The Role of Discoidin Domain Receptor 2 in Tooth Development

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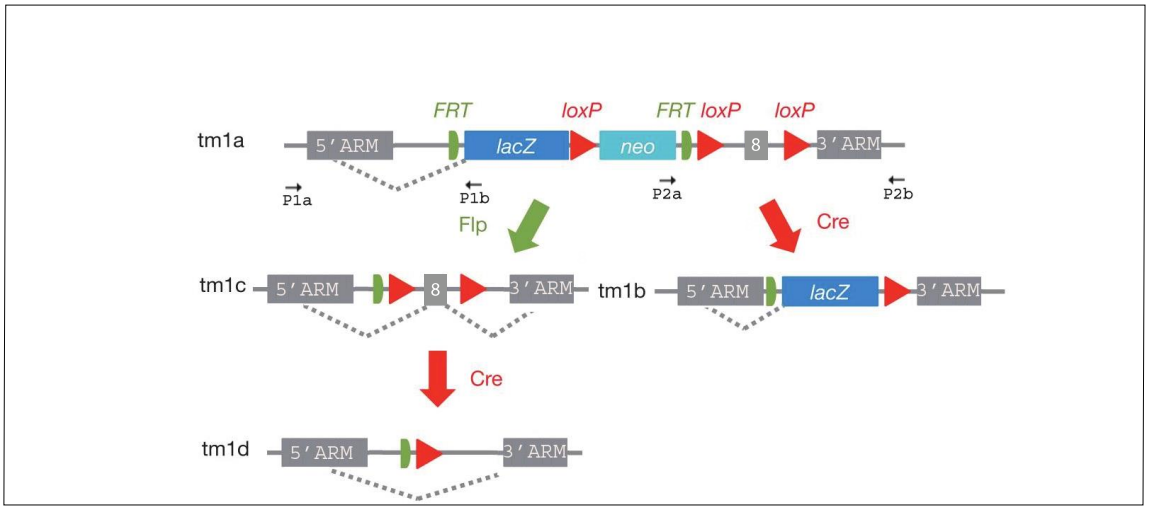
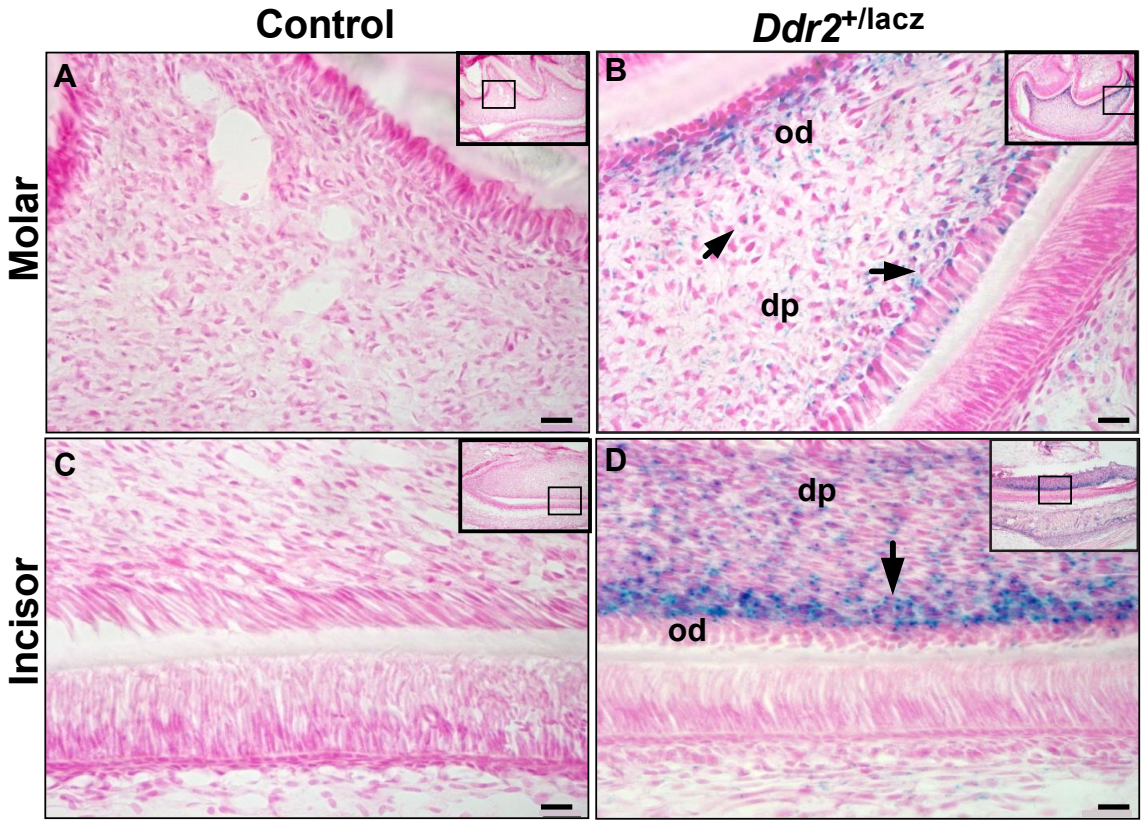
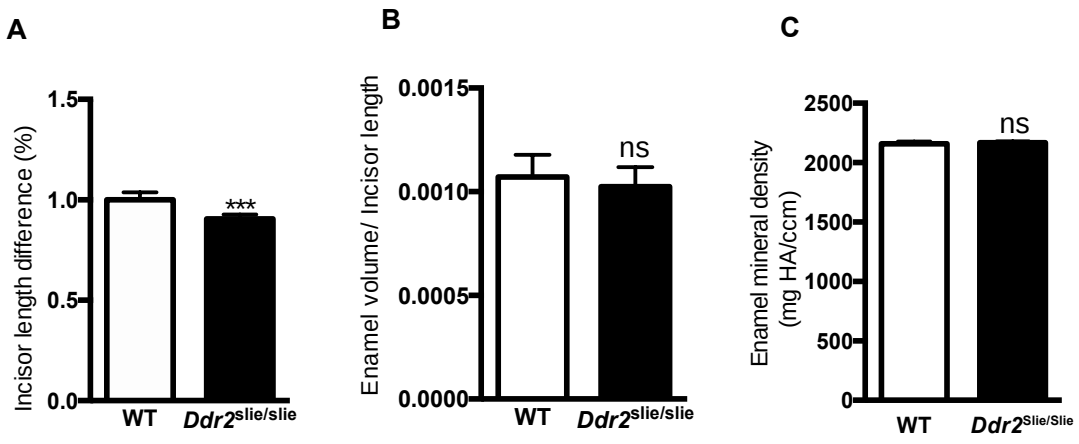
Appendix



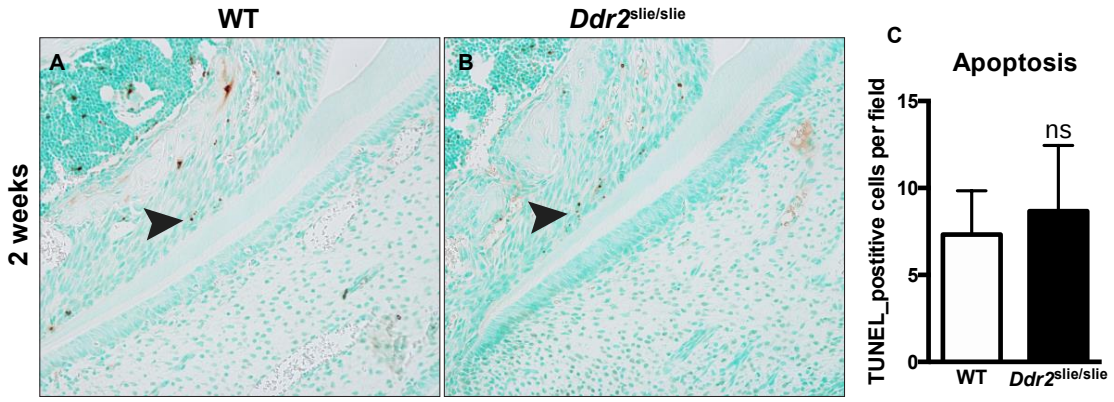
Appendix Figure 1: Development of *Ddr2*-LacZ-knockin mice and *Ddr2* conditional knockout (Ddr2$^{fl/fl}$) mice. To generate a *Ddr2* knockout first mouse line (tm1a), ES cell transplantation was performed by the University of Michigan Transgenic Model core. To generate *Ddr2*$^{fl/fl}$ mice, knockout first mice were crossed with FLPO mice to remove LacZ-Neo elements (tm1c). Mice were bred into a C57BL6 background for at least 6 generations. Treatment with adenoCre removes exon 8 (tm1d).



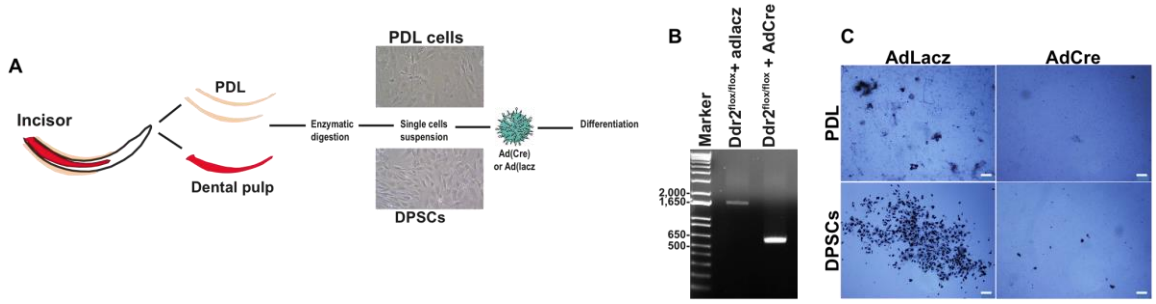
Appendix Figure 2. Specificity of *Ddr2*-lacZ staining in dental tissues. (**A,C**) No X-Gal staining in the *Ddr2*$^{+/+}$ control molar and incisor teeth at P1. (**B,D**) X-Gal staining of *Ddr2*$^{+/LacZ}$ mice in the molar and incisor teeth at P1. Odontoblasts (od), dental pulp (dp). Arrows point to the LacZ staining. Scale bar: 20 μm in (A-D).



Appendix Figure 3. (A-C) Quantification the incisor length, enamel volume and mineral density. ****P*<0.001, ns: not significant



Appendix Figure 4. (**A,B**) Representative images of TUNEL staining of the first molars in *Ddr2*$^{slie/slie}$ and WT mice. (**C**) Quantification of TUNEL-positive cells (brown) per field. Cell nuclei were stained with methyl green (green), *n=3* mice per group. ns: not significant



Appendix Figure 5. Isolation and adenoCre treatment of PDL and DPSCs. (**A**) Experimental design of cell isolation, viral transduction, and differentiation in vitro. Ten mandibular incisors were extracted from 3-month old *Ddr2*$^{fl/fl}$ mice and flushed with Dulbecco's modified Eagles' medium (DMEM, Invitrogen). A complete flush of dental pulp was checked under a dissection microscope (Nikon SMZ 745T). Dissected tissues from PDL and dental pulp were digested with Collagenase P (Roche) for preparation of primary cultures. PDL and dental pulp stromal cells (DPSCs) were plated in 12 well plates at a density of 1x10^5 cells for adenovirus infection and differentiation experiments. (**B**) PCR analysis showing Cre-mediated recombination of the floxed *Ddr2* alleles resulting in a PCR product of 600bp after treatment with AdCre. (**C**) Bright-field images of von Kossa staining of PDL and DPSC cells after differentiation for 21 days in vitro, scale bar: 200 µm in (C).