than one reporter line  
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6 546 to understand the expression pattern of any CreER line used to delete or overexpress a  
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9 547 gene. In addition, it would be wise to use immunostaining to confirm that the gene  
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11 548 deletion/overexpression pattern matches the reporter data. Overall, the best method for  
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14 549 CreER induction when using the *ROSA26<sup>tdTomato</sup>* reporter appears to be one injection of  
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16 550 tamoxifen at 3 mg/40 g given at P0 because it induces similar CreER activity as two  
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19 551 injections without the increased risk of toxicity. Depending on the goal of the study,  
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21 552 either the *ROSA26<sup>tdTomato</sup>* reporter or *CAG-eGFP* reporter is valuable, however the  
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24 553 *CAG-eGFP* labeled fewer cells with all CreER lines investigated.  
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39  
40  
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## 54 55 566 **Conflict of Interest**

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59 567 All authors declare no conflict of interest on the present manuscript.  
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## References

- Abrashkin KA, Izumikawa M, Miyazawa T, Wang CH, Crumling MA, Swiderski DL, Beyer LA, Gong TW, Raphael Y (2006) The fate of outer hair cells after acoustic or ototoxic insults. *Hear Res* 218:20-29.
- Anttonen T, Belevich I, Kirjavainen A, Laos M, Brakebusch C, Jokitalo E, Pirvola U (2014) How to bury the dead: elimination of apoptotic hair cells from the hearing organ of the mouse. *J Assoc Res Otolaryngol* 15:975-992.
- Arnold K, Sarkar A, Yram MA, Polo JM, Bronson R, Sengupta S, Seandel M, Geijsen N, Hochedlinger K (2011) Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem cell* 9:317-329.
- Birmingham-McDonogh O, Oesterle EC, Stone JS, Hume CR, Huynh HM, Hayashi T (2006) Expression of Prox1 during mouse cochlear development. *J Comp Neurol* 496:172-186.
- Boettger T, Hubner CA, Maier H, Rust MB, Beck FX, Jentsch TJ (2002) Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter Kcc4. *Nature* 416:874-878.
- Bramhall NF, Shi F, Arnold K, Hochedlinger K, Edge AS (2014) Lgr5-positive supporting cells generate new hair cells in the postnatal cochlea. *Stem Cell Rep* 2:311-322.
- Burns JC, Kelly MC, Hoa M, Morell RJ, Kelley MW (2015) Single-cell RNA-Seq resolves cellular complexity in sensory organs from the neonatal inner ear. *Nat Commun* 6:8557.
- Cai T, Seymour ML, Zhang H, Pereira FA, Groves AK (2013) Conditional deletion of Atoh1 reveals distinct critical periods for survival and function of hair cells in the organ of Corti. *J Neurosci* 33:10110-10122.
- Chai R, Xia A, Wang T, Jan TA, Hayashi T, Birmingham-McDonogh O, Cheng AG (2011) Dynamic expression of Lgr5, a Wnt target gene, in the developing and mature mouse cochlea. *J Assoc Res Otolaryngol* 12:455-469.
- Colvin JS, Bohne BA, Harding GW, McEwen DG, Ornitz DM (1996) Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. *Nature Genetics* 12:390-397.
- Corwin JT, Warchol ME (1991) Auditory hair cells: structure, function, development, and regeneration. *Annu Rev Neurosci* 14:301-333.
- Cox BC, Liu Z, Lagarde MM, Zuo J (2012) Conditional gene expression in the mouse inner ear using Cre-loxP. *J Assoc Res Otolaryngol* 13:295-322.
- Cox BC, Chai R, Lenoir A, Liu Z, Zhang L, Nguyen D, Chalasani K, Steigelman KA, Fang J, Rubel EW, Cheng AG, Zuo J (2014) Spontaneous hair cell regeneration in the neonatal mouse cochlea in vivo. *Development* 141:816-829.
- Dabdoub A, Puligilla C, Jones JM, Fritsch B, Cheah KS, Pevny LH, Kelley MW (2008) Sox2 signaling in prosensory domain specification and subsequent hair cell differentiation in the developing cochlea. *Proc Natl Acad Sci U S A* 105:18396-18401.

1  
2  
3  
4 611 Denman-Johnson K, Forge A (1999) Establishment of hair bundle polarity and  
5 612 orientation in the developing vestibular system of the mouse. *J Neurocytol*  
6 613 28:821-835.  
7  
8 614 Doerflinger NH, Macklin WB, Popko B (2003) Inducible site-specific recombination in  
9 615 myelinating cells. *Genesis* 35:63-72.  
10 616 Driver EC, Sillers L, Coate TM, Rose MF, Kelley MW (2013) The Atoh1-lineage gives  
11 617 rise to hair cells and supporting cells within the mammalian cochlea. *Dev Biol*  
12 618 376:86-98.  
13  
14 619 Feil R, Brocard J, Mascrez B, LeMeur M, Metzger D, Chambon P (1996) Ligand-  
15 620 activated site-specific recombination in mice. *Proc Natl Acad Sci U S A*  
16 621 93:10887-10890.  
17  
18 622 Feltri ML, D'Antonio M, Previtali S, Fasolini M, Messing A, Wrabetz L (1999) P0-Cre  
19 623 transgenic mice for inactivation of adhesion molecules in Schwann cells. *Ann N Y*  
20 624 *Acad Sci* 883:116-123.  
21 625 Flores-Otero J, Xue HZ, Davis RL (2007) Reciprocal regulation of presynaptic and  
22 626 postsynaptic proteins in bipolar spiral ganglion neurons by neurotrophins. *J*  
23 627 *Neurosci* 27:14023-14034.  
24  
25 628 Furness DN, Hulme JA, Lawton DM, Hackney CM (2002) Distribution of the  
26 629 glutamate/aspartate transporter GLAST in relation to the afferent synapses of  
27 630 outer hair cells in the guinea pig cochlea. *J Assoc Res Otolaryngol* 3:234-247.  
28  
29 631 Fuss B, Mallon B, Phan T, Ohlemeyer C, Kirchhoff F, Nishiyama A, Macklin WB (2000)  
30 632 Purification and analysis of in vivo-differentiated oligodendrocytes expressing the  
31 633 green fluorescent protein. *Dev Biol* 218:259-274.  
32 634 Gomez-Casati ME, Murtie J, Taylor B, Corfas G (2010) Cell-specific inducible gene  
33 635 recombination in postnatal inner ear supporting cells and glia. *J Assoc Res*  
34 636 *Otolaryngol* 11:19-26.  
35  
36 637 Hayashi S, McMahon AP (2002) Efficient recombination in diverse tissues by a  
37 638 tamoxifen-inducible form of Cre: a tool for temporally regulated gene  
38 639 activation/inactivation in the mouse. *Developmental biology* 244:305-318.  
39 640 Hayashi T, Cunningham D, Bermingham-McDonogh O (2007) Loss of Fgfr3 leads to  
40 641 excess hair cell development in the mouse organ of Corti. *Dev Dyn* 236:525-533.  
41 642 Hertzano R, Puligilla C, Chan SL, Timothy C, Depireuz DA, Ahmed Z, Wolf J, Eisenman  
42 643 DJ, Friedman TB, Riazuddin S, Kelley MW, Strome SE (2010) CD44 is a marker  
43 644 for the outer pillar cells in the early postnatal mouse inner ear. *J Assoc Res*  
44 645 *Otolaryngol* 11(3):407-18.  
45  
46 646 Huang T, Cheng AG, Stupak H, Liu W, Kim A, Staecker H, Lefebvre PP, Malgrange B,  
47 647 Kopke R, Moonen G, Van De Water TR (2000) Oxidative stress-induced  
48 648 apoptosis of cochlear sensory cells: otoprotective strategies. *Int J Dev Neurosci*  
49 649 18:259-270.  
50  
51 650 Hudspeth AJ, Corey DP (1977) Sensitivity, polarity, and conductance change in the  
52 651 response of vertebrate hair cells to controlled mechanical stimuli. *Proc Natl Acad*  
53 652 *Sci U S A* 74:2407-2411.  
54  
55 653 Hume CR, Bratt DL, Oesterle EC (2007) Expression of LHX3 and SOX2 during mouse  
56 654 inner ear development. *Gene Expr Patterns* 7:798-807.  
57  
58 655 Kellendonk C, Tronche F, Casanova E, Anlag K, Opherck C, Schutz G (1999) Inducible  
59 656 site-specific recombination in the brain. *J Mol Biol* 285:175-182.  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 657 Kelley MW (2007) Cellular commitment and differentiation in the organ of Corti. *Int J*  
5 658 *Dev Biol* 51:571-583.  
6  
7 659 Kiernan AE, Pelling AL, Leung KK, Tang AS, Bell DM, Tease C, Lovell-Badge R, Steel  
8 660 KP, Cheah KS (2005) Sox2 is required for sensory organ development in the  
9 661 mammalian inner ear. *Nature* 434:1031-1035.  
10 662 Kikuchi T, Kimura RS, Paul DL, Takasaka T, Adams JC (2000) Gap junction systems in  
11 663 the mammalian cochlea. *Brain Res Brain Res Rev* 32:163-166.  
12  
13 664 Kuhn R, Schwenk F, Aguet M, Rajewsky K (1995) Inducible gene targeting in mice.  
14 665 *Science* 269:1427-1429.  
15 666 Lefebvre PP, Malgrange B, Lallemend F, Staecker H, Moonen G, Van De Water TR  
16 667 (2002) Mechanisms of cell death in the injured auditory system: otoprotective  
17 668 strategies. *Audiol Neurootol* 7:165-170.  
18  
19 669 LeMasurier M, Gillespie PG (2005) Hair-cell mechanotransduction and cochlear  
20 670 amplification. *Neuron* 48:403-415.  
21 671 Lewis J, Davies A (2002) Planar cell polarity in the inner ear: how do hair cells acquire  
22 672 their oriented structure? *J Neurobiol* 53:190-201.  
23  
24 673 Liu Z, Fang J, Dearman J, Zhang L, Zuo J (2014) In vivo generation of immature inner  
25 674 hair cells in neonatal mouse cochleae by ectopic atoh1 expression. *PloS one*  
26 675 9:e89377.  
27 676 Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD,  
28 677 Hawrylycz MJ, Jones AR, Lein ES, Zeng H (2010) A robust and high-throughput  
29 678 Cre reporting and characterization system for the whole mouse brain. *Nature*  
30 679 *Neurosci* 13:133-140.  
31  
32 680 Madisen L et al. (2015) Transgenic mice for intersectional targeting of neural sensors  
33 681 and effectors with high specificity and performance. *Neuron* 85:942-958.  
34  
35 682 Mallon BS, Shick HE, Kidd GJ, Macklin WB (2002) Proteolipid promoter activity  
36 683 distinguishes two populations of NG2-positive cells throughout neonatal cortical  
37 684 development. *J Neurosci* 22:876-885.  
38  
39 685 McDowell B, Davies S, Forge A (1989) The effect of gentamicin-induced hair cell loss  
40 686 on the tight junctions of the reticular lamina. *Hear Res* 40:221-232.  
41 687 Mellado Lagarde MM, Cox BC, Fang J, Taylor R, Forge A, Zuo J (2013) Selective  
42 688 ablation of pillar and deiters' cells severely affects cochlear postnatal  
43 689 development and hearing in mice. *J Neurosci* 33:1564-1576.  
44  
45 690 Mellado Lagarde MM, Wan G, Zhang L, Gigliello AR, McInnis JJ, Zhang Y, Bergles D,  
46 691 Zuo J, Corfas G (2014) Spontaneous regeneration of cochlear supporting cells  
47 692 after neonatal ablation ensures hearing in the adult mouse. *Proc Natl Acad Sci U*  
48 693 *S A* 111:16919-16924.  
49  
50 694 Montgomery SC, Cox BC (2016) Whole Mount Dissection and Immunofluorescence of  
51 695 the Adult Mouse Cochlea. *J Vis Exp.* 107: e53561.  
52 696 Morris JK, Maklad A, Hansen LA, Feng F, Sorensen C, Lee KF, Macklin WB, Fritzsche B  
53 697 (2006) A disorganized innervation of the inner ear persists in the absence of  
54 698 ErbB2. *Brain Res* 1091:186-199.  
55 699 Mueller KL, Jacques BE, Kelley MW (2002) Fibroblast growth factor signaling regulates  
56 700 pillar cell development in the organ of corti. *J Neurosci* 22:9368-9377.

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64  
65

701 Nakamura T, Colbert MC, Robbins J (2006) Neural crest cells retain multipotential  
702 characteristics in the developing valves and label the cardiac conduction system.  
703 *Circ Res* 98:1547-1554.

704 Oesterle EC, Campbell S, Taylor RR, Forge A, Hume CR (2008) Sox2 and JAGGED1  
705 expression in normal and drug-damaged adult mouse inner ear. *J Assoc Res*  
706 *Otolaryngol* 9:65-89.

707 Peters K, Ornitz D, Werner S, Williams L (1993) Unique expression pattern of the FGF  
708 receptor 3 gene during mouse organogenesis. *Dev Biol* 155:423-430.

709 Pirvola U, Ylikoski J, Palgi J, Lehtonen E, Arumae U, Saarna M (1992) Brain-derived  
710 neurotrophic factor and neurotrophin 3 mRNAs in the peripheral target fields of  
711 developing inner ear ganglia. *Proc Natl Acad Sci U S A* 89:9915-9919.

712 Pirvola U, Cao Y, Oellig C, Suoqiang Z, Pettersson RF, Ylikoski J (1995) The site of  
713 action of neuronal acidic fibroblast growth factor is the organ of Corti of the rat  
714 cochlea. *Proc Natl Acad Sci U S A* 92:9269-9273.

715 Pirvola U, Ylikoski J, Trokovic R, Hebert JM, McConnell SK, Partanen J (2002) FGFR1  
716 is required for the development of the auditory sensory epithelium. *Neuron*  
717 35:671-680.

718 Raphael Y, Altschuler RA (1991a) Scar formation after drug-induced cochlear insult.  
719 *Hear Res* 51:173-183

720 Raphael Y, Altschuler RA (1991b) Reorganization of cytoskeletal and junctional proteins  
721 during cochlear hair cell degeneration. *Cell Motil Cytoskeleton* 18:215-227.

722 Raphael Y, Altschuler RA (2003) Structure and innervation of the cochlea. *Brain Res*  
723 *Bull* 60:397-422.

724 Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessarlis N,  
725 Richardson WD (2008) PDGFRA/NG2 glia generate myelinating  
726 oligodendrocytes and piriform projection neurons in adult mice. *Nature Neurosci*  
727 11:1392-1401.

728 Rubel EW, Dew LA, Roberson DW (1995) Mammalian vestibular hair cell regeneration.  
729 *Science* 267:701-707.

730 Sauer B, Henderson N (1989) Cre-stimulated recombination at loxP-containing DNA  
731 sequences placed into the mammalian genome. *Nucleic Acids Res* 17:147-161.

732 Shi F, Kempfle JS, Edge AS (2012) Wnt-responsive Igr5-expressing stem cells are hair  
733 cell progenitors in the cochlea. *J Neurosci* 32:9639-9648.

734 Spicer SS, Schulte BA (1996) The fine structure of spiral ligament cells relates to ion  
735 return to the stria and varies with place-frequency. *Hear Res* 100:80-100.

736 Srinivasan RS, Dillard ME, Lagutin OV, Lin FJ, Tsai S, Tsai MJ, Samokhvalov IM, Oliver  
737 G (2007) Lineage tracing demonstrates the venous origin of the mammalian  
738 lymphatic vasculature. *Genes Dev* 21:2422-2432.

739 Sugawara M, Murtie JC, Stankovic KM, Liberman MC, Corfas G (2007) Dynamic  
740 patterns of neurotrophin 3 expression in the postnatal mouse inner ear. *J Comp*  
741 *Neurol* 501:30-37.

742 Taylor RR, Nevill G, Forge A (2008) Rapid hair cell loss: a mouse model for cochlear  
743 lesions. *J Assoc Res Otolaryngol* 9:44-64.

744 Tritsch NX, Bergles DE (2010) Developmental regulation of spontaneous activity in the  
745 Mammalian cochlea. *J Neurosci* 30:1539-1550.

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4 746 Tritsch NX, Yi E, Gale JE, Glowatzki E, Bergles DE (2007) The origin of spontaneous  
5 747 activity in the developing auditory system. *Nature* 450:50-55.  
6 748 Waldhaus J, Durruthy-Durruthy R, Heller S (2015) Quantitative High-Resolution Cellular  
7 749 Map of the Organ of Corti. *Cell Rep* 11:1385-1399.  
8 750 Walters BJ, Yamashita T, Zuo J (2015) Sox2-CreER mice are useful for fate mapping of  
9 751 mature, but not neonatal, cochlear supporting cells in hair cell regeneration  
10 752 studies. *Sci Rep* 5:11621.  
11 753 Woods C, Montcouquiol M, Kelley MW (2004) Math1 regulates development of the  
12 754 sensory epithelium in the mammalian cochlea. *Nature Neurosci* 7:1310-1318.  
13 755 Yang H, Xie X, Deng M, Chen X, Gan L (2010) Generation and characterization of  
14 756 Atoh1-Cre knock-in mouse line. *Genesis* 48:407-413.  
15 757 Young KM, Mitsumori T, Pringle N, Grist M, Kessar N, Richardson WD (2010) An  
16 758 Fgfr3-iCreER(T2) transgenic mouse line for studies of neural stem cells and  
17 759 astrocytes. *Glia* 58:943-953.  
18 760 Yu Y, Weber T, Yamashita T, Liu Z, Valentine MB, Cox BC, Zuo J (2010) In vivo  
19 761 proliferation of postmitotic cochlear supporting cells by acute ablation of the  
20 762 retinoblastoma protein in neonatal mice. *J Neurosci* 30:5927-5936.  
21 763 Zuccotti A, Kuhn S, Johnson SL, Franz C, Singer W, Hecker D, Geisler HS, Kopschall I,  
22 764 Rohbock K, Gutsche K, Dlugaiczyk J, Schick B, Marcotti W, Ruttiger L,  
23 765 Schimmang T, Knipper M (2012) Lack of brain-derived neurotrophic factor  
24 766 hampers inner hair cell synapse physiology, but protects against noise-induced  
25 767 hearing loss. *J Neurosci* 32:8545-8553.

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38 **Figure 1. *Fgfr3-iCreER<sup>T2</sup>* is expressed in the majority of PCs/DCs. A,** Cross-  
39 sectional view of the mouse organ of Corti with SC subtypes highlighted in different  
40 colors. The tunnel of Corti is partially open to represent the immature state of the  
41 771 cochlea. GER, greater epithelial ridge; SGN, spiral ganglion neurons. **B,**  
42 772 Quantification of total reporter-positive SCs in *Fgfr3-iCreER<sup>T2/+</sup>::ROSA26<sup>tdTomato/+</sup>* mice  
43 773 or *Fgfr3-iCreER<sup>T2/+</sup>::CAG-eGFP<sup>+loxP</sup>* mice given different doses of tamoxifen (TAM).  
44 774 Cells were quantified in two randomly chosen 200 μm regions per cochlear turn,  
45 775 averaged, and expressed as a percentage of labeled cells compared to total cells.  
46 776 (\*\**P*<0.01, \*\*\**P*<0.001 as determined by a two-way ANOVA *F*(3,24)=274.5 with a  
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779 Tukey's post-hoc test) (N =3-4). Representative confocal images of tdTomato (red)  
780 expression in *Fgfr3-iCreER<sup>T2/+</sup>::ROSA26<sup>tdTomato/+</sup>* controls without tamoxifen (C-E) and  
781 those given various doses of tamoxifen (F-H"). Representative confocal images of  
782 eGFP (green) expression in *Fgfr3-iCreER<sup>T2/+</sup>::CAG-eGFP<sup>+/-loxP</sup>* controls without  
783 tamoxifen (I-K) and those given various doses of tamoxifen (L-N"). Optical cross  
784 sections showing that the location of tdTomato (H'-H'") and eGFP (N'-N'") expression  
785 is primarily in SCs. Scale bar in C: 25  $\mu$ m and in H' and N': 6.25  $\mu$ m.

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787 **Figure 2. *Fgfr3-iCreER<sup>T2/+</sup>::ROSA26<sup>tdTomato/+</sup>* is robustly expressed in OHCs in an**  
788 **apical to basal gradient. A,** For quantification with the *ROSA26<sup>tdTomato/+</sup>* reporter line,  
789 the entire cochlea was imaged, measured, and divided into six equal sections. A, apex  
790 and B, base. **B,** Quantification of reporter-positive OHCs in *Fgfr3-*  
791 *iCreER<sup>T2/+</sup>::ROSA26<sup>tdTomato/+</sup>* mice given different doses of tamoxifen (TAM). All cells  
792 were quantified in each of the six segments, averaged, and expressed as a percentage  
793 of labeled cells compared to total cells. (N =3-4). Representative confocal images of  
794 tdTomato (red) expression in *Fgfr3-iCreER<sup>T2/+</sup>::ROSA26<sup>tdTomato/+</sup>* controls without  
795 tamoxifen (C-E) and those given various doses of tamoxifen (F-K). **L-M,** higher  
796 magnification images from regions marked in H and K respectively. Scale bars: 25  $\mu$ m.

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798 **Figure 3. *Fgfr3-iCreER<sup>T2/+</sup>::CAG-eGFP<sup>+/-loxP</sup>* labels fewer HCs except for the apical**  
799 **tip of the cochlea. A,** For quantification with the *CAG-eGFP* reporter line, the cochlea  
800 was divided into two sections: the apical most 250  $\mu$ m and the rest of the cochlea. A,



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801 apex and B, base. **B**, Quantification of reporter-positive OHCs in *Fgfr3-iCreERT2/+::CAG-*  
802 *eGFP+/loxP* mice given different doses of tamoxifen (TAM). All cells were quantified in  
803 the two segments, averaged, and expressed as a percentage of labeled cells compared  
804 to total cells. (\* $P \leq 0.0101$ , as determined by a paired Student's *t* test  $t(2) = 9.867$ ) (N  
805 =3-4). Representative confocal images of eGFP (green) expression in *Fgfr3-*  
806 *iCreERT2/+::CAG-eGFP+/loxP* controls without tamoxifen (**C-E**) and those given various  
807 doses of tamoxifen (**F-K**). **L-M**, higher magnification images from regions marked in H  
808 and K respectively. Scale bars: 25  $\mu\text{m}$ .

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**Figure 4. Sox2<sup>CreERT2</sup> is expressed in all SCs within the organ of Corti. A,**  
810 Quantification of total reporter-positive SCs in *Sox2<sup>CreERT2+/-::ROSA26<sup>tdTomato</sup></sup>* mice or  
811 *Sox2<sup>CreERT2+/-::CAG-eGFP+/loxP</sup>* mice given different doses of tamoxifen (TAM). Cells  
812 were quantified in two randomly chosen 200  $\mu\text{m}$  regions per cochlear turn, averaged,  
813 and expressed as a percentage of labeled cells compared to total cells. (\*\*\*) $P < 0.0001$  as  
814 determined by a two-way ANOVA  $F(4,33) = 462.3$  with a Tukey's post-hoc test) (N =3-4).  
815 Representative confocal images of tdTomato (red) expression in *Sox2<sup>CreERT2+/-</sup>*  
816 *::ROSA26<sup>tdTomato</sup>* controls without tamoxifen (**B-D**) and those given various doses of  
817 tamoxifen (**E-G**”). Representative confocal images of eGFP (green) expression in  
818 *Sox2<sup>CreERT2+/-::CAG-eGFP+/loxP</sup>* controls without tamoxifen (**H-J**) and those given various  
819 doses of tamoxifen (**K-M**”). Optical cross sections showing location of tdTomato (**G'-**  
820 **G**”) and eGFP (**M'-M**”) expression is in SCs and some HCs. Scale bar in B: 25  $\mu\text{m}$   
821 and in G' and M': 6.25  $\mu\text{m}$ .

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**Figure 5. *Sox2<sup>CreERT2+/-</sup>::ROSA26<sup>tdTomato</sup>* is expressed in a large number of HCs in a descending apical to basal gradient. A,** Quantification of reporter-positive HCs in *Sox2<sup>CreERT2+/-</sup>::ROSA26<sup>tdTomato</sup>* mice given different doses of tamoxifen (TAM). All cells were quantified in each of the six segments labeled in Figure 2A, averaged, and expressed as a percentage of labeled cells compared to total cells. (For comparison of OHCs  $***P = 0.00005$  as determined by a Pearson correlation coefficient  $r^2 = 0.994$ , for comparison of IHCs  $***P = 0.0003$  as determined by a Pearson correlation coefficient  $r^2 = 0.985$ ) (N =3-4). Representative confocal images of tdTomato (red) expression in *Sox2<sup>CreERT2+/-</sup>::ROSA26<sup>tdTomato</sup>* controls without tamoxifen (**B-D**) and those given various doses of tamoxifen (**E-J**). **K-L**, higher magnification images from regions marked in G and J respectively. Scale bars: 25  $\mu$ m.

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**Figure 6. *Sox2<sup>CreERT2+/-</sup>::CAG-eGFP<sup>+/-loxP</sup>* labels fewer HCs except for the most apical tip of the cochlea. A,** Quantification of reporter-positive HCs in *Sox2<sup>CreERT2+/-</sup>::CAG-eGFP<sup>+/-loxP</sup>* mice given different doses of tamoxifen (TAM). All cells were quantified in the two segments labeled in Figure 3A, averaged, and expressed as a percentage of labeled cells compared to total cells. (N =3-4). Representative confocal images of eGFP (green) expression in *Sox2<sup>CreERT2+/-</sup>::CAG-eGFP<sup>+/-loxP</sup>* controls without tamoxifen (**B-D**) and those given various doses of tamoxifen (**E-J**). **K-L**, higher magnification images from regions marked in G and J respectively. Scale bars: 25  $\mu$ m.

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**Figure 7. Targeting of *Plp-CreER<sup>T2</sup>* to IPhCs/BCs increased when the *CAG-eGFP* reporter was used.** **A**, Quantification of total reporter-positive SCs in the three cochlear turns of *Plp-CreER<sup>T2/+</sup>::ROSA26<sup>tdTomato</sup>* or *Plp-CreER<sup>T2/+</sup>::CAG-eGFP<sup>+/-loxP</sup>* mice given different doses of tamoxifen (TAM). Cells were quantified in two randomly chosen 200  $\mu$ m regions per cochlear turn, averaged, and expressed as a percentage of labeled cells compared to total cells. Representative confocal images of tdTomato (red) expression in *Plp-CreER<sup>T2/+</sup>::ROSA26<sup>tdTomato</sup>* controls without tamoxifen (**B-D**) and those given various doses of tamoxifen (**E-J**). Representative confocal images of eGFP (green) expression in *Plp-CreER<sup>T2/+</sup>::CAG-eGFP<sup>+/-loxP</sup>* controls without tamoxifen (**K-M**) and those given various doses of tamoxifen (**N-S**). Scale bar: 25  $\mu$ m. (For comparison among cochlear turns within the same tamoxifen dosing paradigm: 3 mg/40 g tamoxifen dose at P0 only \* $P < 0.05$  as determined by a one-way ANOVA  $F(1.016, 2.031) = 7.435$  with a Tukey's post-hoc test, for the 3 mg/40 g tamoxifen dose at P0/P1 \*\* $P < 0.01$  as determined by a one-way ANOVA  $F(1.346, 4.038) = 129.5$  with a Tukey's post-hoc test. For comparison across tamoxifen dosing paradigms: \* $P < 0.05$  and \*\*\* $P < 0.001$  as determined by a two-way ANOVA  $F(6,44) = 40.9$  with a Tukey's post-hoc test.) (N = 3-4).

**Figure 8. Quantification of reporter expression among SC subtypes in *Plp-CreER<sup>T2</sup>* mice.** SC subtypes that expressed the tdTomato or eGFP reporter were quantified as described in 7A. **A**, IPhCs/BCs (For comparison among cochlear turns within the same tamoxifen dosing paradigm: *ROSA26<sup>tdTomato</sup>* reporter with tamoxifen at P0/P1 \*\*\* $P < 0.001$  as determined by a one-way ANOVA  $F(1.313, 3.940) = 71.03$  with a Tukey's post-hoc test; and *CAG-eGFP<sup>+/-loxP</sup>* reporter with tamoxifen at P0 \* $P < 0.05$ ,

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4 868 \*\*\* $P < 0.001$  as determined by a one-way ANOVA  $F(1,012, 2,025)=268.8$  with a Tukey's  
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6 869 post-hoc test. For comparison across tamoxifen dosing paradigms: \*\* $P < 0.01$  and  
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9 870 \*\*\* $P < 0.001$  as determined by a two-way ANOVA  $F(6,45)=97.06$  with a Tukey's post-hoc  
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11 871 test). **B**, IPCs (For comparison across tamoxifen dosing paradigms: \* $P < 0.05$  as  
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13 872 determined by a two-way ANOVA  $F(6,45)=6.017$  with a Tukey's post-hoc test). **C**, OPCs  
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15 873 (For comparison across tamoxifen dosing paradigms: \* $P < 0.05$  as determined by a two-  
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17 874 way ANOVA  $F(6, 60)=8.524$  with a Tukey's post-hoc test). **D**, DCs (For comparison  
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19 875 among cochlear turns within the same tamoxifen dosing paradigm: *ROSA26<sup>tdTomato</sup>*  
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21 876 reporter with tamoxifen at P0/P1 \* $P < 0.05$  as determined by a one-way ANOVA  $F(1,193,$   
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23 877  $3.579)=39.29$  with a Tukey's post-hoc test and *ROSA26<sup>tdTomato</sup>* reporter with tamoxifen  
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25 878 at P1 \* $P < 0.05$  as determined by a one-way ANOVA  $F(2, 6)=5.189$  with a Tukey's post-  
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27 879 hoc test. For comparison across tamoxifen dosing paradigms: \* $P < 0.05$  and \*\* $P < 0.01$  as  
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29 880 determined by a two-way ANOVA  $F(6,45)=6.313$  with a Tukey's post-hoc test) (N =3-4).  
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42 882 **Figure 9. Targeting of *Prox1<sup>CreERT2</sup>* to PCs and DCs was not altered with changes**  
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44 883 **in tamoxifen induction paradigm. A**, Quantification of total reporter-positive SCs in  
45 884 the three cochlear turns of *Prox1<sup>CreERT2+/-::ROSA26<sup>tdTomato</sup></sup>* or *Prox1<sup>CreERT2+/-::CAG-</sup>*  
46 885 *eGFP<sup>+/-loxP</sup>* mice given different doses of tamoxifen (TAM). Cells were quantified in two  
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48 886 randomly chosen 200  $\mu\text{m}$  regions per cochlear turn, averaged, and expressed as a  
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50 887 percentage of labeled cells compared to total cells. (For comparison among cochlear  
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52 888 turns within the same tamoxifen dosing paradigm: 3 mg/40g tamoxifen dose at P0/P1  
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54 889 \* $P < 0.05$  as determined by a one-way ANOVA  $F(1,018, 2,036)=37.08$  with a Tukey's  
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56 890 post-hoc test; and 5 mg/40g tamoxifen dose \* $P < 0.05$  and \*\* $P < 0.01$  as determined by a  
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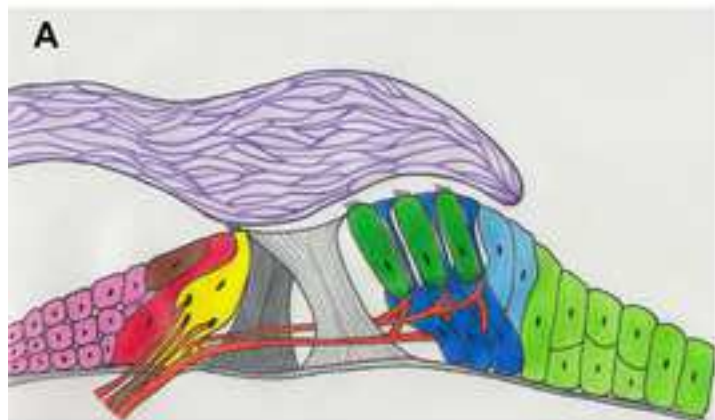
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4 891 one-way ANOVA  $F(1,2)=144.1$  with a Tukey's post-hoc test. For comparison across  
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7 892 tamoxifen dosing paradigms:  $**P<0.01$  and  $***P<0.001$  as determined by a two-way  
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9 893 ANOVA  $F(4,30)=151.8$  with a Tukey's post-hoc test) (N =3-4). Representative confocal  
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12 894 images of tdTomato (red) expression in *Prox1<sup>CreERT2+/-</sup>::ROSA26<sup>tdTomato</sup>* controls without  
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14 895 tamoxifen (**B-D**) and those given various doses of tamoxifen (**E-M**). **N-P**, SC subtypes  
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16 896 that expressed the tdTomato reporter were quantified as described in A. **N**, IPCs (For  
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19 897 comparison among cochlear turns within the same tamoxifen dosing paradigm: 3  
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21 898 mg/40g tamoxifen dose at P0/P1  $**P<0.01$  as determined by a one-way ANOVA  
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24 899  $F(1.021, 2.042)=35.11$  with a Tukey's post-hoc test; and 5 mg/40g tamoxifen dose  
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26 900  $*P<0.05$  as determined by a one-way ANOVA  $F(1.445, 2.890)=47.77$  with a Tukey's  
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29 901 post-hoc test. For comparison across tamoxifen dosing paradigms:  $*P<0.05$ ,  $**P<0.01$ ,  
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31 902 and  $***P<0.001$  as determined by a two-way ANOVA  $F(4,30)=24.63$  with a Tukey's  
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34 903 post-hoc test). **O**, OPCs (For comparison among cochlear turns within the same  
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36 904 tamoxifen dosing paradigm: 5 mg/40g tamoxifen dose  $*P<0.05$  as determined by a one-  
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38 905 way ANOVA  $F(1.427, 2.854)=37.35$  with a Tukey's post-hoc test. For comparison  
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41 906 across tamoxifen dosing paradigms:  $**P<0.01$  and  $***P<0.001$  as determined by a two-  
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43 907 way ANOVA  $F(4,30)=139.5$  with a Tukey's post-hoc test). **P**, DCs (For comparison  
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46 908 among cochlear turns within the same tamoxifen dosing paradigm: 5 mg/40g tamoxifen  
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48 909 dose  $*P<0.05$ ,  $**P<0.01$  as determined by a one-way ANOVA  $F(1.156, 2.911)=94.69$   
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51 910 with a Tukey's post-hoc test. For comparison across tamoxifen dosing paradigms:  
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53 911  $***P<0.001$  as determined by a two-way ANOVA  $F(4,30)=231.2$  with a Tukey's post-hoc  
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55 912 test). (N =3-4). **O-Q**, Representative confocal images of a tdTomato+ HC (arrow) in

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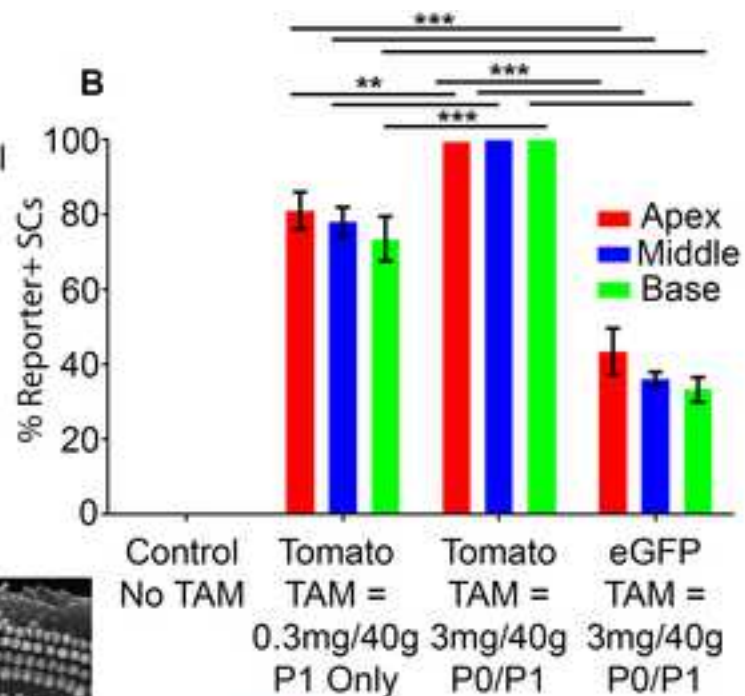
913 *Prox1*<sup>CreERT2+/-::Rosa26<sup>tdTomato</sup></sup> mice given the highest dose of tamoxifen (5mg/40g,  
914 P0/P1). Scale bars: 25 μm.

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916 **Table 1: Summary of reporter labeled HC and SC subtypes in *Fgfr3-iCreERT2/+*,**  
917 ***Sox2*<sup>CreERT2+/-</sup>, *Plp-CreERT2*, and *Prox1*<sup>CreERT2+/-</sup> mice.** Quantification of tdTomato-  
918 positive or eGFP-positive cells was performed as described in Figures 1B, 2A, and 3A.

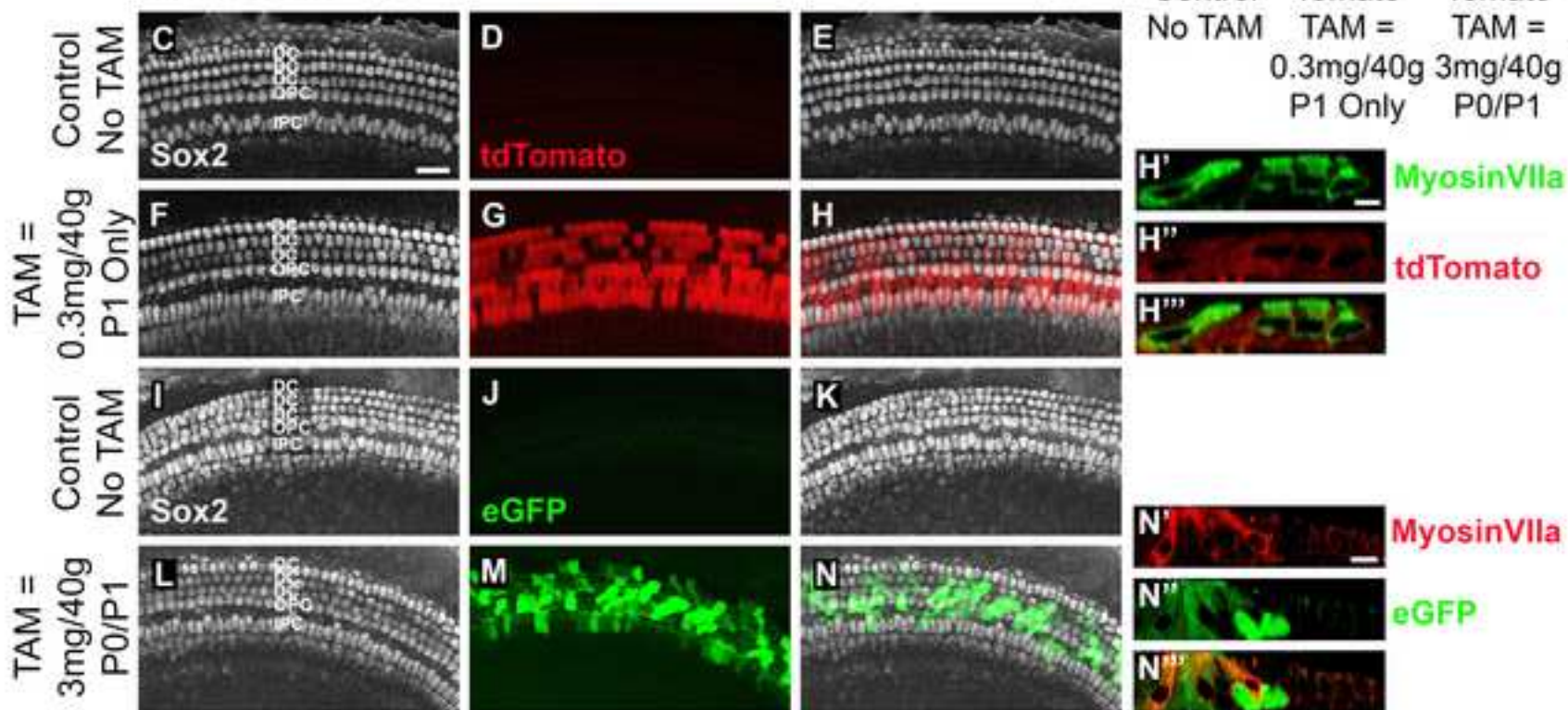
Mouse Line	Dose	Cell Type	% Labeled Cells						Whole cochlea
			Apex		Middle		Base		
			Segment 1	Segment 2	Segment 3	Segment 4	Segment 5	Segment 6	
Fgfr3-iCreER <sup>T2/+</sup> ::ROSA26 <sup>tdTomato/+</sup>	0.3mg/40g P0	SCs	81.2% ± 5.0%		78.3% ± 3.8%		73.6% ± 5.9%		77.7% ± 4.9%
		OHCs	14.6% ± 10.6%	2.9% ± 2.5%	0.3% ± 0.3%	0.3% ± 0.2%	0.0% ± 0.0%	0.2% ± 0.2%	3.9% ± 2.2%
	3mg/40g P0-P1	SCs	97.3% ± 2.3%		92.3% ± 4.3%		93.43% ± 6.6%		94.4% ± 5.5%
		OHCs	98.5% ± 0.7%	77.3% ± 3.8%	50.5% ± 5.1%	19.8% ± 3.5%	7.6% ± 0.9%	13.2% ± 3.1%	43.8% ± 2.9%
Sox2 <sup>CreERT2/+</sup> ::ROSA26 <sup>tdTomato/+</sup>	3mg/40g P0	SCs	99.9% ± 0.1%		99.9% ± 0%		100% ± 0%		99.9% ± 0.0%
		HCs	97.2% ± 1.6%	68.3% ± 11.0%	25.1 ± 10.0%	7.6% ± 2.2%	2.6% ± 1.8%	6.0% ± 2.7	34.5% ± 4.7%
	3mg/40g P0-P1	SCs	100% ± 0%		100% ± 0%		100% ± 0%		100.0% ± 0.0%
		HCs	97.2% ± 1.7%	66.9% ± 10.2%	31.7% ± 11.2%	10.2% ± 3.8%	3.2% ± 1.2%	2.7% ± 1.2%	35.3% ± 4.8%
Plp-CreER <sup>T2/+</sup> ::ROSA26 <sup>tdTomato/+</sup>	3mg/40g P0	IPhCs/BCs	46.5% ± 4.7%		80.3% ± 4.6%		91.5% ± 2.6%		72.8% ± 5.4%
		IPCs	6.7% ± 2.0%		6.6% ± 3.3%		8.5% ± 2.4%		7.3% ± 0.7%
		OPCs	9.3% ± 2.8%		13.9% ± 2.2%		3.5% ± 2.4%		8.9% ± 1.3%
		DCs	5.5% ± 1.1%		13.6% ± 4.9%		13.7% ± 5.0%		10.9% ± 2.8%
	3mg/40g P1	IPhCs/BCs	56.8% ± 11.7%		71.0% ± 13.7%		87.1% ± 2.6%		71.7% ± 4.3%
		IPCs	11.0% ± 4.1%		11.4% ± 4.7%		7.2% ± 4.4%		9.9% ± 2.6%
		OPCs	16.4% ± 5.2%		5.9% ± 2.3%		2.0% ± 1.1%		8.1% ± 2.8%
		DCs	4.6% ± 1.3%		7.3% ± 3.2%		14.5% ± 1.7%		8.8% ± 2.0%
	3m/40g P0-P1	IPhCs/BCs	48.2% ± 4.7%		79.9% ± 2.5%		89.2% ± 3.7%		72.4% ± 3.1%
		IPCs	15.6% ± 5.5%		6.7% ± 3.8%		18.2% ± 5.2%		13.5% ± 4.6%
		OPCs	12.3% ± 3.7%		15.7% ± 3.4%		3.9% ± 1.0%		10.6% ± 2.0%
		DCs	5.8% ± 1.9%		9.8% ± 2.8%		29.3% ± 4.7%		15% ± 2.9%
Prox1 <sup>CreERT2/+</sup> ::ROSA26 <sup>tdTomato/+</sup>	0.75mg/40g P0	IPCs	17.2% ± 2.8%		30% ± 11.3%		3.2% ± 1.7%		16.8% ± 4.0%
		OPCs	46.3% ± 4.0%		32.5% ± 13.3%		29.5% ± 4.7%		37.9% ± 2.5%
		DCs	51.6% ± 2.5%		27.4% ± 10.9%		28.0% ± 8.7%		41.5% ± 2.4%
	3mg/40g P0	IPCs	41.9% ± 5.5%		33.4% ± 6.8%		28.3% ± 5.1%		33.4% ± 2.4%
		OPCs	84.3% ± 1.3%		76.2% ± 5.4%		72.7% ± 5.6%		77.7% ± 3.4%
		DCs	88.5% ± 2.2%		81.4% ± 3.9%		75.5% ± 1.7%		77.9% ± 4.2%
	3mg/40g P0-P1	IPCs	45.9% ± 1.6%		22.2% ± 6.5%		7.4% ± 1.2%		25.1% ± 2.9%
		OPCs	77.7% ± 3.3%		69.0% ± 4.4%		63.8% ± 4.8%		70.1% ± 2.6%
		DCs	89.6% ± 3.0%		80.7% ± 0.6%		61.7% ± 4.0%		76.1% ± 0.1%
	5mg/40g P0-P1	IPCs	47.9% ± 3.5%		14.5% ± 2.6%		11.1% ± 0.7%		24.5% ± 0.9%
		OPCs	88.4% ± 1.8%		69.0% ± 2.9%		56.8% ± 2.6%		71.4% ± 1.3%
		DCs	93.6% ± 0.8%		75.4% ± 0.8%		69.3% ± 1.6%		79.5% ± 0.4%
Mouse Line	Dose	Cell Type	% Labeled Cells			Whole cochlea			
			Apex		Middle	Base			
			Apical Tip	Rest of Cochlea					
Fgfr3-iCreER <sup>T2/+</sup> ::CAG-eGFP <sup>+/loxP</sup>	3mg/40g P0-P1	SCs	43.6% ± 6.1%		36.3% ± 1.8%	33.4% ± 3.3%	37.8% ± 3.4%		
		OHCs	40.5% ± 6.1%	4.4% ± 2.5%			5.8% ± 2.6%		
Sox2 <sup>CreERT2/+</sup> ::CAG-eGFP <sup>+/loxP</sup>	3mg/40g P1	SCs	74.0% ± 4.9%		65.7% ± 9.0%	58.4% ± 5.7%	66.0% ± 5.9%		
		HCs	44.8% ± 10.8%	1.6% ± 0.5%			3.2% ± 0.4%		
Plp-CreER <sup>T2/+</sup> ::CAG-eGFP <sup>+/loxP</sup>	3mg/40g P0	IPhCs/BCs	10.1% ± 4.5%		24.3% ± 5.9%	43.8% ± 3.9%	26.0% ± 4.8%		
		IPCs	0.0% ± 0.0%		0.9% ± 0.9%	0.0% ± 0.0%	0.3% ± 0.3%		
		OPCs	0.8% ± 0.8%		0.0% ± 0.0%	0.0% ± 0.0%	0.3% ± 0.3%		
		DCs	0.9% ± 0.9%		1.1% ± 0.8%	5.9% ± 4.8%	2.6% ± 2.1%		
	3mg/40g P0-P1	IPhCs/BCs	21.8% ± 10.9%		28.2% ± 2.1%	38.1% ± 3.9%	29.4% ± 3.7%		
		IPCs	8.8% ± 7.9%		9.1% ± 7.5%	7.3% ± 5.8%	8.4% ± 7.1%		
		OPCs	10.2% ± 8.9%		8.4% ± 7.5%	5.8% ± 5.8%	8.1% ± 7.4%		
		DCs	5.1% ± 5.1%		12.0% ± 11.4%	9.8% ± 9.4%	9.0% ± 8.6%		



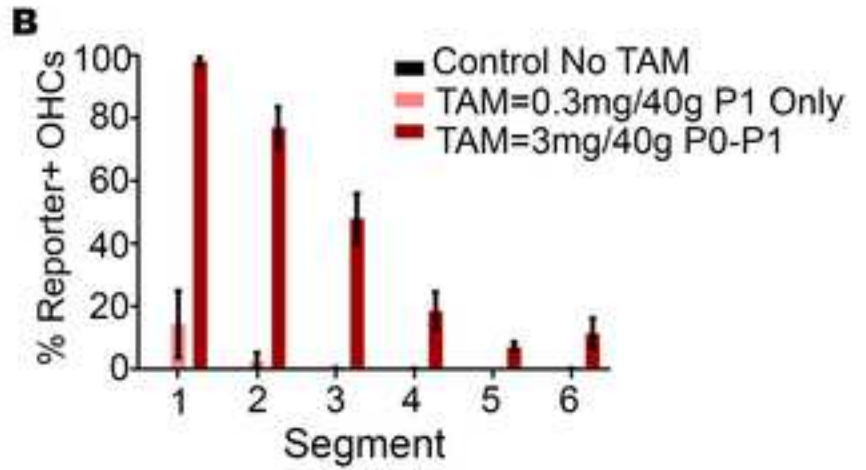
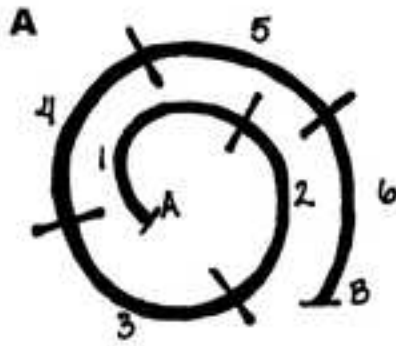
- Tectorial Membrane
- GER
- Border Cell
- Inner Phalangeal Cell
- Inner Hair Cell
- Inner Pillar Cell
- Outer Pillar Cell
- SGN Fibers
- Deiters' Cells
- Outer Hair Cells
- Hensen Cells
- Claudius Cells



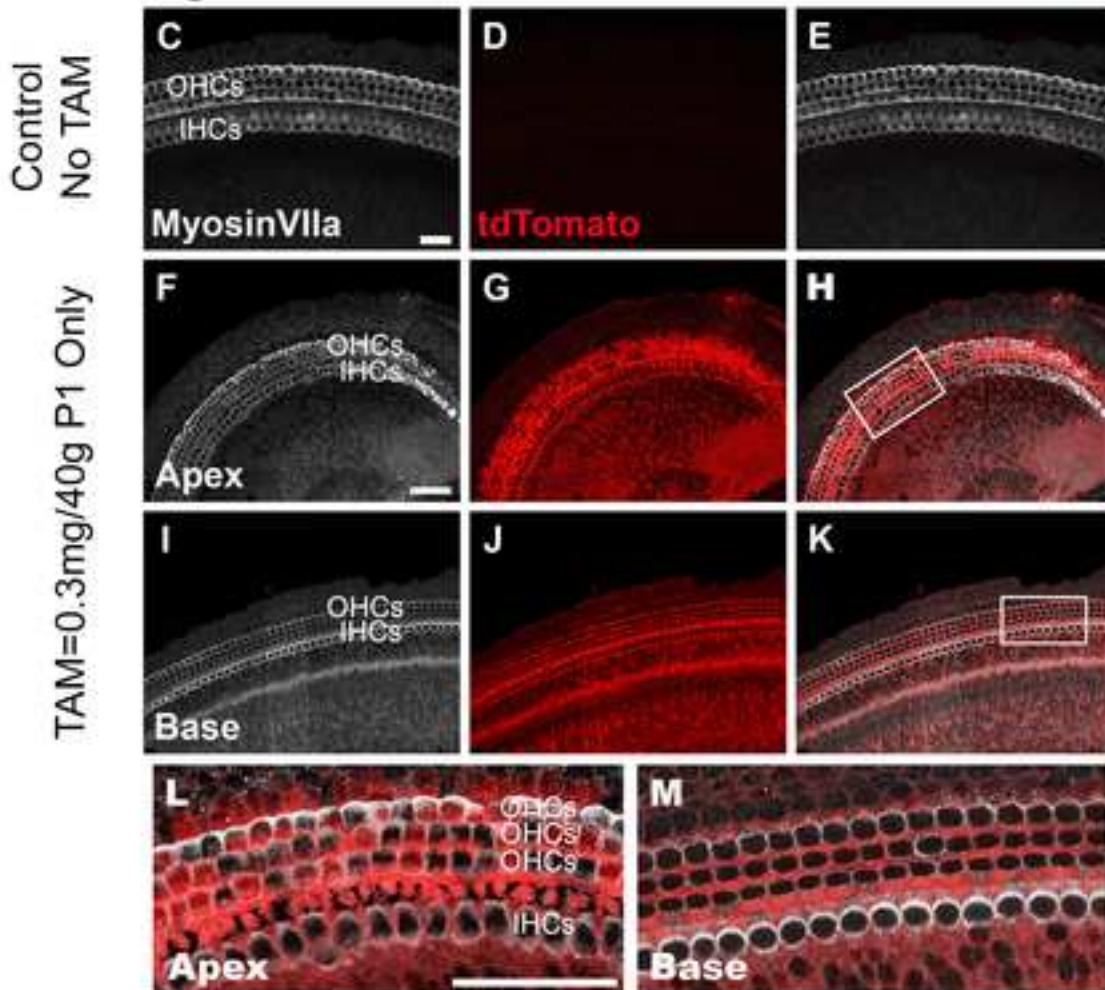
***Fgfr3-iCreER<sup>T2</sup>***



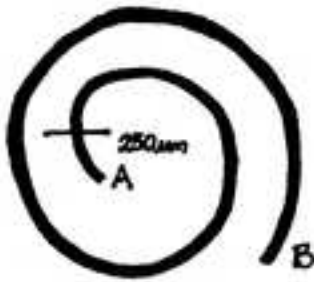




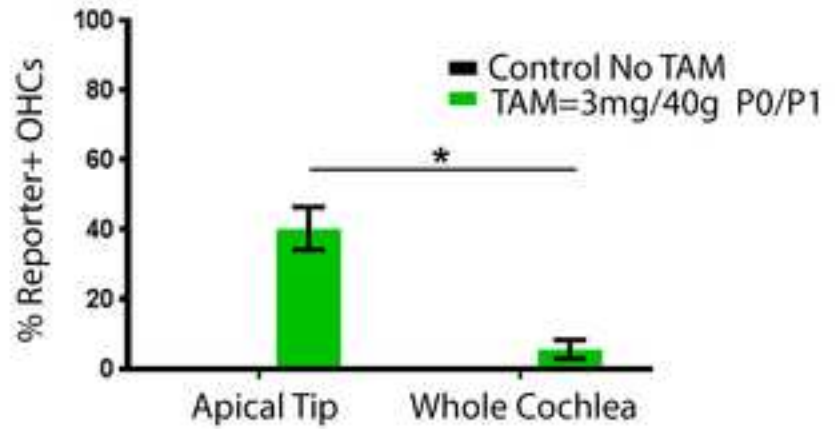
*Fgfr3-iCreER<sup>T2</sup>::ROSA26<sup>tdTomato</sup>*



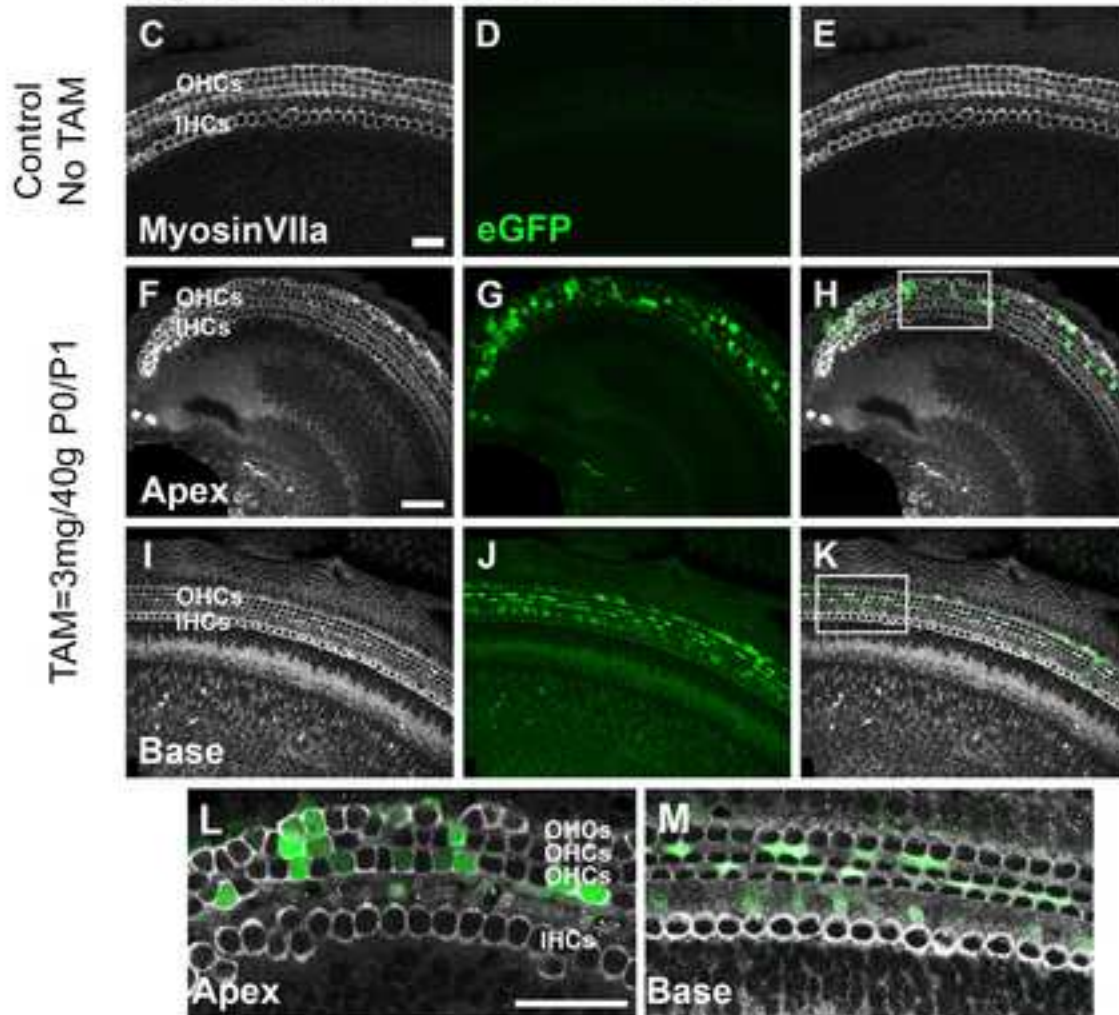
A



B

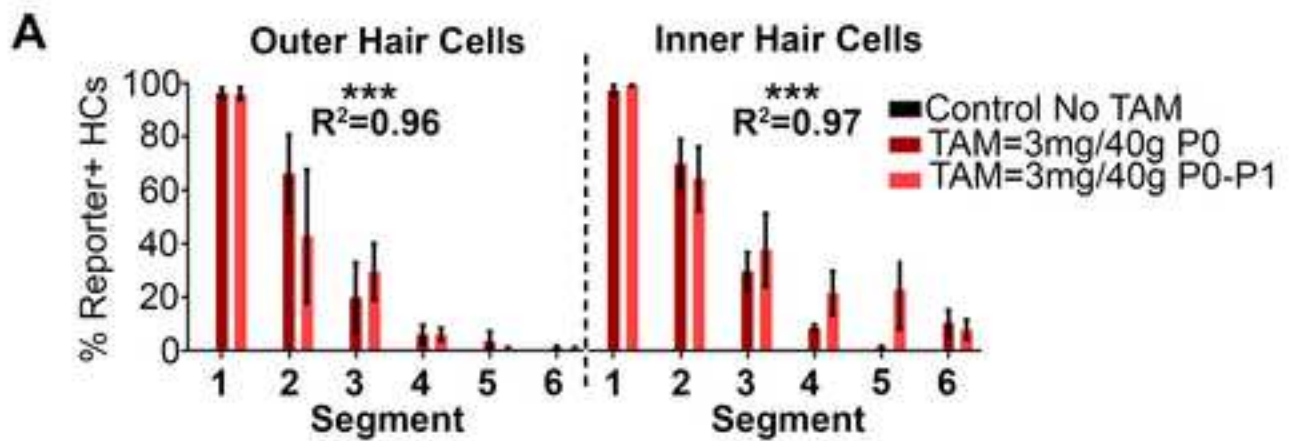


### *Fgfr3-iCreER<sup>T2</sup>::CAG-eGFP*

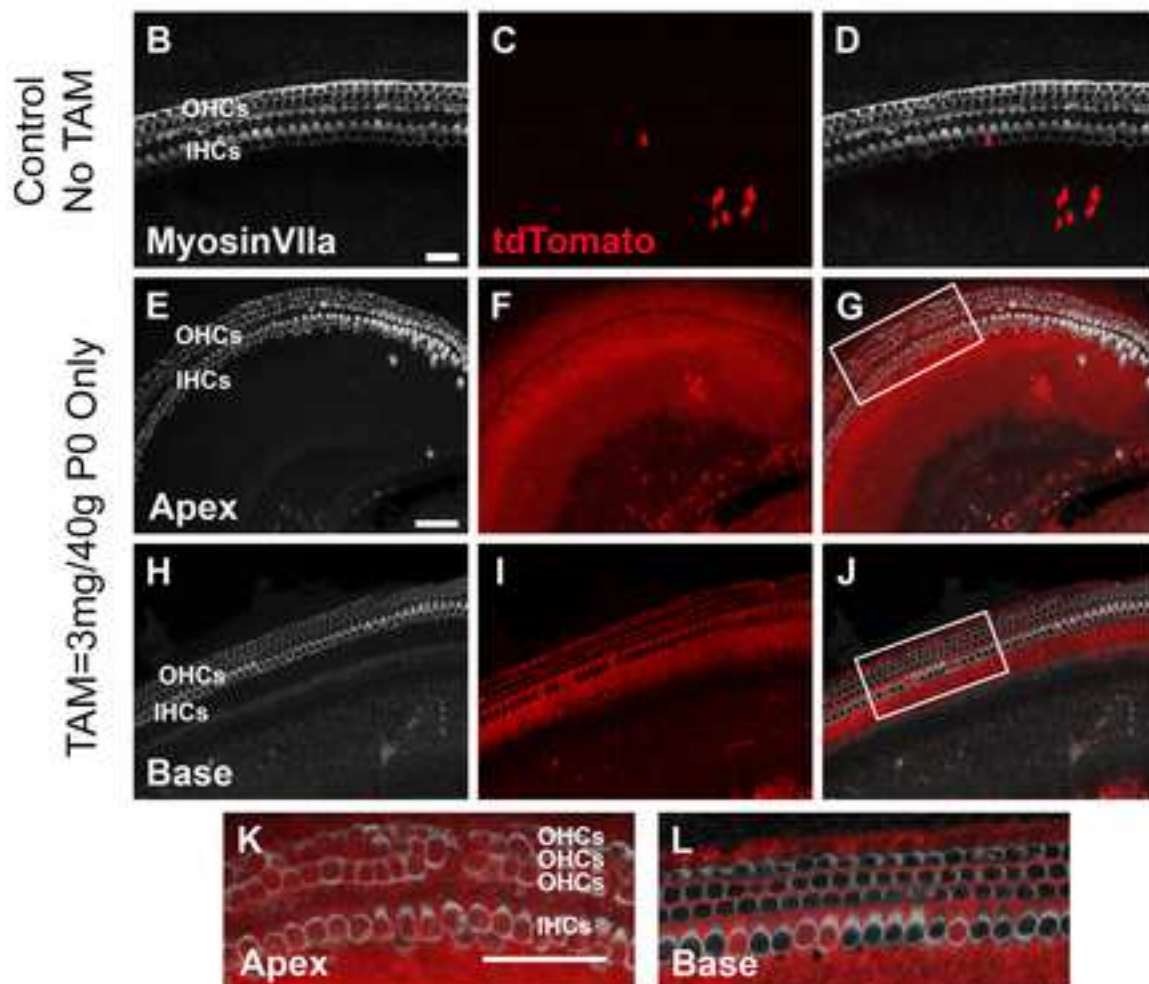


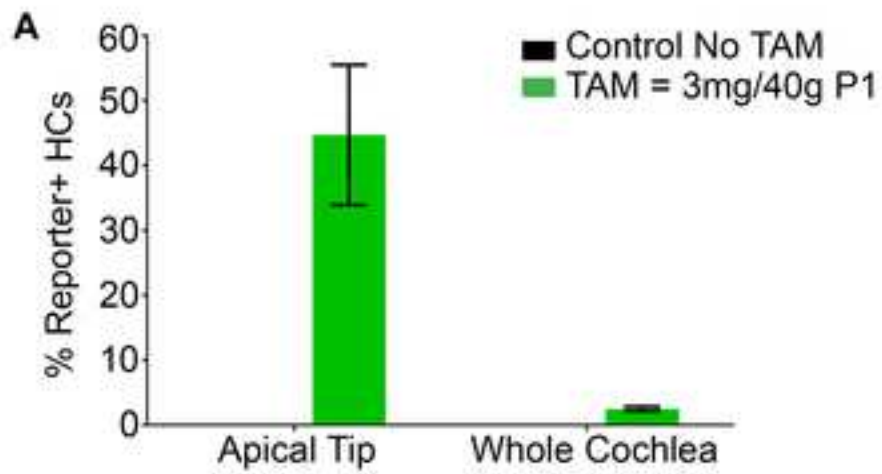




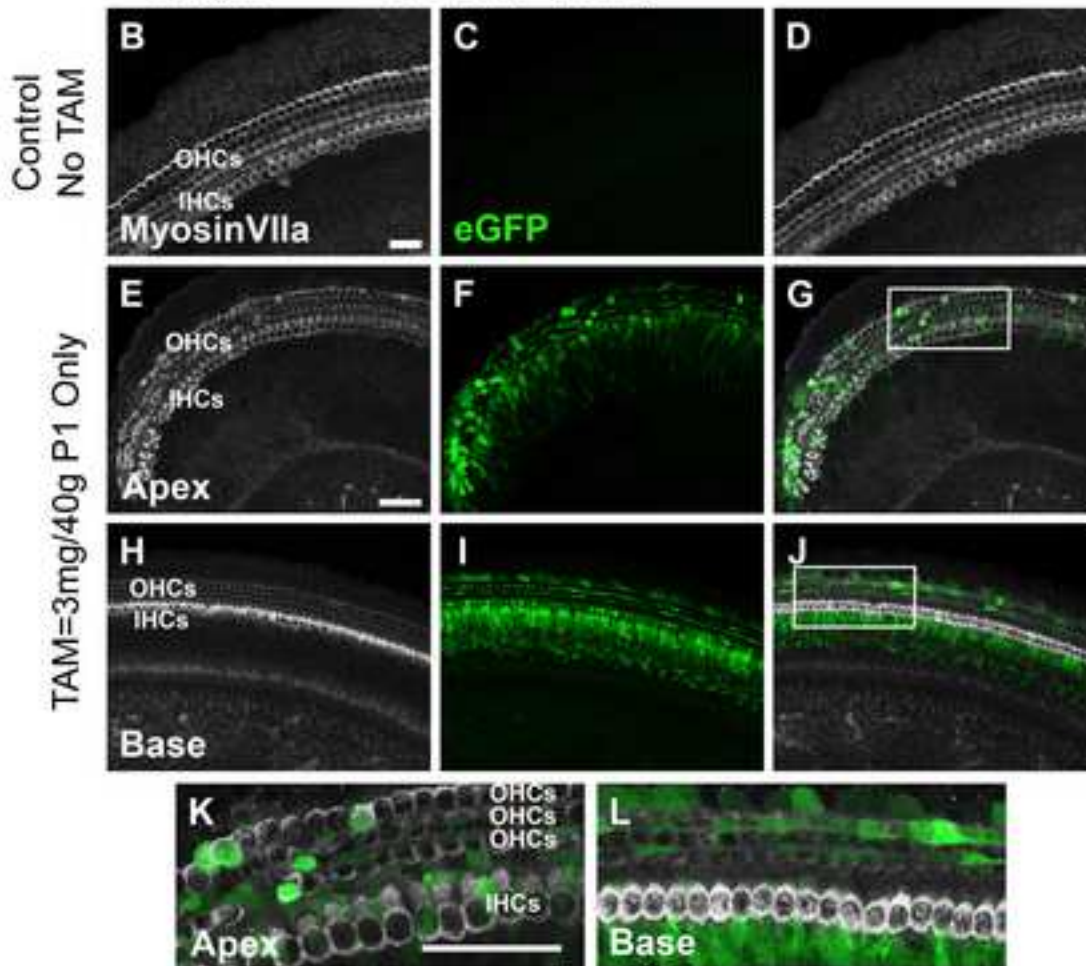


*Sox2<sup>CreERT2</sup>::ROSA26<sup>tdTomato</sup>*

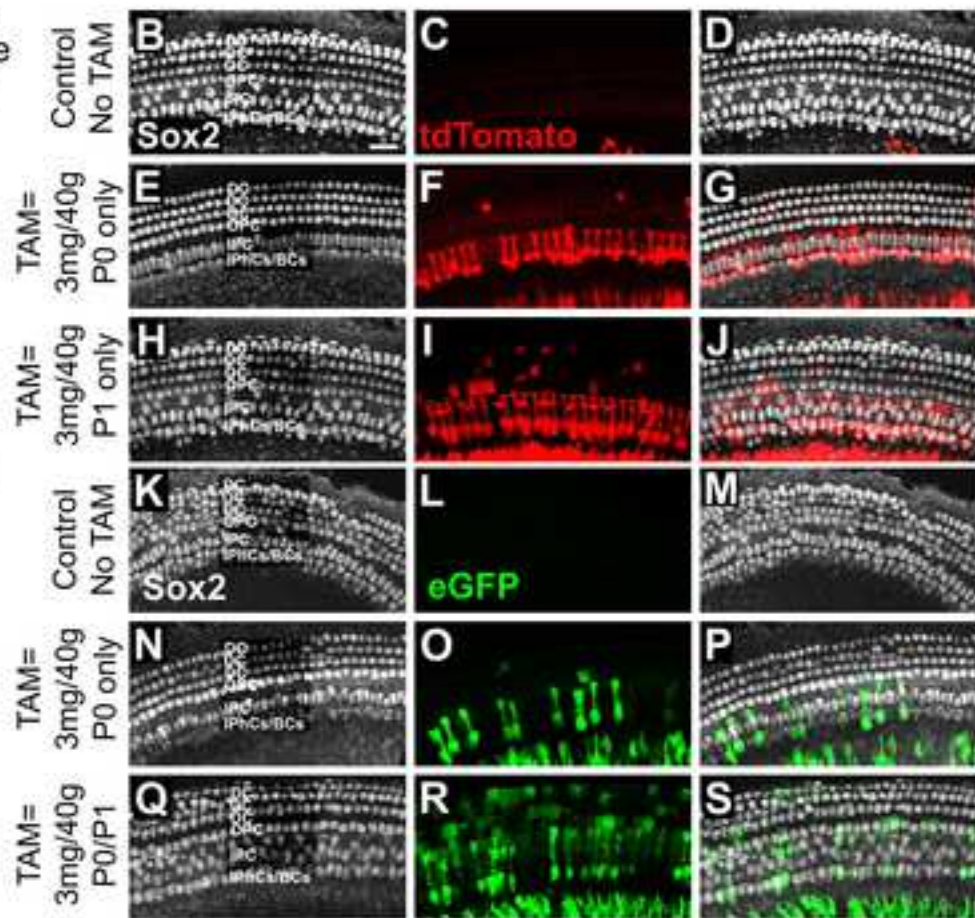
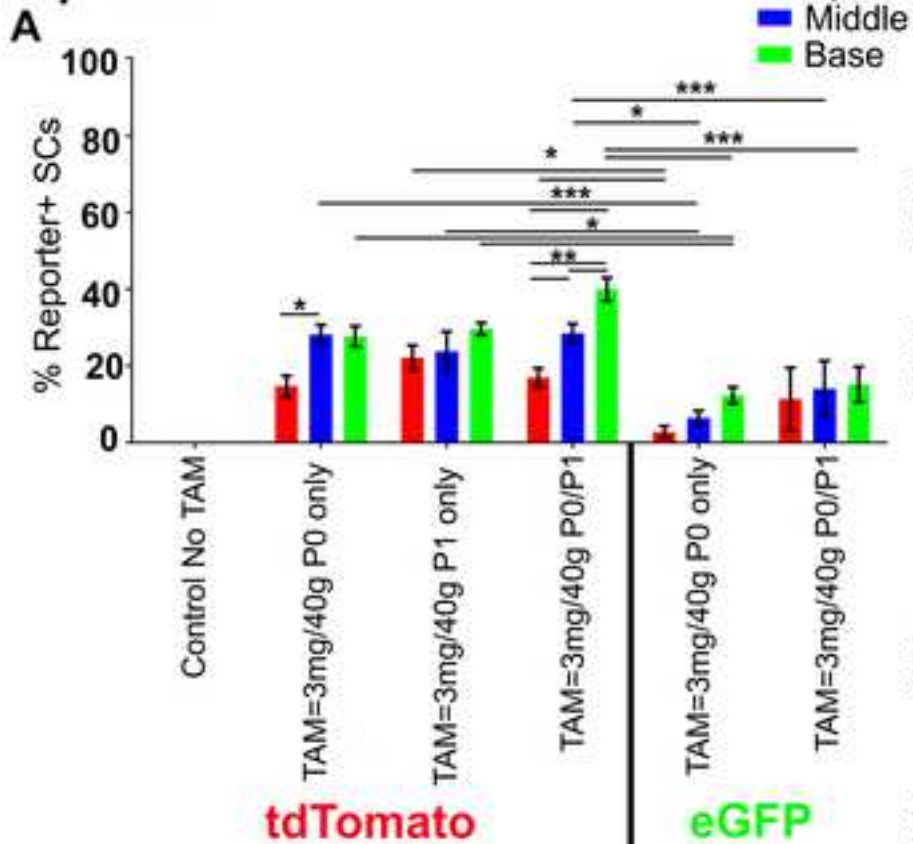




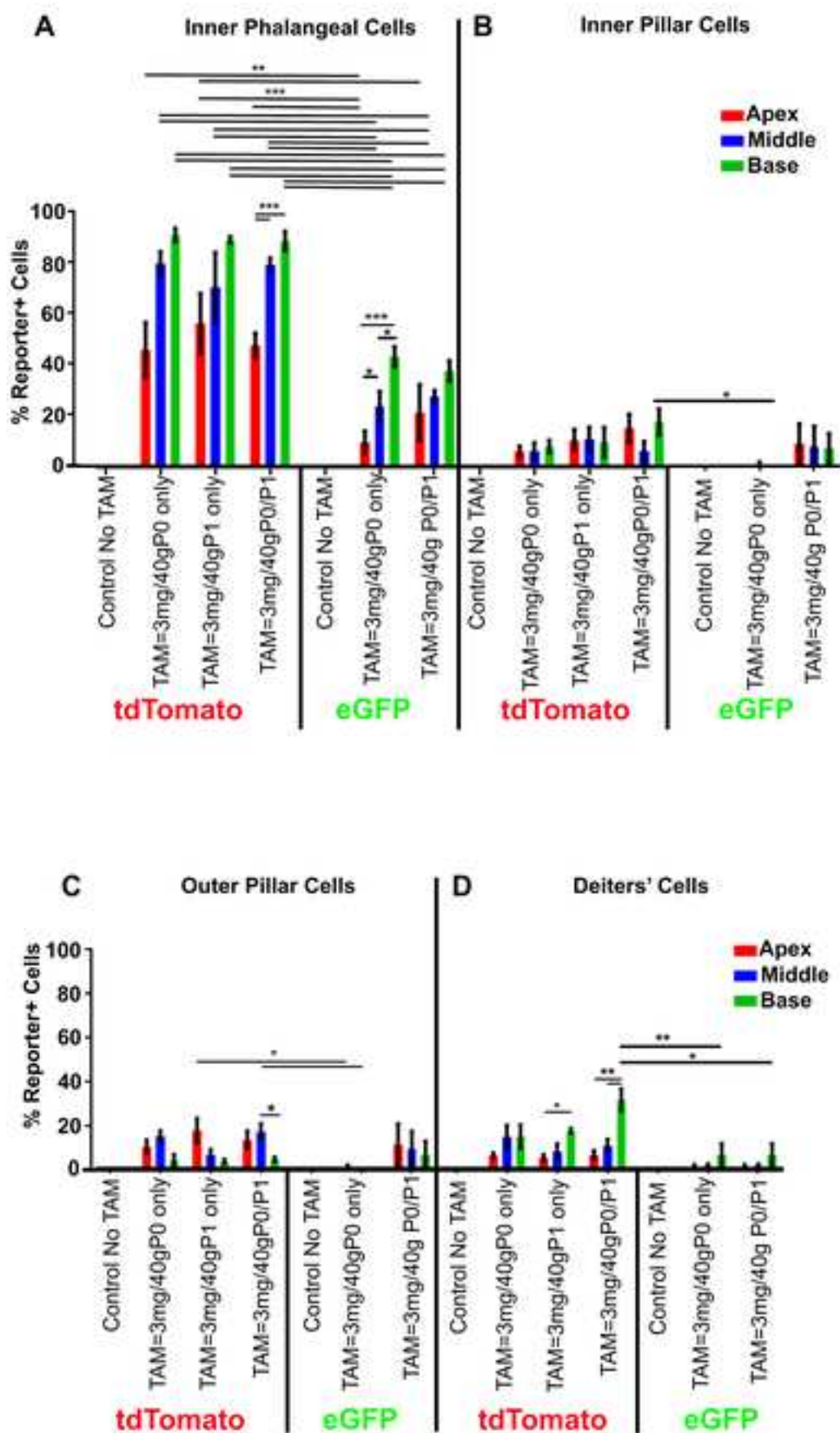
**Sox2<sup>CreERT2</sup>::CAG-eGFP**

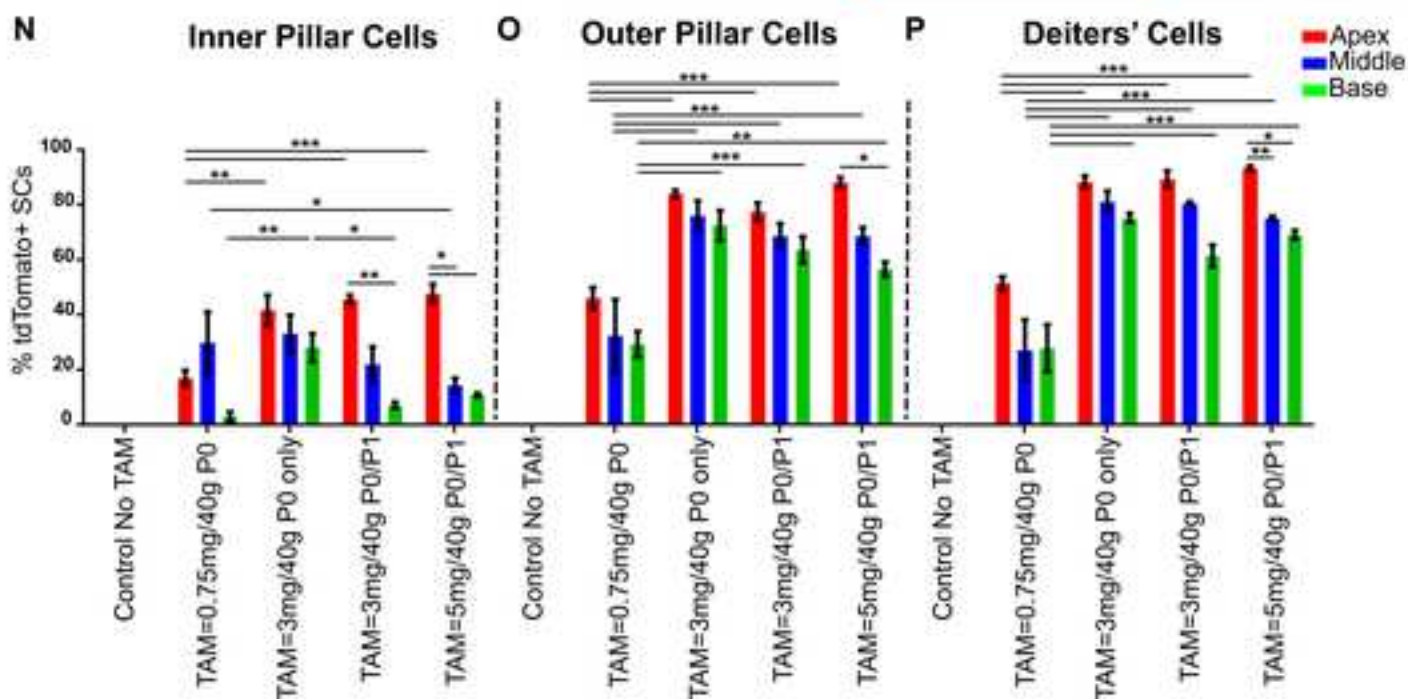
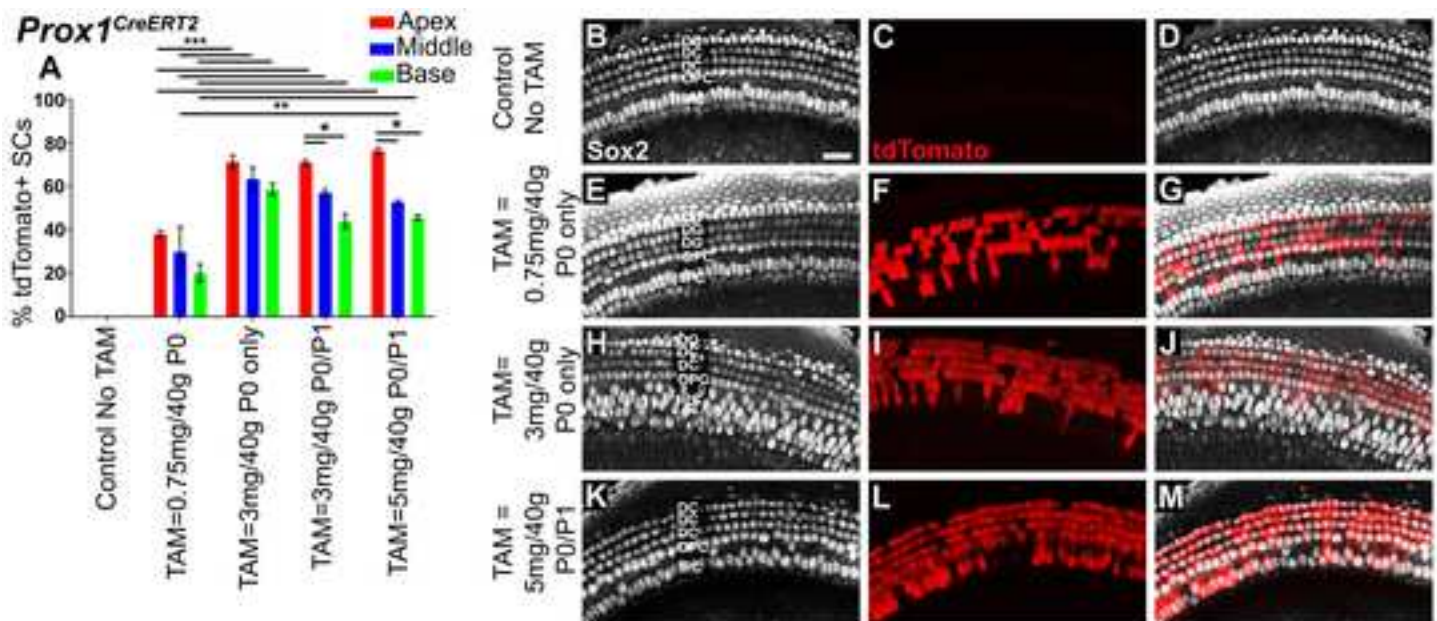


*Plp-CreER<sup>T2</sup>*











Authors do not have any professional or financial affiliations that may be perceived as a conflict of interest.