Supplementary file

Materials and Methods

Mice, Tamoxifen Administration and Cyclopamine Injection

Cre-ERT2 mice carry the Cre recombinase gene fused to the estrogen receptor T2 cassette inserted into the Rosa 26 locus (Ventura et al. 2007). Consequently, recombination takes place in a ubiquitous manner after administration of tamoxifen (Hayashi and McMahon 2002). *Rdh10*$^{flox/flox}$ embryos were used to obtain control samples throughout the study.

All animal experiments were performed in accordance with the guidelines of the Animal Care and Use Committee of the Osaka University Graduate School of Dentistry.

Embryo Imaging, Immunohistochemistry and TUNEL Staining

Whole-mount nuclear fluorescent “pseudo-SEM” imaging (Sandell et al. 2012) was performed to examine embryonic morphological features. Frozen sections were immunostained as previously described (Kurosaka et al. 2017). Antibodies against

phospho-HISTONE-H3 (#05806, 1:200, Millipore), E-cadherin (#3195, 1:200, Cell Signaling Technology), and SOX2 (#97959, 1:100, Abcam) were used with appropriate secondary antibodies tagged with Alexa Fluor 546/488 (1:500, Invitrogen). Apoptotic cells were detected using an *in situ cell death detection* kit (Roche, #11684795910) following the manufacturer´s instructions. Three sections were counted for each of the three samples. The results from cellular analysis are presented as the percentage of PHH3-positive and TUNEL-positive cells among Dapi-labeled nuclei at the same anteroposterior levels in different samples. Comparisons between the two groups were performed using the Student's t-test.

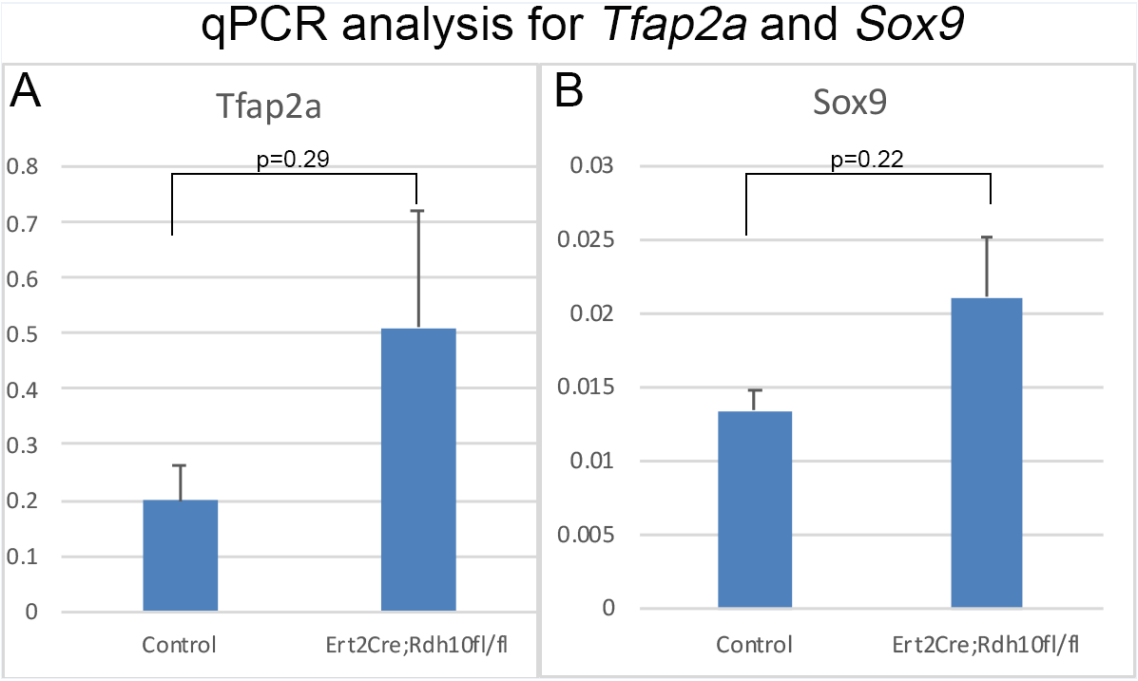
Histological Analysis

The embryos were fixed overnight in 4% paraformaldehyde. Paraffin embedding and preparation of 7-µm-thick histological sections, followed by hematoxylin and eosin staining, were performed according to standard histological protocols.

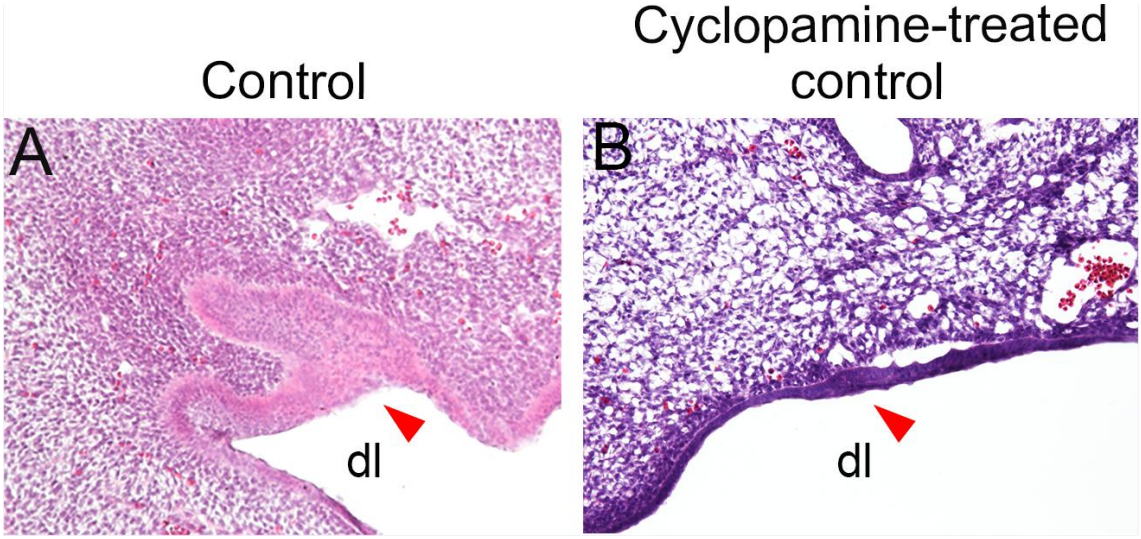
Statistical analysis

Statistical significance in Figures 2, 4, and 5 was assessed using the Student´s *t*-test in Microsoft Excel. *P* values of less than 0.05, were defined as significant in all experiments.

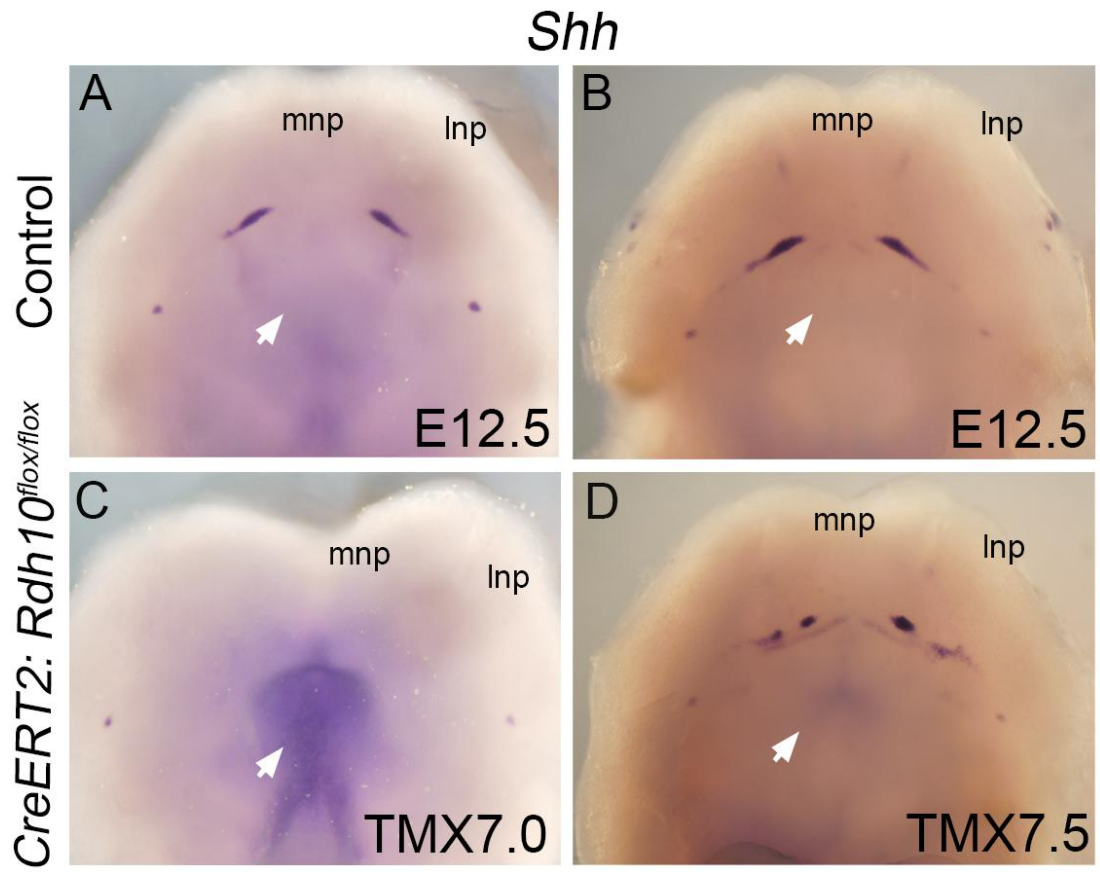
Appendix Figure and Legends



Appendix Figure 1. *Tfap2a* and *Sox9* expression levels were quantified using RT-qPCR. **(A-B)** *Tfap2a* and *Sox9* mRNA expression levels in the craniofacial region tended to increase without significant differences between *CreERT2: Rdh10*$^{flox/flox}$ embryos and control embryos at E9.5.



Appendix Figure 2. Comparison of the morphology of the upper teeth in control and cyclopamine-treated control embryos at E13.5. **(A, B)** Hematoxylin and eosin-stained frontal sections of the upper incisors. The red arrowheads in A and B indicate the dental lamina. dl, dental lamina.



Appendix Figure 3. Differentially disrupted patterns of *Shh* expression following tamoxifen treatment at different time points **(A-D)** Whole-mount *in situ* hybridization of *Shh* in control (A-B) and *CreERT2: Rdh10*$^{flox/flox}$ embryos (C-D). Ventral views of *Shh* expression with tamoxifen treatment at E7.0 (A, C) and E7.5 (B, D). The white arrows in A-D indicate an expansion of *Shh* expression in the ventral forebrain.

Appendix References

Hayashi S, McMahon AP. 2002. Efficient recombination in diverse tissues by a tamoxifen-inducible form of cre: A tool for temporally regulated gene activation/inactivation in the mouse. Dev Biol. 244(2):305-318.

Sandell LL, Kurosaka H, Trainor PA. 2012. Whole mount nuclear fluorescent imaging: Convenient documentation of embryo morphology. Genesis. 50(11):844-850.

Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T. 2007. Restoration of p53 function leads to tumour regression in vivo. Nature. 445(7128):661-665.