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Supplemental Information

Sox2+ Stem Cells Contribute to All Epithelial Lineages of the Tooth via *Sfrp5*+ Progenitors

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Supplemental Inventory

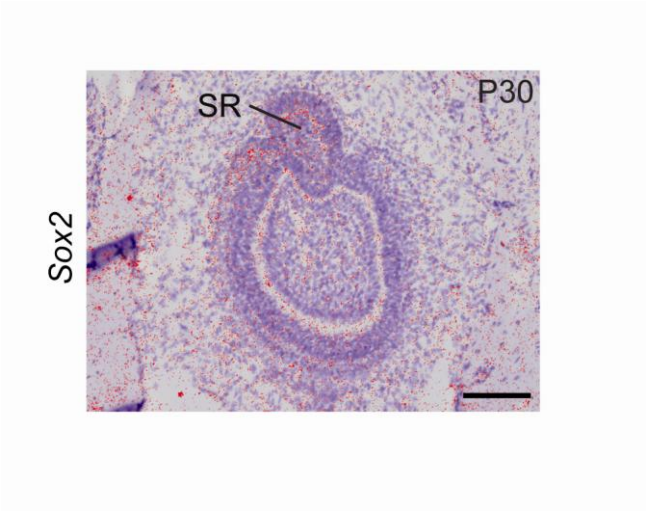

Figure S1.

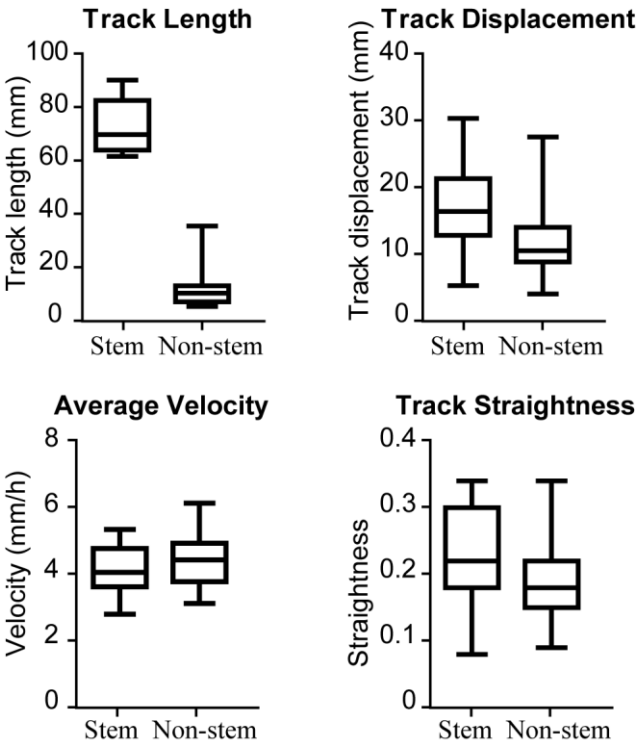

Figure S2.

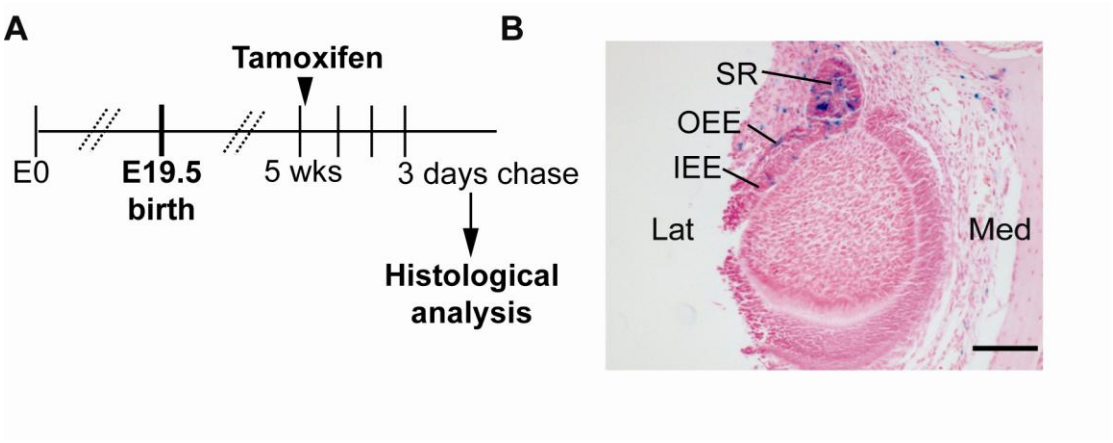

Figure S3.

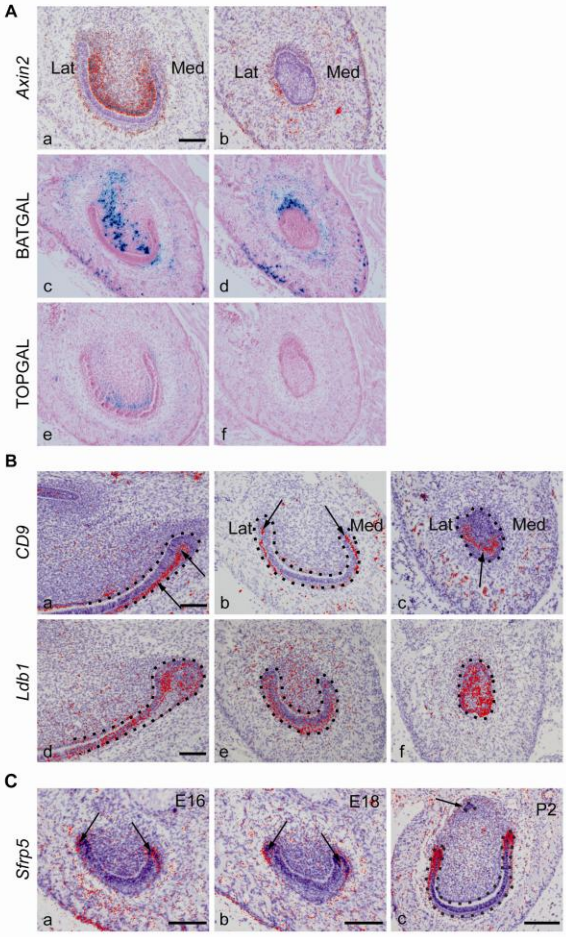

Figure S4.

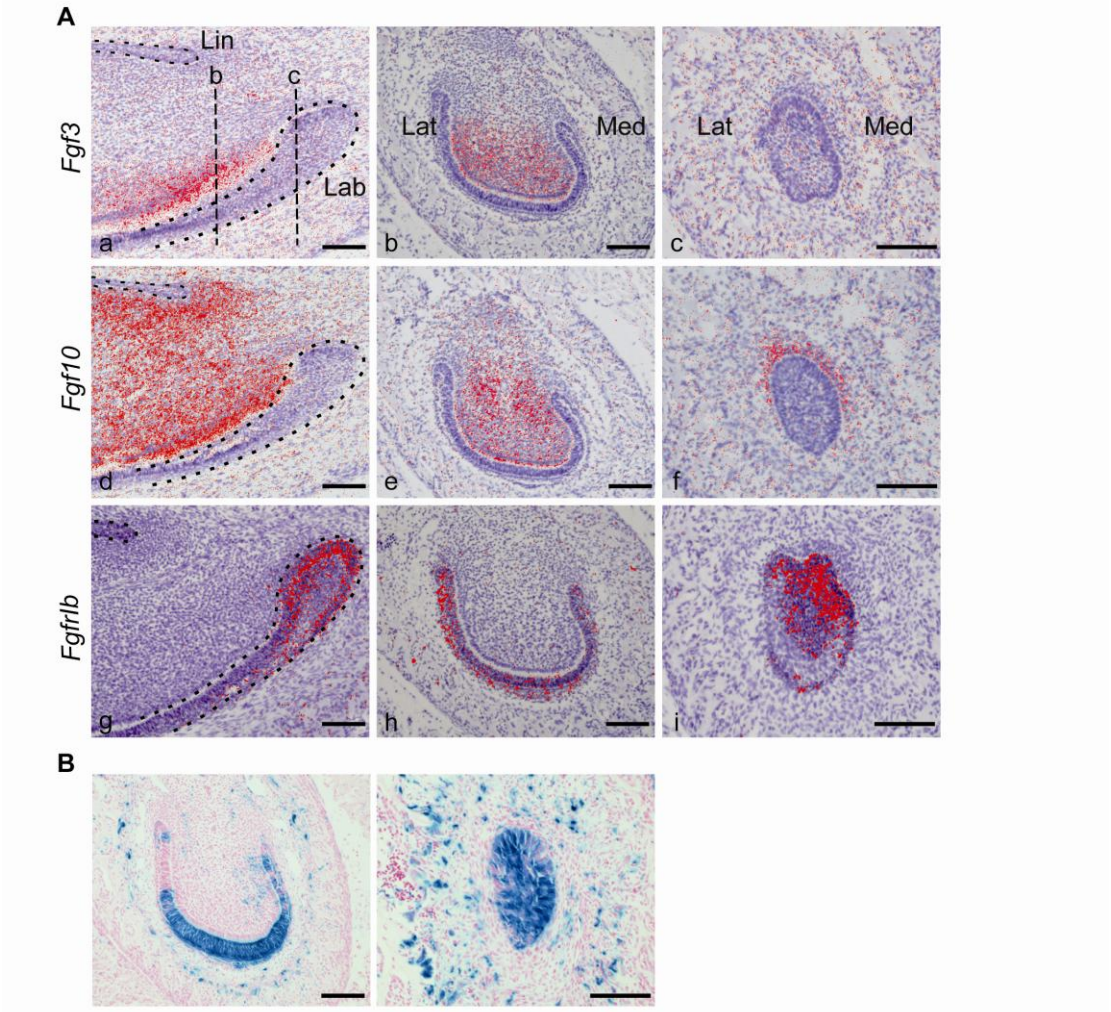

Figure S5.

Figure S1. Sox2 Is Expressed in the Labial Cervical Loop in Adult Incisor

In situ hybridization on frontal section of P30 mouse incisor shows *Sox2* expression in SR of the labial cervical loop. Scale bar equals 100 μm.

Figure S2. Stem Cell Movement in the Labial CL

The track length in groups *Sox2*+ SR cell population and *Sox2*-negative SR cell population was 72.8 μm and 12.1 μm in follow-up of 8 h; the distribution in the two groups differed significantly (Mann-Whitney U, n_1=13 n_2=20, $P < 0.0001$, two tailed). The displacement differed significantly being 17.1 and 12 (Mann-Whitney U, n_1=13 n_2=20, $P < 0.005$, two tailed). The average velocity and straightness did not differ in the two groups. Box and whiskers plot represented as minimum, 25% percentile, median, 75% percentile and maximum values for dataset. Statistical analysis used was non-parametric Mann-Whitney U-test.

Figure S3. *Sox2*+ Stem Cells Are Present in Adult Incisor

(A) Timing of tamoxifen administration in (B). (B) *LacZ*+ cells are detected in SR, OEE, and IEE compartments after 3 days.

Scale bar represents 100 μm.

Figure S4. Absence of Wnt Reporter Activity And Expression of Wnt Inhibitors *CD9* And *Ldb1* in P2 Labial CL

(A) Frontal sections of P2 labial CL show strong *Axin2* expression in the mesenchyme (a) but the expression is absent from the epithelium and the SR (b). X-gal staining of P2 incisors of BATGAL (c, d) and TOPGAL mice (e, f) reporting canonical Wnt activity indicates Wnt signaling activity only in the mesenchyme.

(B) *In situ* hybridization of Wnt inhibitors *CD9* and *Ldb1* in sagittal and frontal sections of P2 incisors. In sagittal section, *CD9* appears in the SR and stratum

intermedium in the labial CL (arrows) (a). Expression is intense in the tip of the epithelial ridge at IEE/OEE junction (arrows) (b). *CD9* expression is also detected in the SR in the labial CL in the stem cell area (arrow, c). Similarly, *Ldb1* expression covers the IEE and OEE (e) and also SR (f). Faint expression is detected in the dental mesenchyme (d, e).

(C) (a) Sagittal section of P2 incisor showing Sfrp5 expression surrounding the labial CL. (b and c) In frontal sections of E16 and E18 incisor *Sfrp5* expression is detected in the IEE/OEE ridge (arrows). (c) Frontal section of wild type P2 incisor showing the absence of *Sfrp5* expression (arrow) in the tip of the lingual CL.

Scale bar equals 100 μm. Med, medial; Lat, lateral

Figure S5. Expression of *Fgf3, Fgf10,* And *FgfrIb* in the Labial CL of P2 Incisor

(A) We performed a detailed analysis of *Fgf3*, *Fgf10*, and *Fgfr1b* expression pattern using frontal sections in addition to sagittal sections used in previous studies (Harada et al., 2002). *Fgf3* is expressed in the mesenchyme adjacent to the region of TA cells in the labial side of the incisor (a, b) while its expression is absent in the mesenchyme surrounding the posterior part of the labial CL (c). *Fgf10* is expressed throughout the dental mesenchyme between the lingual and labial CL (d, e) and it extends in the posterior part of the labial CL (f). *Fgfr1b*, the receptor for Fgf10, is expressed in the epithelium of the labial CL (g), in the IEE/OEE (h), and in the lingual side of SR (i). (B) Frontal sections of P5 Shh-Cre;Rosa26R incisor shows the efficiency of Cre recombinase. Most of the epithelial cells exhibit LacZ staining demonstrating the proper recombination events during incisor development.

Scale bar equals 100 μm.

Table S1.
(see accompanying Excel spreadsheet)

Table S1A. The Microarray Gene Expression Profiling of the Labial CL

The microarray gene expression profiling uncovers 515 genes that are at least 50% more expressed in the labial CL compared to the entire incisor basal area. Cut p-value 0.005; cut fold value 1.5.

Table S1B. The GOP analysis of the microarray results

The GOP analysis of the microarray results point out 95 biological processes enriched in the labial CL (in red compared) to incisor basal area (in blue). Among them are several processes specific to ectodermal biological functions. (p-value <0.001; fold >2.5)

Supplemental Experimental Procedures

Luciferase Reporter Assay

Mouse miR-720 and miR-200b precursor expression clones were constructed in non-viral expression vector (pEZX-MR04). Luciferase reporter constructs containing the 3'-UTR of Sox2 and Fgf8 were designed in pEZX-MT01 vector (GeneCopoeia). For transfection, HEK293 were plated in triplicate into 6-well plates and cotransfected with 1 µg of pEZX-MT01 (empty or containing the studied 3'-UTR) and 0.5 µg of pEZX-MR04 (containing miR-720, miR-200b, or miR-scramble as negative control) by using FuGene (Roche). Transfection efficiency was normalized on the basis of the Renilla luciferase activity. Firefly and Renilla luciferase activities were measured by using the Dual Luciferase Reporter Assay System kit (Promega), according to the manufacturer's and GeneCopoeia's instructions. For each triplicate the standard deviation was calculated and the Student t-test was used to determine p-values and $p<0.01$ was set as the level of significance.

Wnt Reporters and X-Gal Staining

TOPGAL mice were from Jackson Laboratories (DasGupta and Fuchs, 1999) and BATGAL mice were kindly provided by Stefano Piccolo (Maretto et al., 2003). X-Gal staining and further processing of tissues was performed as previously described (Suomalainen and Thesleff, 2010).

Radioactive *In Situ* Hybridization

Radioactive *in situ* hybridization was performed according to standard protocols on 7 µm frontal and sagittal sections. ^{35}S (Amersham) labeled RNA probes were used to detect the expression of *Axin2* (Lustig et al., 2002), *Fgf3* (Kettunen et al., 2000), *Fgf10* (Kettunen et al., 2000), *Fgfr1b* (Kettunen et al., 1998b), and *Ldb1* (Zhao et al.,

2007). To detect *CD9* expression a 671 bp probe was cloned in pCRII-TOPO vector using 5'CAAGTGCATCAAATACCTGCTC and 5'TTGGGCAGACTCTAGACCATTT.

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