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Foxi3^{GFP} and *Foxi3*^{CreER} mice allow identification and lineage labeling of pharyngeal arch ectoderm and endoderm, and tooth and hair placodes

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Abstract

Background: FOXI3 is a forkhead family transcription factor that is expressed in the progenitors of craniofacial placodes, epidermal placodes, and the ectoderm and endoderm of the pharyngeal arch region. Loss of *Foxi3* in mice and pathogenic *Foxi3* variants in dogs and humans cause a variety of craniofacial defects including absence of the inner ear, severe truncations of the jaw, loss or reduction in external and middle ear structures, and defects in teeth and hair.

Results: To allow for the identification, isolation, and lineage tracing of Foxi3-expressing cells in developing mice, we targeted the *Foxi3* locus to create *Foxi3*^{GFP} and *Foxi3*^{CreER} mice. We show that *Foxi3*^{GFP} mice faithfully recapitulate the expression pattern of *Foxi3* mRNA at all ages examined, and *Foxi3*^{CreER} mice can trace the derivatives of pharyngeal arch ectoderm and endoderm, the pharyngeal pouches and clefts that separate each arch, and the derivatives of hair and tooth placodes.

Conclusions: *Foxi3*^{GFP} and *Foxi3*^{CreER} mice are new tools that will be of use in identifying and manipulating pharyngeal arch ectoderm and endoderm and hair and tooth placodes.

Keywords

Pharyngeal arch; pharyngeal pouch; pharyngeal cleft; tooth; hair follicle; CreER and reporter mice

INTRODUCTION

Pharyngeal arches are transient embryonic structures that give rise to many craniofacial elements such as the lower jaw, middle and external ears, and endodermal structures such as

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the thymus, thyroid and parathyroid [1–3]. Each arch is lined on its outside by ectoderm, on the inside by endoderm, and is populated by neural crest cells and endothelial precursors. Each pharyngeal arch is separated from its neighbor by constrictions of the ectoderm and endoderm that comprise the pharyngeal clefts and pouches respectively. In addition to demarcating each arch, the pouch and cleft regions can serve as signaling centers that sculpt arch development [3]. A number of different human syndromes involving pharyngeal arch defects include DiGeorge syndrome, Pierre Robin Syndrome, Goldenhar syndrome and Treacher-Collins Syndrome [4–7], as well as cleft lip/palate.

Many previous studies report that signaling molecules or the transcription factors present in pharyngeal arch ectoderm or endoderm are essential for pharyngeal arch patterning [8–16]. FOXI3, a member of the Forkhead transcription factor class I (Foxi) family plays a crucial role in early craniofacial development. *Foxi3* mRNA is expressed in the ectoderm and endoderm of the developing pharyngeal arches, gradually localizing to the pouch/cleft regions between the arches before being down-regulated as arch derivatives differentiate [17–19]. Loss of *Foxi3* resulted in craniofacial abnormalities, including absence of the outer, middle and inner ears, a misshapen Meckel's cartilage, a shortened mandible fused with the maxilla, and thymic, parathyroid and aortic arch defects [18,20]. Although *Foxi3* is not expressed in neural crest cells migrating into the pharyngeal arches, *Foxi3* is nevertheless required for the survival of migrating crest. Loss of *Foxi3* causes migrating neural crest cells entering the pharyngeal arches to undergo significant cell death, due in part from the loss of FGF8 from arch ectoderm [18]. *Foxi3* is also expressed in ectodermal appendage progenitors such as hair and whisker follicles, and tooth and mammary buds during embryonic development [21,22]. Loss of *Foxi3* in these structures leads to defects in tooth and hair development [21,23], and a semidominant neomorphic mutation in dog *Foxi3* leads to partial or complete loss of hair and tooth defects in three hairless dog breeds [24–26]. Variants or deletions of the *FOXI3* locus in humans can also lead to craniofacial abnormalities such as microtia and craniofacial microsomia [27–29].

The specific and transient expression of *Foxi3* in pharyngeal arches and the progenitors of ectodermal appendages suggests it may be a useful gene to direct the expression of reporter proteins or Cre recombinase for developmental studies of these tissues. In this study, we introduce two mouse models that can be used to alter the specific genes in ectodermal or endodermal derived tissues in the pharyngeal arches, teeth, and hair/whisker follicles. We developed $Foxi3^{GFP}$ mice in which Venus fluorescent protein is targeted downstream of the *Foxi3* coding region. GFP labeling in these mice reveals a very similar expression pattern to *Foxi3* mRNA expression previously reported in pharyngeal arches and the progenitors of ectodermal appendages [18,22,24]. We created a second line of mice in which the *Foxi3* coding region was replaced by a *CreER* construct encoding a Cre recombinase fused to a modified estrogen receptor. We used these mice to trace the lineage of *Foxi3*-expressing cells and labeled cells in cranial epidermis, the ear pinna and ear canal (ectodermal derivatives), and in the Eustachian tube, oropharynx, glottis, epiglottis, thyroid, parathyroid and thymus (endodermal derivatives), as well as hair/whisker follicles and tooth placodes. No *Foxi3* lineage-labeled cells were present in neural crest- or mesodermal-derived tissues such as cartilages and muscles. Both mouse lines will be useful tools for investigators seeking to

identify, purify and manipulate progenitors of the pharyngeal arches, their derivatives and the progenitors of teeth and hair follicles.

RESULTS AND DISCUSSION

Foxi3^{GFP} mice show expression in the pharyngeal arches along anterior to posterior axis

We targeted the *Foxi3* locus to insert a Venus fluorescent protein downstream of the *Foxi3* coding region, separated by a GSG-P2A sequence to allow bicistronic expression of FOXI3 and the fluorescent reporter (Figure 1A). To validate the expression of our *Foxi3*^{GFP} mice, we analyzed GFP expression in *Foxi3*^{GFP} embryos at three consecutive stages E8.5, E9.5 and E10.5 (Figure 2). We previously reported the expression of *Foxi3* mRNA in both ectoderm and endodermal regions of the pharyngeal arches at E8.5, with the expression becoming restricted to the clefts and pouches in an anterior-posterior direction [18,19]. At E8.5 and E9.5, GFP is expressed continuously along the surface ectoderm of maxillary and mandibular regions of the first arch (Figure 2A, B, E, arrowheads). The first and second pharyngeal clefts and pharyngeal pouches at these stages show very strong GFP expression (Figure 2B, C, F G, arrowheads). By E9.5, GFP could be detected in the third pharyngeal cleft and pharyngeal pouch (Figure 2H, arrowheads). At E10.5, GFP expression along the surface ectoderm decreased in the first pharyngeal arch (Figure 2J), its expression becoming restricted to the pharyngeal pouches and clefts (Figure 2K, L). The duct arising from the third pharyngeal pouch is the primordia of thymus and parathyroid tissue and strongly expresses GFP (Figure 2M). We used light sheet microscopy to visualize these expression patterns in whole mounts of the embryonic head at E10.5 (Supplementary Movies 1–3). Together, these results show that our *Foxi3*^{GFP} mice faithfully recapitulate the expression of *Foxi3* mRNA at all stages of arch development and can thus be used as a reporter and as a tool to isolate *Foxi3*-expressing progenitors.

Fate mapping reveals contribution of *Foxi3*-expressing cells to the pharyngeal arches

To map the fates of *Foxi3*-expressing cells, we targeted the *Foxi3* locus and replaced the both *Foxi3* coding exons and the intervening intron with a construct containing a tamoxifen-inducible *CreER* fusion construct, a GSG-P2A sequence and an EGFP sequence (Figure 1B). We employed the EGFP sequence to visualize *Foxi3*-expressing cells; however, we found that although the CreER protein was expressed and active, the low level of expression from the construct did not allow detection of the EGFP protein, either by native fluorescence or by immunostaining with GFP antibodies (data not shown). To determine the contribution of *Foxi3*-expressing cells to the pharyngeal arch region, we crossed *Foxi3*^{CreER} mice to ROSA-Ai9 Cre reporter mice, administered a single moderate dose of tamoxifen (75μg/g body weight) at 7.5 days post-coitum (dpc) and harvested the embryos at E10.5. We observed a line of *Foxi3* lineage-labeled cells, revealed by expression of the tdTomato reporter, in the surface ectoderm of the maxillary portion of the first pharyngeal arch (Figure 2O). *Foxi3* lineage-labeled cells were also located specifically in the first, second and third pharyngeal clefts and pouches (Figure 2P–R). Additionally, we observed labeled derivatives in the cranial ganglia, otocyst and the endodermally-derived thymus-parathyroid primordia (Figure 2P–R). These results demonstrate the contribution of *Foxi3* lineage-labeled cells to the ectoderm- and endodermally derived pharyngeal pouches and clefts. *Foxi3* is also

expressed in the pre-placodal region that gives rise to all craniofacial placodes[17,19], and we also observed labeled cells in some placodal derivatives such as the trigeminal ganglion (Figure 2P) and the otic vesicle and its associated vestibulo-acoustic ganglion (Figure 2Q).

To assess the contribution of *Foxi3*-expressing progenitors to pharyngeal arch derivatives more fully, we analyzed *Foxi3*^{CreER} embryos at later developmental stages. To examine the presence of *Foxi3* lineage-labeled cells in the first two pharyngeal arches at later stages, we administered a single dose of tamoxifen at 7.5 dpc and harvested embryos at E14.5. We identified developing cartilage in the cranial region with immunostaining for Sox9. *Foxi3* lineage-labeled cells were found in the ectodermal regions of the ear pinna, groove, and ear canal regions of the outer ear (Figure 3A–C). However, no *Foxi3* lineage-labeled cells were seen in the regions of six auricular hillock cartilages of the pinna (Figure 3D). In the middle ear region, no *Foxi3* lineage-labeled cells were found in the malleus or incus (the middle ear ossicles derived from the first arch), although small numbers of endodermally-derived *Foxi3* lineage-labeled descendants could be seen in the region of the Eustachian tube (Figure 3F–I). Similarly, in the second pharyngeal arch region, the stapes (the third middle ear ossicle; Figure 3I) did not contain any *Foxi3*^{CreER} labeled cells. These results suggest that *Foxi3*-expressing cells give rise to tissues in the first two pharyngeal arches derived from ectodermal and endodermal origins, but not to other derivatives derived from migrating neural crest cells or mesodermal cells, which do not express *Foxi3* during development. Previous studies have suggested a link between some forms of craniofacial microsomia and defects of the stapedial artery leading to hemorrhage [4,30]. We did not observe *Foxi3* lineage-labeled cells in the stapedial artery or endothelium any other vessels of the head (not shown), suggesting that cases of craniofacial microsomia with a vascular etiology may not be directly due to *FOXI3* pathogenic variants. In sum, *Foxi3*^{CreER} mice can be used to follow the fate of pharyngeal ectoderm and endoderm derivatives, and to manipulate gene expression in these structures at different ages.

Foxi3-expressing cells contribute to the posterior pharyngeal arches

Tissues derived from the posterior pharyngeal arches include elements of the cricoid, arytenoid, and thyroid cartilages which are located surrounding the glottis region, the trachea (which is of mesodermal origin) and the endodermally-derived thymus, parathyroid and thyroid tissues. We mapped the fates of *Foxi3*-expressing cells by administering tamoxifen at 7.5 dpc and analyzing at E14.5. *Foxi3* lineage-labeled cells are present in all endodermally-derived tissues in this region including the oropharynx, glottis, epiglottis, thyroid, parathyroid and thymus tissues (Figure 4A-G). However, no *Foxi3* lineage-labeled cells were found in any neural crest-derived, Sox9 -expressing cartilage elements. Thus, our results show that *Foxi3* lineage-labeled cells contribute mainly to endodermal derivatives in posterior pharyngeal arches, and that Foxi3^{CreER} mice can be used to label and isolate or manipulate the derivatives, thyroid, parathyroid and thymus tissues.

Foxi3^{GFP} and *Foxi3*^{CreER} mice reveal the fates of *Foxi3*- expressing progenitors in ectodermal appendages

Previous studies reported that *Foxi3* is expressed in the epithelium of all hair and whisker follicles, and in dental epithelium throughout tooth morphogenesis [22,24]. We showed that

our *Foxi3*^{GFP} reporter was strongly expressed in ectodermal appendages including whisker and hair follicles and tooth placodes at E12.5 and E14.5. Specifically, GFP expression was strongly observed in the tooth and hair placodes at E12.5, and the epithelial compartments of invaginating whisker and hair follicles and in the tooth germs at E14.5 (Figure 5A–E) similar to what has been reported for *Foxi3* mRNA in previous studies [22]. GFP is also expressed in the oral epithelium near the tooth placodes (Figure 5D and E). We performed lineage analysis to locate *Foxi3* lineage-labeled cells during hair follicle and tooth development. For these experiments, we administered tamoxifen at 11.5 dpc and harvested the mice at E14.5 (for hair follicle and tooth derivatives) or E18.5 (for hair follicle derivatives). Consistent with a role for *Foxi3* in tooth and hair development[21,23–26], we found *Foxi3* lineage-labeled cells in the dental epithelium (Figure 5G, H) and the inner root sheet of hair and whisker follicles (Figure 5F, I). *Foxi3*^{GFP} and *Foxi3*^{CreER} mice are thus likely to be useful and specific tools to label, isolate and manipulate hair and tooth progenitors in late embryonic and postnatal animals.

EXPERIMENTAL PROCEDURES

Generation of *Foxi3*^{GFP} mice

Foxi3^{GFP} reporter mice were created by the Genetically Engineered Rodent Models core at Baylor College of Medicine. The targeting strategy is shown in Figure 1A. Mouse zygotes were injected with 100ng/μl of Cas9 mRNA, 20ng/μl of a guide RNA targeting the end of the *Foxi3* coding region (CCC CCGGGATGGCTCTGATATA) and 50ng/μl of a targeting ssDNA sequence. This single stranded DNA molecule contained 150 bases of the 3’ end of the *Foxi3* coding region (upstream homology arm) containing two base pair alterations to prevent cleavage by the gRNA (Figure 1A), 66 bases comprising a GSG-P2A sequence, the coding region for mVenus fluorescent protein and a final 115 bases of the 3’ end of the *Foxi3* gene downstream from the stop codon (downstream homology arm). Founder mice were genotyped using a forward primer (5’-TTC TCC ACA CTC CAT GCA GC-3’) and a reverse primer (5’-CCC TTT CCA AGA CGC TAA GCT-3’). A correctly targeted band was 1069bp in length; the wild type band was 285bp in length. Positive founders were outcrossed to wild type mice for five generations before the characterization described in this paper.

Generation of *Foxi3*^{CreER} mice

Foxi3^{CreER} mice were created by the Genetically Engineered Rodent Models core at Baylor College of Medicine. The targeting strategy is shown in Figure 1B. We developed a targeting construct for the *Foxi3* locus containing 1456bp upstream of the *Foxi3* start codon, a CreER fusion gene, a GSG-P2A sequence, an EGFP sequence, a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), polyA signal, a PGK-neo resistance cassette, and 1200bp downstream from the *Foxi3* stop codon. This was targeted to mouse ES cells by homologous recombination. To enhance targeting, AB2.2 ES cells were electroporated with the linearized targeting construct (2μg) were electroporated with 20μg of a pX330 plasmid (Addgene # 42230) expressing Cas9 and a sgRNA sequence to target the 3’ end of the *Foxi3* gene (CCC CCGGGATGGCTCTGATATA). Neomycin-resistant colonies were expanded and screened for correct targeting, and three correctly targeted clones were expanded and injected into 129 blastocysts to create chimeras, which were then bred to C57Bl6 mice to

establish germline founders. Genotyping was performed using a forward primer (5'-AAA GCC GCT GCC GCT CTG CA-3') and a reverse primer (5'-TTG GTC GTG GCA GCC CGG AC-3').

Maintenance and breeding of mouse lines and tamoxifen administration

All the mice were maintained according to IACUC policies (Baylor College of Medicine). We bred *Foxi3*^{CreER} mice with homozygous *Rosa-Ai9* mice [31] that express tdTomato after Cre recombination. *Foxi3*^{GFP} mice were maintained as homozygotes and with ICR female mice to generate embryos carrying the *Foxi3*^{GFP} allele. For *Foxi3*^{CreER} mouse lineage tracing, a single dose of 3mg tamoxifen and 3mg progesterone per 40gm body weight (75μg/g) was administered to pregnant mice by oral gavage at 6.5, 7.5 or 11.5 days post-coitum (dpc). Embryos from both lines were harvested at embryonic day (E) 8.5, 9.5, 10.5, 12.5, 14.5 and 18.5 respectively. For both lines, at least 10 embryos were analyzed at each embryonic age described in the Results section.

Preparation of embryos

Embryos at were harvested and fixed in 4% paraformaldehyde for overnight at 4°C, dehydrated in 30% sucrose in PBS overnight at 4°C, and finally embedded in cryomolds (Tissue-Tek, 4565) using OCT compound (Tissue-Tek, 4583) by freezing in liquid nitrogen. The tissues were sectioned at 12μm thickness using a Leica CM 1850 cryostat with sections collected on Superfrost Plus slides (Fisher scientific, 12-550-15). Sections were stored at −20°C.

Immunohistochemistry

The primary antibodies used in study were rabbit anti-GFP (1:1000, Thermofisher, A-11122), rabbit anti-Sox9 (1:1000, Millipore, AB5535), and rabbit anti-Runx2 (1:1000, Cell Signaling Technology, 12556). The secondary antibodies used for this study were: goat anti-rabbit Alexa 488 (1: 1000, Invitrogen, A11008), and goat anti-mouse Alexa 488 (1:1000, Invitrogen, A11029). Immunohistochemistry was performed based on standard protocols. Briefly, sections were dried at room temperature for 10 minutes and washed with 1X PBS to dissolve the OCT compound. The sections were blocked with blocking solution containing 1X PBS, 10% normal goat serum and 0.1% Triton X-100 at room temperature, then treated with diluted primary antibodies overnight at 4°C. After rinsing with 1% Triton X-100 in PBS at room temperature three times, they were treated with diluted secondary antibodies at room temperature for at least 1 hour, washed thoroughly with 1% Triton X-100 in PBS, followed by a 1X PBS wash, and incubation with DAPI for 5–10 minutes at room temperature. The slides were mounted with Fluoromount-G (Southern Biotech, 0100–01). The slides were imaged with a Zeiss Axio Observer microscope with an Apotome structured illumination attachment and an Axiocam using Zen 3.0 software (Blue edition).

Light Sheet Microscopy

Embryos were harvested at E10.5 from ICR females crossed with *Foxi3*^{GFP} homozygous mice and were fixed in 4% paraformaldehyde overnight at 4°C. Samples were washed with 1X PBS and incubated in blocking solution (1% Triton-X 100, 1% BSA, 10% Horse serum

in 1X PBS) overnight at room temperature, treated with primary GFP antibody diluted at 1:500 in antibody solution (0.1% Triton-X 100, 1% BSA, 5% Horse serum in 1X PBS) for at least three days at room temperature, washed with 0.1% Triton-X100 in 1X PBS, treated with secondary antibody (Goat anti-rabbit Alexa 488) diluted at 1:1000 in antibody solution for at least three days, washed with 0.1% Triton-X100 in 1X PBS, incubated in DAPI for another overnight at room temperature. The samples were washed with 1X PBS for three times and processed for tissue clearing using a modified EZ Clear protocol[32]. Embryos were incubated in EZ Clear solution (~80% Nycodenz - Accurate Chemical & Scientific #100334–594, 0.05% sodium azide prepared in 0.02 M sodium phosphate; refractive index = ~1.46) for 5+ days and then mounted in 1% agarose using a 1ml syringe to make an agarose cylinder with the sample suspended within. The sample in the syringe was partially ejected into the EZ Clear solution (to be used as imaging buffer) for 3+ days. The samples were imaged using a Zeiss Lightsheet Z.1 microscope. ZEN software (Zeiss), Stitchy (Translucence Biosystems), and Imaris software (Oxford Instruments) were used for data processing to render videos.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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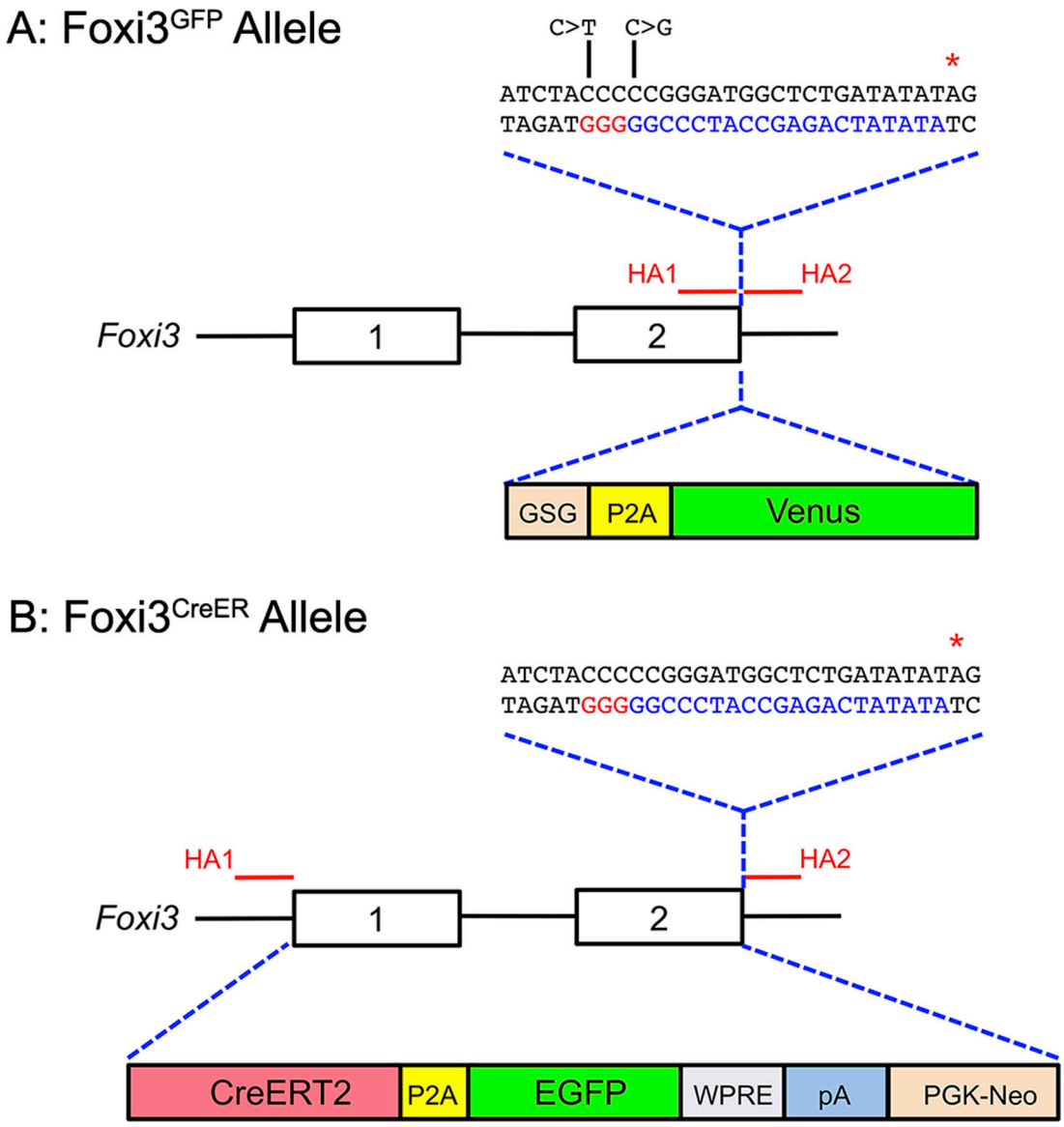

Figure 1: Generation of *Foxi3*^{GFP} and *Foxi3*^{CreER} mice.

(A) Diagram showing the *Foxi3* locus, including the position of the two homology arms (HA1 and HA2) used to target the locus by CRISPR-mediated homology-directed repair. The homology arms enclosed a DNA sequence coding for a GSG-P2A-Venus fluorescent protein, followed by a terminating stop codon (red asterisk). The sequence corresponding to the gRNA targeting the 3' end of *Foxi3* is shown in blue letters, with the PAM sequence shown in red letters. The DNA used for homologous recombination contained two nucleotide changes (C>T and C>G; indicated in the diagram) to prevent the inserted sequence from being targeted by the *Foxi3* gRNA.

(B) Diagram showing the *Foxi3* locus, including the position of the two homology arms (HA1 and HA2) used to target either end of the *Foxi3* gene and insert CreER by homologous recombination in ES cells. Exons 1 and 2 and the intervening intron were replaced by an insert containing a CreER^{T2} fusion cDNA, a P2A sequence followed by EGFP, a

woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), a polyA signal, and a PGK-neo resistance cassette. Homologous recombination in ES cells was enhanced by CRISPR-mediated cleavage of the 3' end of the *Foxi3* locus using the same gRNA as in (A) above.

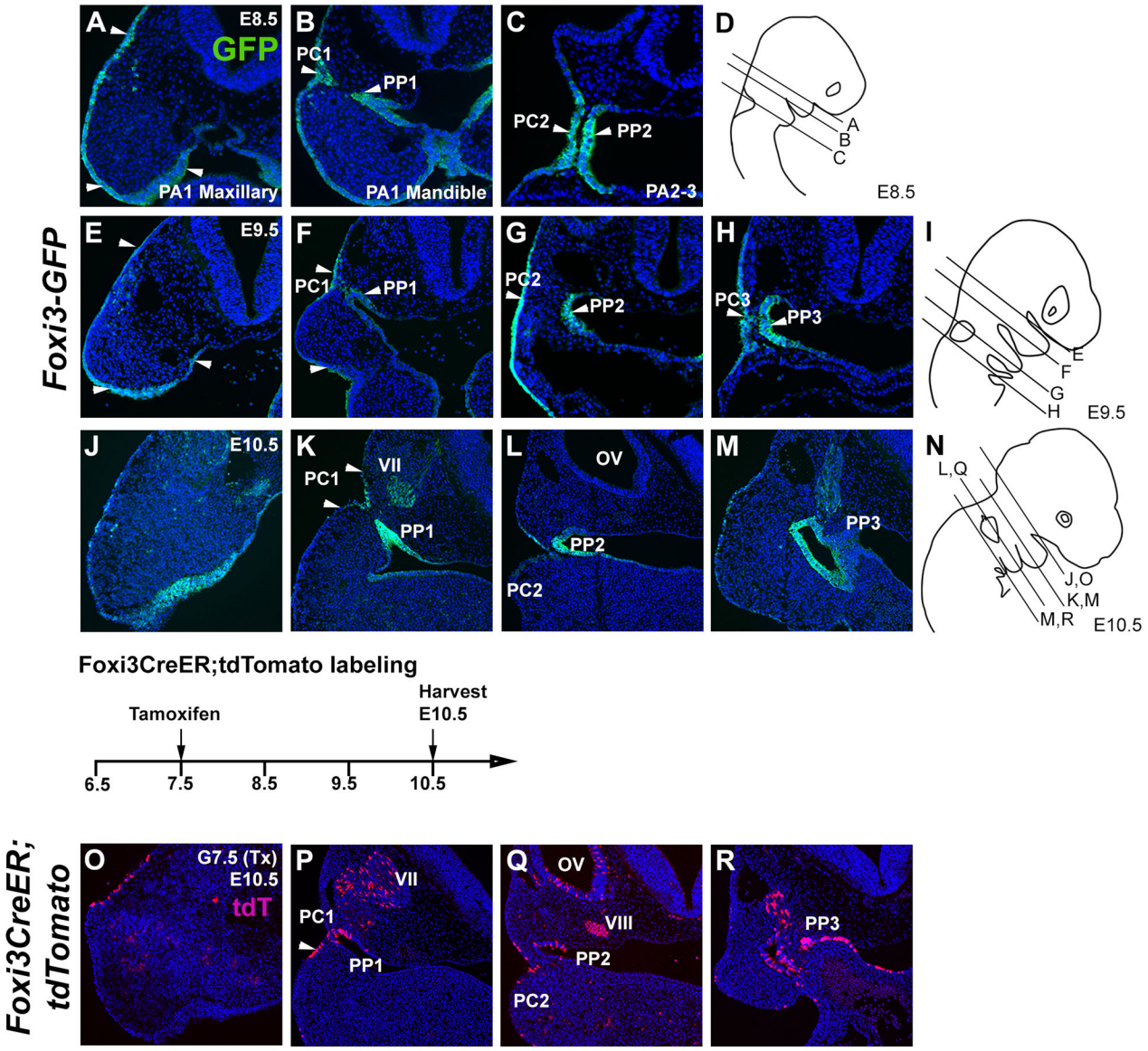

Figure 2: Activity of *Foxi3*^{GFP} and *Foxi3*^{CreER} mice in the first and second pharyngeal arches.

(A-N): Expression of the *Foxi3*^{GFP} reporter in the first and second pharyngeal arches at E8.5, E9.5 and 10.5. Panels D, I and N illustrate the approximate planes of section for each panel at the three ages. (A-C) *Foxi3*^{GFP} is expressed in the ectoderm of the mandible and maxilla of the first pharyngeal arch (PA1) at E8.5, and in the pharyngeal cleft and pharyngeal pouch between the first and second arches (PC1 and PP1). (E-H) *Foxi3*^{GFP} expression begins to be down-regulated from the first arch ectoderm and endoderm by E9.5 but remains expressed in the cleft and pouch regions. Expression is also seen in the second and third cleft and pouch regions (PC2, PC3, PP2, PP3). (J-M) *Foxi3*^{GFP} continues to be down-regulated from the ectoderm of the first and second pharyngeal arches but persists in the first three cleft and pouch regions. (O-R). *Foxi3*^{CreER} mice were mated with *ROSA-Ai9* Cre reporter mice and the pregnant females received a single dose of tamoxifen at 7.5 dpc. At E10.5, tdTomato-expressing cells can be observed in the ectoderm and endoderm of the

first three cleft and pouch regions. Labeled cells can also be seen in the geniculate ganglion (VII, panel P), the otic vesicle (OV) and vestibulo-acoustic ganglion (VIII; panel Q) which are derived from *Foxi3* lineage-labeled progenitors in the pre-placodal region.

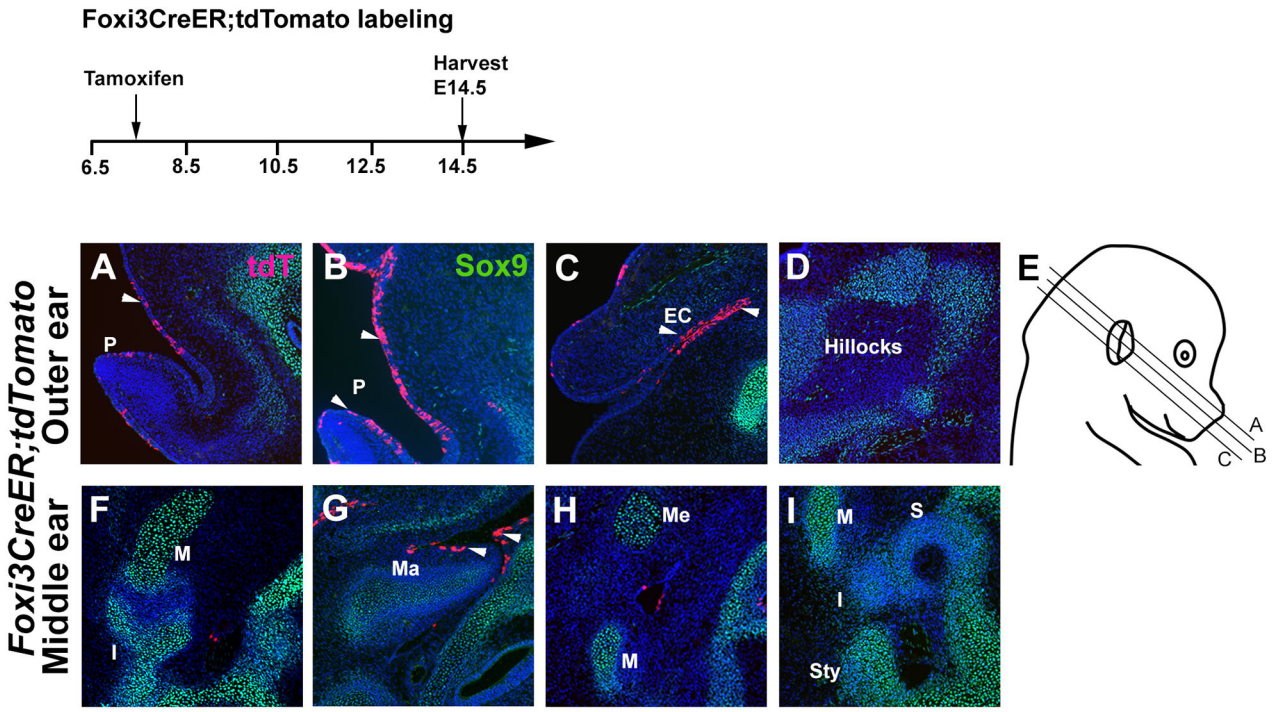

Figure 3: *Foxi3*-expressing cells contribute to the external and middle ear, but not the middle ear ossicles.

Foxi3^{CreER} mice were mated with *ROSA-Ai9* Cre reporter mice and the pregnant females received a single dose of tamoxifen at 7.5 dpc. Mice were analyzed for tdTomato expression at E14.5, together with Sox9 to show developing cartilage. (A-C) *Foxi3* lineage-labeled derivatives can be seen in the ectoderm of the external ear pinna (P) and ear canal (EC), but not the mesenchymal auricular hillocks that label with Sox9 (D). (F-I) Although some cells can be observed in the endoderm of the Eustachian tube, the middle ear ossicles – the malleus (M), incus (I) and stapes (S) – are all unlabeled, as are Meckel's cartilage (Me) and the styloid process (Sty)

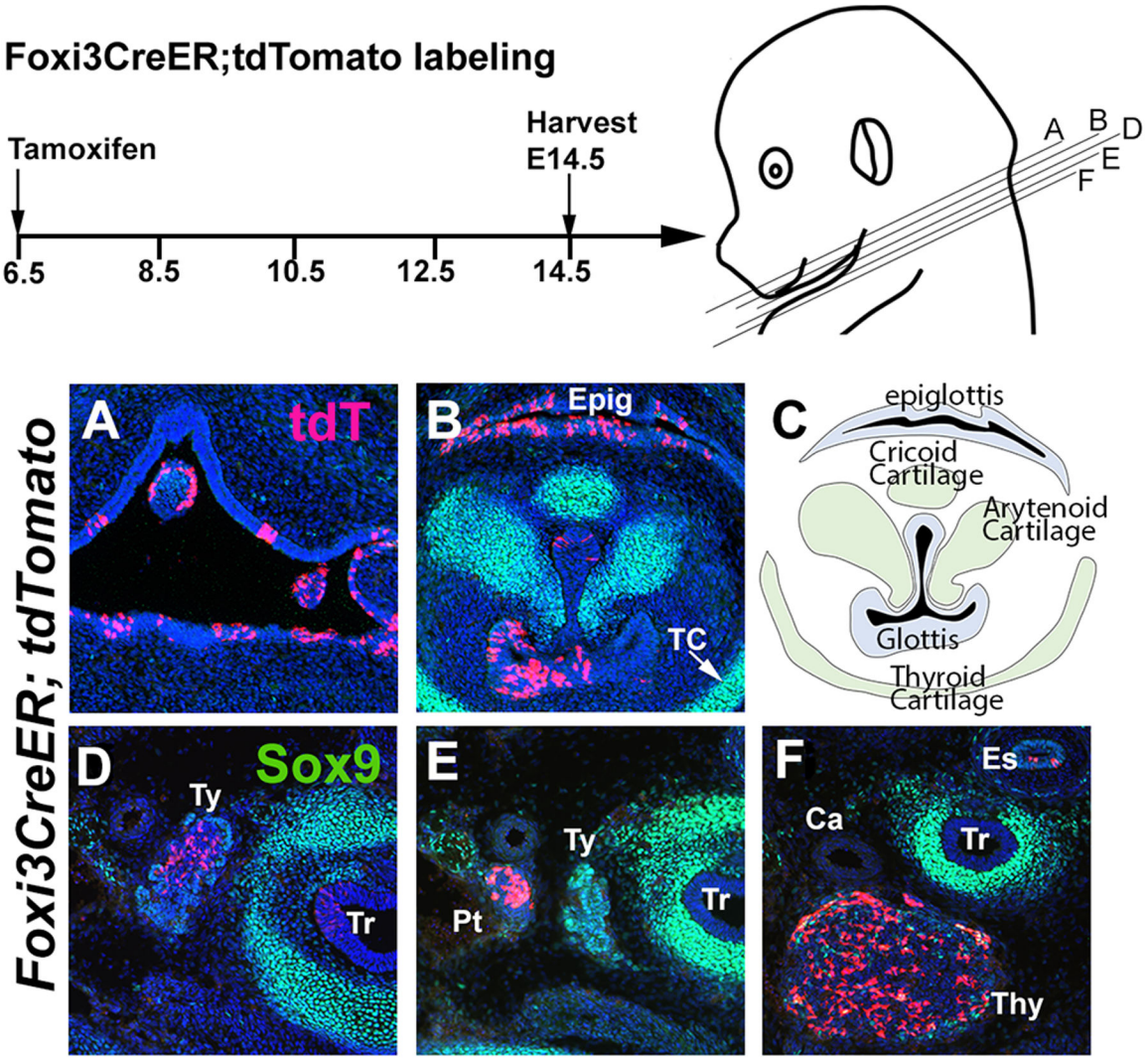

Figure 4: *Foxi3*-expressing cells contribute to endodermal derivatives of the third and fourth pharyngeal arches.

Foxi3^{CreER} mice were mated with *ROSA-Ai9* Cre reporter mice and the pregnant females received a single dose of tamoxifen at 6.5 dpc. Mice were analyzed for tdTomato expression at E14.5, together with Sox9 to show developing cartilage. TdTomato cells can be observed in the oropharynx (A), glottis and epiglottis (B), but not the cricoid, arytenoid, or thyroid cartilages (TC; B, C). (D-F) Labeled cells can also be seen in the developing thyroid (Ty), parathyroid (Pt) and thymus (Thy) tissues, as well as the esophagus (Es) and trachea (Tr).

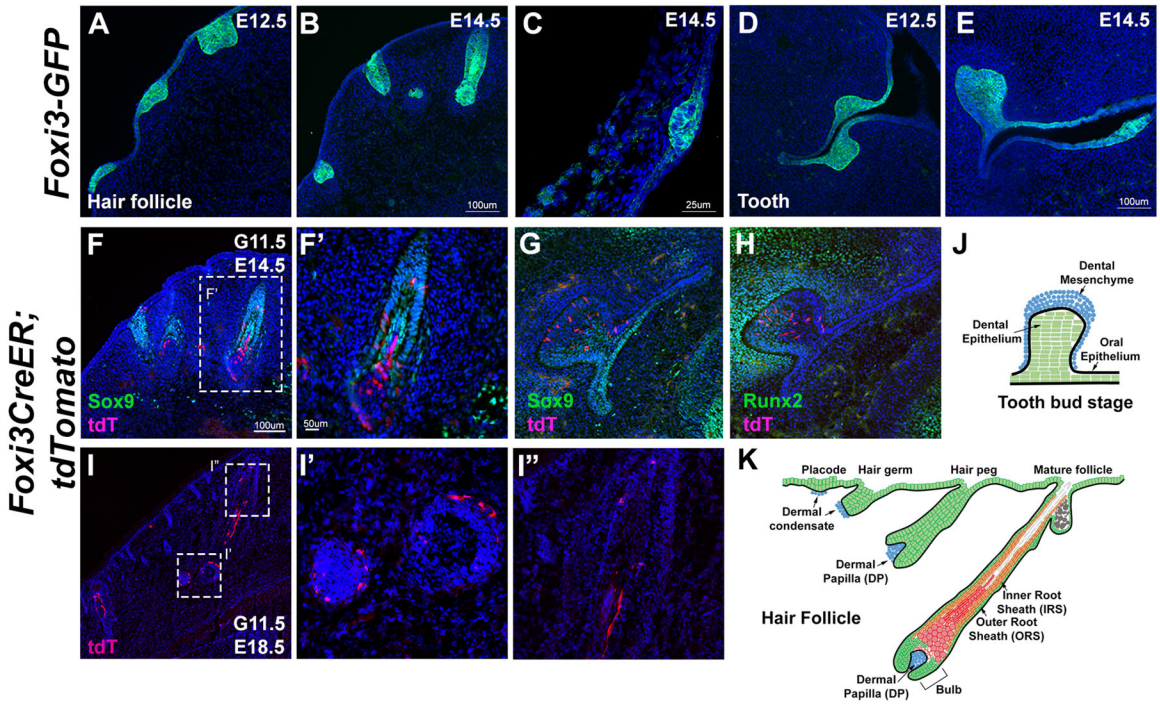

Figure 5: *Foxi3*-expressing cells contribute to hair and tooth placodes.

(A-E) *Foxi3*^{GFP} reporter expression is seen in the progenitors of whisker follicles at E12.5 and 14.5 (A, B), hair follicles from dissected rump skin at E14.5 (C) and tooth placodes at E12.5 and 14.5 (D,E). (F-K) To label the descendants of these populations, *Foxi3*^{CreER} mice were mated with *ROSA-Ai9* Cre reporter mice and the pregnant females received a single dose of tamoxifen by gavage at 11.5 dpc (G11.5). Mice were analyzed for tdTomato expression at E14.5 and E18.5. Cells could be observed in the outer root sheath, cortex, and dermal papilla of the whisker follicle (F, F', I, I', I'', K) and in the dental and oral epithelium (G, H, J)