Supplemental Information

Figures

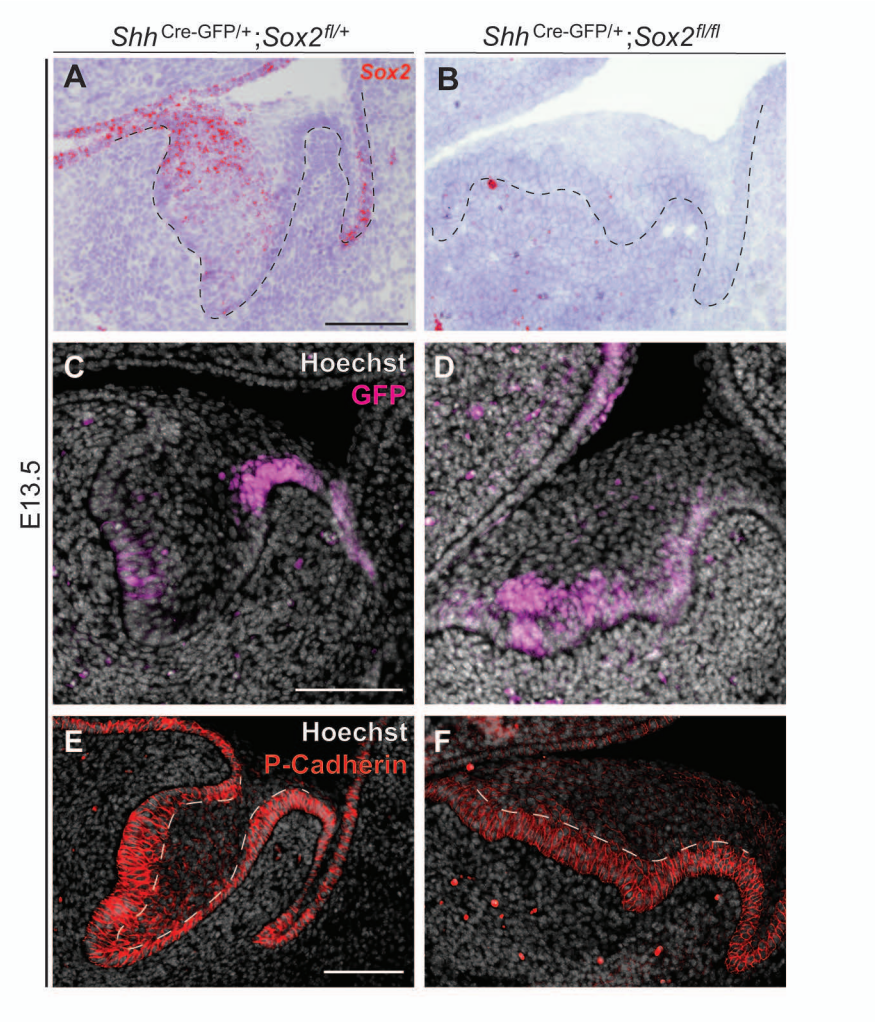

Figure S1. *Sox2* expression is efficiently knocked out from the dental epithelium of *Sox2*^{cKO} embryos. Expansion of the *Shh* domain in *Sox2*^{cKO}, but similar patterns of P-Cadherin in *Sox2*^{cKO} and control incisors.

(A) *Sox2* transcripts (red) are present in the dental epithelium at E13.5 (lingual CL, laCL, enamel knot, oral epithelium and vestibular lamina). **(B)** *Sox2* expression is not detected in the *Sox2*^{cKO}. The mutant incisor cap exhibits a wider dental lamina than the control littermates. **(C, D)** *Shh* expression pattern is expanded in the *Sox2*^{cKO} incisor buds at E13.5. **(E, F)** Sagittal sections of *Sox2*^{cKO} and control incisors at E13.5 show similar patterns of P-Cadherin.

Scale bars: 100μm.

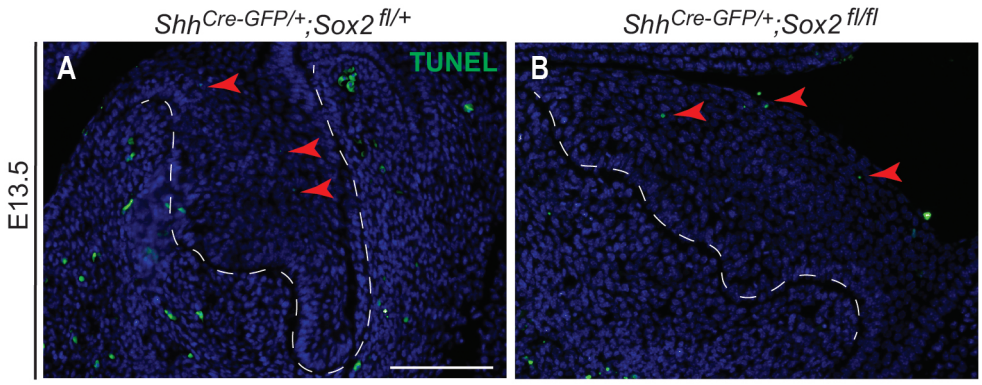

Figure S2. Cell death is unchanged in *Sox2*^{cKO} incisors at E13.5.

Cell death detection via TUNEL assay reveals that a similarly small number of apoptotic cells in both control **(A)** and *Sox2*^{cKO} **(B)** incisor. Red arrowheads point at TUNEL+ staining.

Scale bar: 100μm.

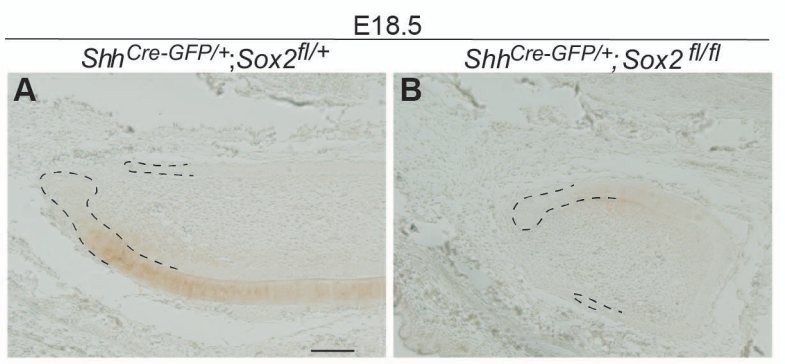

Figure S3. SHH expression pattern in *Sox2*^{cKO} incisors.

Immunodetection of SHH in E18.5 incisor in control **(A)** and *Sox2*^{cKO} **(B).** liCL and laCL are contoured with a dotted black line.

Scale bar: 100µm.

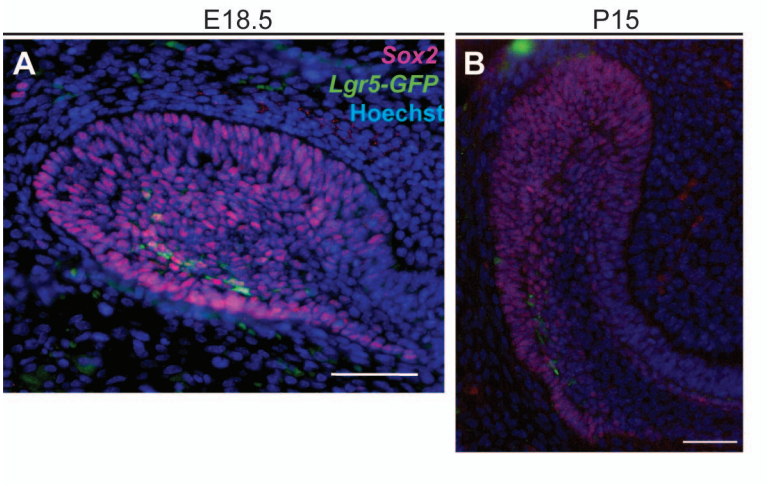

Figure S4. *Lgr5*+ cells are a subpopulation of *Sox2*+ cells.

(A) Immunodetection of Sox2 and Lgr5-GFP proteins at E18.5 shows overlap of both expression patterns. **(B)** Most of the cells in the postnatal laCL express Sox2 protein, while Lgr5-GFP appears in the distal SR and OEE.

Scale bars: 100µm.

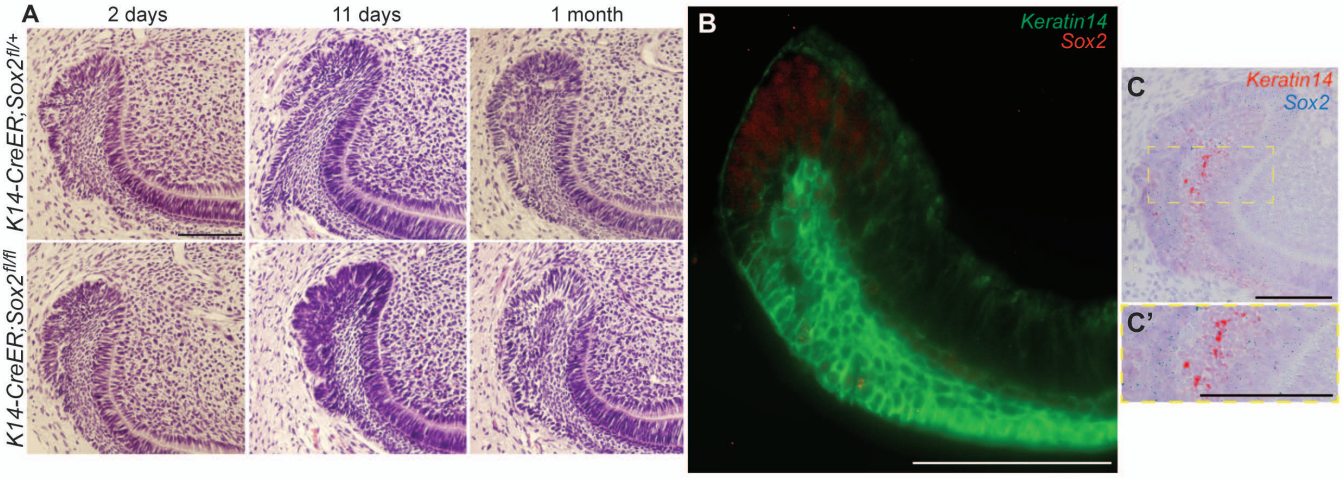

Figure S5. *K14-CreER* is not suitable for Sox2 deletion, due to partial overlap of *K14* and *Sox2* expression patterns.

(A) No abnormal phenotype is observed in the laCL of *K14-CreER;Sox2*$^{fl/fl}$ mice at two days, 11 days or one month after Tamoxifen administration. **(B, C, C')** *Sox2* and *K14* patterns overlap only partially, both at the transcriptomic and protein levels. Majority of *Sox2* and SOX2 expression is found in the enamel epithelium, where low levels of K14 are found. Majority of *K14* expression is localised to the SR.

Scale bars: 100μm.

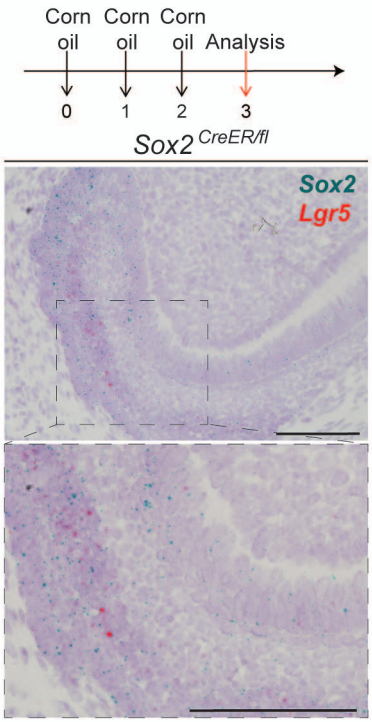

Figure S6. *Sox2*$^{CreER/fl}$ mice injected with Corn oil (Tamoxifen vehicle) as negative controls.

Sox2 and *Lgr5* mRNA detection for *Sox2*$^{CreER/fl}$ laCL 3 dies after the first corn oil injection.

Scale bars: 100μm.

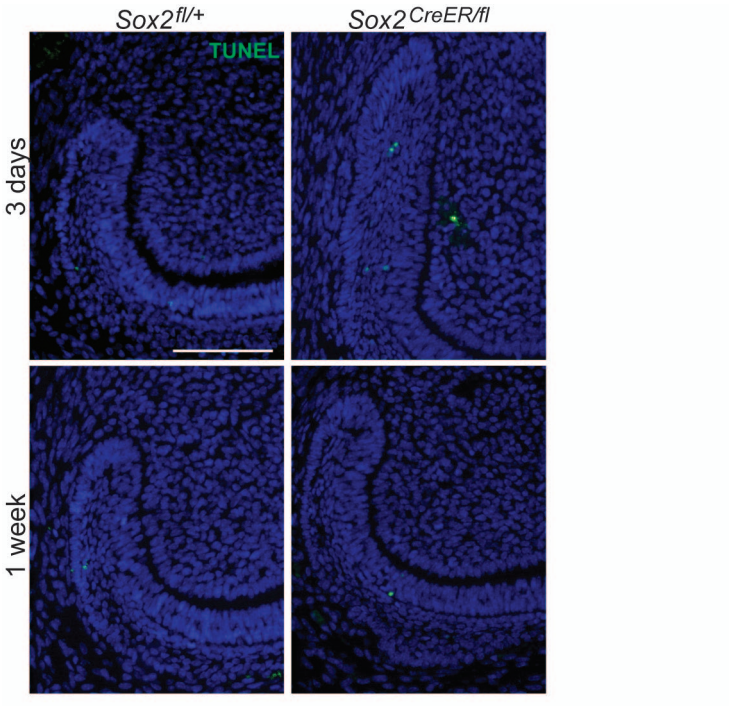

Figure S7. Apoptosis is not the driving force causing the narrowing of the laCL in the *Sox2*$^{CreER/fl}$ mice.

TUNEL assay on *Sox2*$^{CreER/fl}$ incisors, three days and one week after Tamoxifen administration shows low levels of cell death.

Scale bars: 100μm.

Table S1. List of transcripts obtained from the microarray.

All transcripts with the fold change of the following conditions: mESCs vs. Embryonic *Sox2*+ cells, mESCs vs. Adult *Sox2*+ cells, and Embryonic *Sox2*+ cells vs. Adult *Sox2*+ cells.

Click here to Download Table S1

Table S2. List of transcripts differentially expressed between *Sox2*+ cells and mESCs.

WE observed that 2.34% of the signature was enriched in Sox2+ cells and 4% of the signature was downregulated. This list does not contain the fold of change.

Click here to Download Table S2

Table S3. Gene ontology processes (GOP) activated in *Sox2*+ cells compared to mESCs.

From the genes enriched in Sox2 positive cells, listed in table S2, we found that 187 processes were activated over 2.5 fold.

Click here to Download Table S3

Table S4. GOP enriched in Embryonic compared to Adult *Sox2*+ cells.

We observed that 3.54% of the signature was enriched in embryonic cells. Among these, 143 GOP were enriched over 2.5 fold.

Click here to Download Table S4

Table S5. GOP enriched in Adult compared to Embryonic *Sox2*+ cells.

We observed that 2.75% of the signature was enriched in adult cells. Amongst these, 153 GOP were enriched over 2.5 fold.

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Table S6. List of genotyping primers.

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Supplemental Material and Methods

RNAscope protocol

Single channel RNAscope, red kit

Slides containing 5-µm sections were preheated for 1h in +60^{0}C and deparafinized for ten minutes in a series of three xylenes, two minutes in a series of two 94% ethanol with agitation and dried for 30 minutes on a hotplate at 60°C. Samples were incubated with hydrogen peroxide (included in the kit) for 10 minutes. Slides were incubated for 30 minutes in the target retrieval reagent at 85°C, rinsed with distilled water and dried on a hot plate at 60°C for 30 minutes.

A barrier around the sections was drawn with Immedge pen, and were incubated with protease (included in the kit) during 15 minutes. Hybridization of the probe, amplification and detection of the signal steps were done according to the manufacturer's protocol.

Samples were counterstained by incubating for two minutes in 50 % hematoxylin and rinsed with ammonia water. Sections were dehydrated by incubating them at 60°C during 45 minutes and mounted using VectaMount.

Duplex RNAscope, red and blue-green kit

Pretreatment of the samples was done as described for the single channel RNAscope, hybridization of the probe, amplification and detection of the signal steps were done according to the manufacturer's protocol.

Samples were counterstained by incubating for 30 seconds in 50 % hematoxylin and rinsed with water. Sections were dehydrated by incubating them at 60° during 45 minutes and mounted using VectaMount.

Immunofluorescence Protocol

Tissue sections were deparafinized and rehydrated five minutes in a series of four xylenes, two minutes in a series of three absolute ethanol, two minutes in a series of two 94% ethanol, two minutes in 70% ethanol, two minutes in 50% ethanol and two minutes in distilled water. Slides were washed for 10 minutes in 1x PBS-0.3% triton and incubate for two hours in 10mM sodium citrate, inside a pressurized device (2100 retriever; APTUM), for antigen retrieval. Slides were washed for 10 minutes in 1x PBS-0.3% triton. Blocking was performed for one hour in 1x PBS-0.3% Triton, 1% BSA, 10% serum (depending on the secondary antibody). The slides were incubated with the primary antibody in blocking solution overnight.

The following day, slides were washed thrice for five minutes in 1x PBS-0.3% triton. Incubation with the secondary antibody 1:400 and Hoechst 1:2000 in 5% BSA in PBS-0.3% triton was held for two hours at room temperature. Slides were washed for ten minutes in 1x PBS-0.3% Triton, ten minutes in 1x PBS-0.1% Triton and ten minutes in 1xPBS to reduce the background staining. Sections were mounted using Vectashield (Vector).

DAB Immunohistochemistry Protocol

Samples were deparaffinised using the same protocol as for immunofluorescence. Samples were washed three times with 1x PBS-0.3% triton for ten minutes and incubated for two hours in blocking solution (1x PBS-0.3% triton with 2% Skimmed milk powder). Primary antibody was incubated overnight at +4^{0}C. Samples were washed thrice in blocking solution for 10 minutes. Secondary antibody was incubated in blocking solution overnight at +4^{0}C. Samples were washed in blocking solution three times for 10 minutes. DAB colour development was done using the Mouse on Mouse Elite Peroxidase Kit (Vector, PK-2200), following the manufacturer's protocol. Samples were mounted with Immu-Mount (ThermoFisher Scientific, 9990402).

Sample amplification and preparation for microarray

RNA samples from embryonic (E14.5) and adult (P60) *Sox2*+ cells were prepared for whole transcriptome expression analysis (Affymetrix MTA) using the Affymetrix's WT Pico Reagent kit. First, in the reverse transcription, RNA is primed with primers containing a T7 promoter sequence, and a single-stranded cDNA is synthesized. Using this cDNA as a template, a second-strand cDNA is synthesized. Next, the complimentary RNA (cRNA) is synthesized and amplified by *in vitro* transcription of the second-stranded cDNA using T7 RNA polymerase. Unincorporated nucleotides and other debri are removed at this point. Sense-strand cDNA is synthesized by reverse transcription of cRNA using second-cycle primers. The sense-strand cDNA contains dUTP at a fixed ratio relative to dTTP. The secnd-cycle single-stranded cDNA is purified. Next, the sense-strand cDNA is fragmented by uracil-DNA glycosylase (UDG) andapurinic/apyrimidinic endonuclease 1 (APE 1) at the unnatural dUTP residues. The fragmented cDNA is labeled by terminal deoxynucleotidyl transferase (TdT) using the Affymetrix proprietary DNA Labeling Reagent, covalently linked to biotin. A hybridization cocktail containing the target and hybridization controls is prepared. The target is hybridized to the GeneChip array in cartridge format during 17-hour incubation at 45 °C. After hybridization, the array is washed and stained with streptavidin phycoerythrin conjugate using an automated protocol on the GeneChip® Fluidics Station 450, followed by scanning on a GeneChip® Scanner.

Probe combinations for multiplex qPCR

For *K14-CreER;Sox2*$^{fl/fl}$ we used the following combination: Lgr5-TEX (qMmuCEP0053421), Sox2-FAM (qMmuCEP0060283), Sfrp5-HEX (qMmuCEP0053072), Bmi1-Cy5.5 (qMmuCEP0043063) and GAPDH-Cy5 (qMmuCEP0039581).

For *Sox2*^{cKO} gene analysis two combinations of PrimePCR Probe Assay (Bio-Rad) were used: Lgr5-TEX (qMmuCEP0053421), Sox2-FAM (qMmuCEP0060283), Hprt-

HEX (qMmuCEP0054164) and Shh-FAM (qMmuCIP0028772), Sfrp5-HEX (qMmuCEP0053072), GAPDH-Cy5 (qMmuCEP0039581).