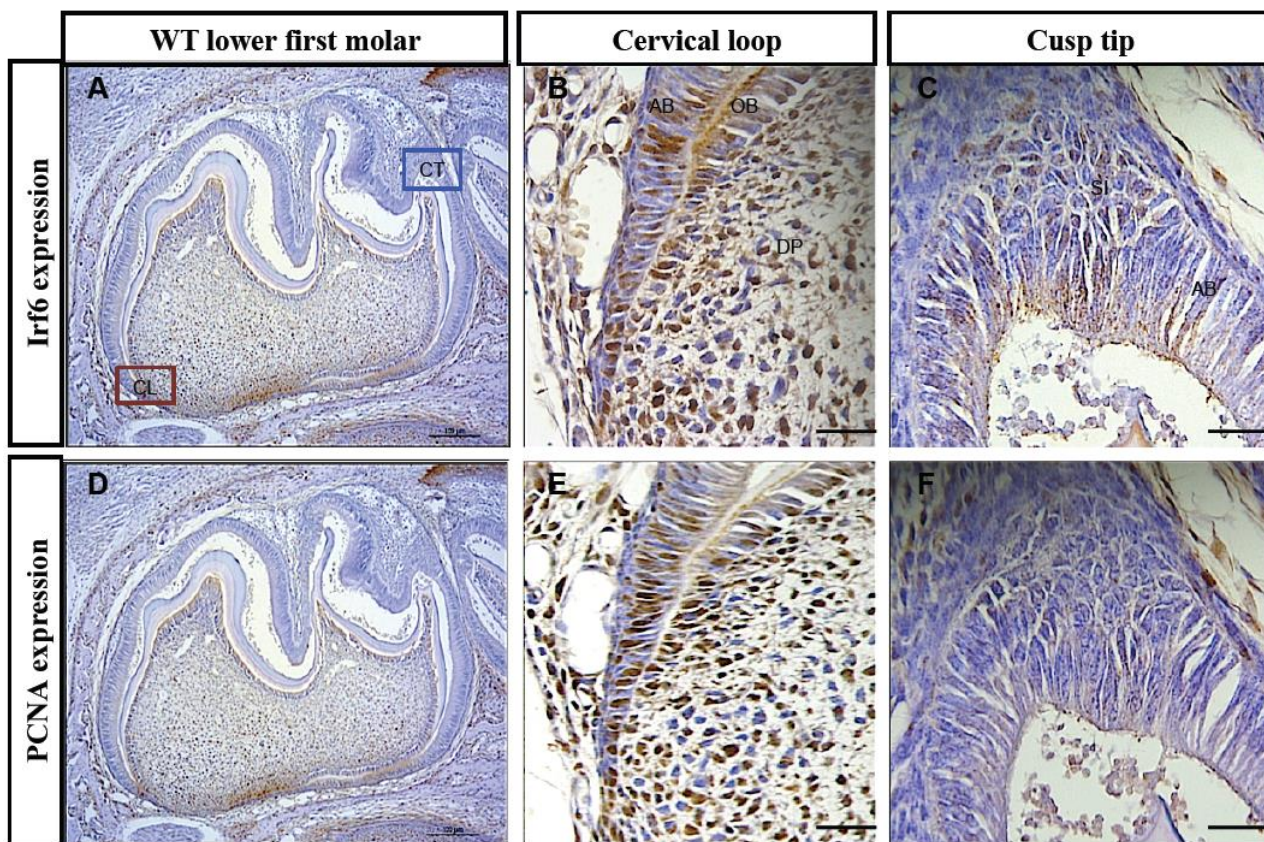


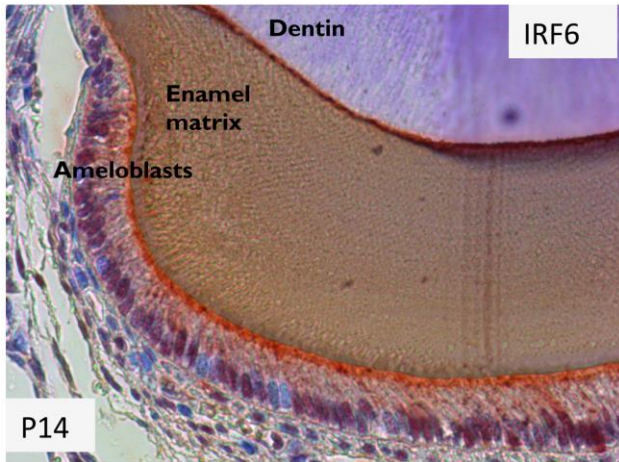
# Full Spectrum of Postnatal Tooth Phenotypes in a Novel *Irf6* Cleft Lip Model

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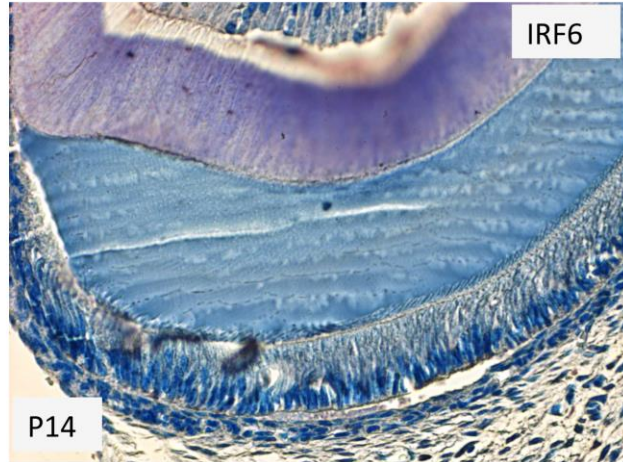
**Appendix Figure 1. Expression of *Irf6* in postnatal dental tissue.** IHC detection of *Irf6* (A-C) and proliferating cell nuclear antigen (PCNA) (D-F) in the lower first molar of a wildtype mouse at postnatal day 3 (P3). Strong nuclear *Irf6* staining is seen in the highly proliferating area of the cervical loop and the presecretory ameloblasts (B), where it colocalized with PCNA (E). At the cusp tip (C, F), staining for *Irf6* (C), but not PCNA (F), was evident. Of note, *Irf6* staining was primarily cytosolic with only relatively light staining in nuclei of the mature ameloblasts and cells of the stratum intermedium. CT: Cusp tip, CL: Cervical loop, AB: ameloblast, OB: Odontoblast, DP: Dental papilla, SI: Stratum intermedium. Scale bars: 50  $\mu\text{m}$  (B, C, E, and F); and 500  $\mu\text{m}$  (A and D). In secretory-stage incisor ameloblasts at P14 (lower panel), strong expression is seen both in the nucleus and at the apical end, both of which are lost in the dental epithelium-specific knockout (*cKO*) mice.



wildtype



*Irf6*-cKO

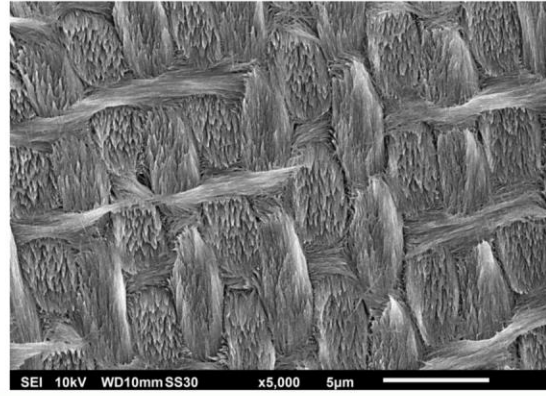
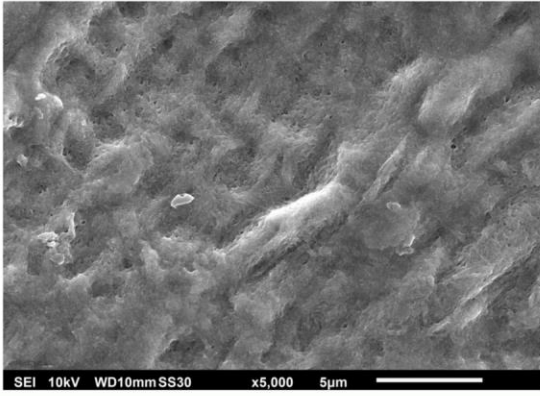


**Appendix Figure 2. SEM of immature and mature enamel from *Irf6-cKO* and control genotypes.**

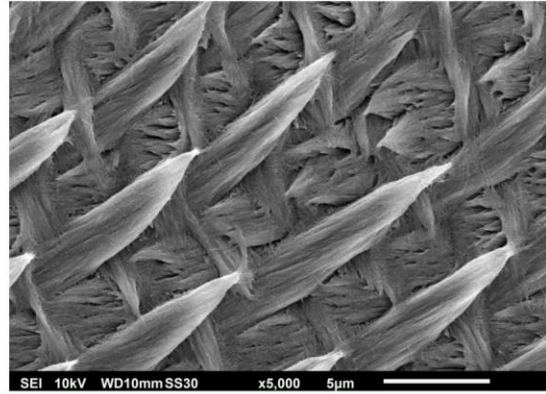
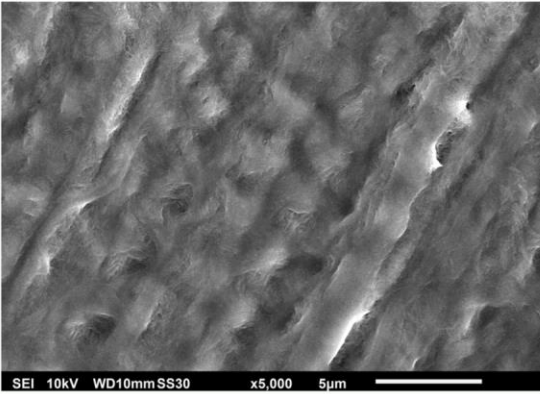
Immature enamel

Mature enamel

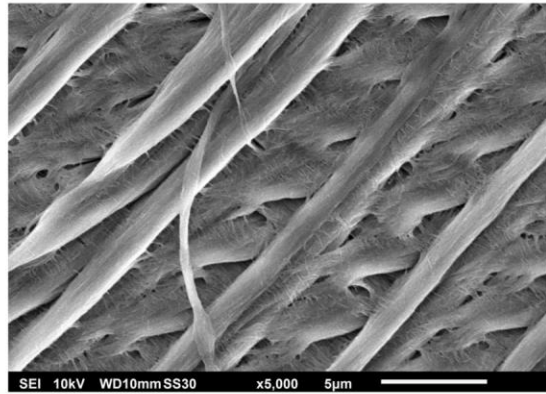
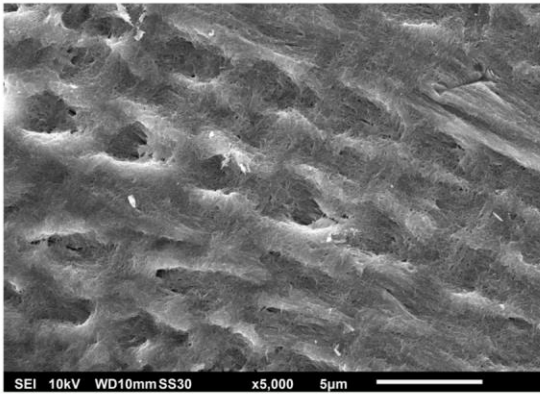
*Pitx2<sup>+/-</sup>; Irf6<sup>fl/+</sup>*



*Pitx2<sup>Cre/+</sup>; Irf6<sup>fl/+</sup>*

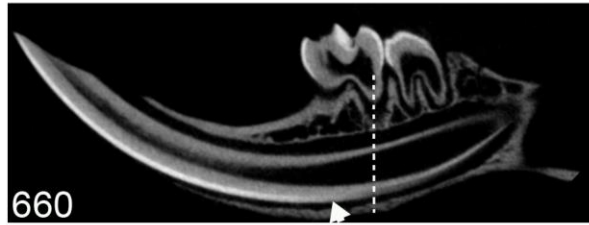
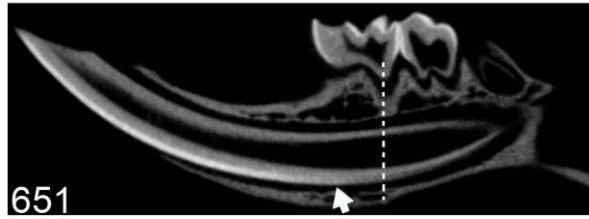


*Irf6-cKO*

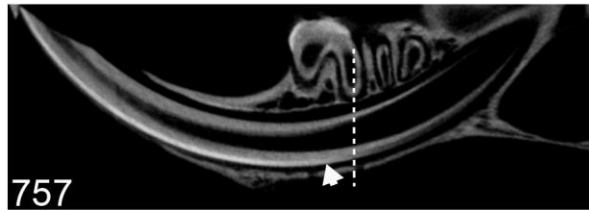


**Appendix Figure 3. Virtual sections from microCT scans comparing *Irf6-cKO* to control genotypes.**

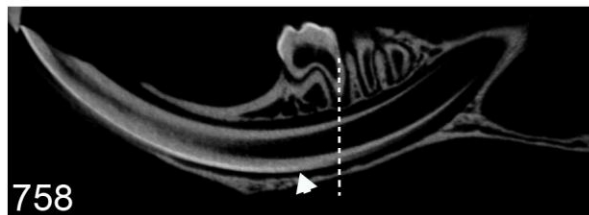
Pitx2<sup>+/+</sup>;  
Irf6<sup>fl/+</sup>



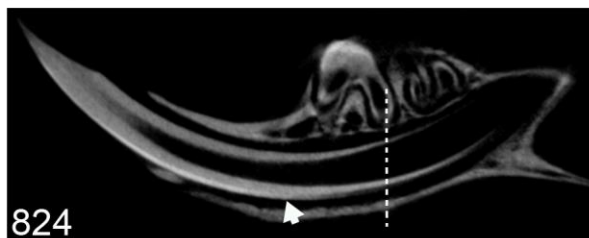
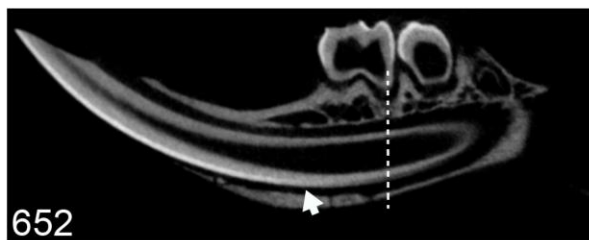
Pitx2<sup>+/+</sup>;  
Irf6<sup>fl/fl</sup>



Pitx2<sup>Cre/+</sup>;  
Irf6<sup>fl/+</sup>



Pitx2<sup>Cre/+</sup>;  
Irf6<sup>fl/fl</sup>



**Appendix Table 1. Primers used in RT-qPCR studies. f: forward, r: reverse**

Gene name	Primer name	Sequence (5' to 3')
<b>Ameloblastin</b>	<i>Ambn-f</i>	TCCCACCGCATAACTCT
	<i>Ambn-r</i>	GATATTGAACGGGCGAT
<b>Amelogenin</b>	<i>Amel-f</i>	ATCGGATCAAGCATCCC
	<i>Amel-r</i>	GGGTTTCGTAACCATAGG
<b>Enamelin</b>	<i>Enam-f</i>	ACTATGATGCGGCCAG
	<i>Enam-r</i>	GGTTGAGGCGTAGTGC
<b>Gapdh</b>	<i>Gapdh-f</i>	ACCACAGTCCATGCCATCAC
	<i>Gapdh-r</i>	TCCACCACCCTGTTGCTGTA
<b>Irf6</b>	<i>IRF6-f</i>	GCGGTGTGAACTCTTGTGC
	<i>IRF6-r</i>	GGTGGAGGGCCATGATCT
<b>Klk4</b>	<i>KLK4-f</i>	GTTCCCTGGGGTGCCTCAT
	<i>KLK4-r</i>	ATCCGGCTGCTGACACTT
<b>Mmp20</b>	<i>MMP20-f</i>	GGAGCCTCAGAAGACCCTTT
	<i>MMP20-r</i>	TTTGGGTAGTCTTTTTCCATTTTC
<b>Wnt10b</b>	<i>WNT10B-f</i>	CGGATTTCTGTCTAGGG
	<i>WNT10B-r</i>	AGGTAGAGAAGACGCTAAC

**Appendix Table 2. Frequency of dental anomalies in *Irf6-cKO* mice compared to control genotypes.**

	Controls			<i>Irf6-cKO</i>
	Pitx2 <sup>+/+</sup> ; Irf6 <sup>fl/+</sup>	Pitx2 <sup>Cre/+</sup> ; Irf6 <sup>fl/+</sup>	Pitx2 <sup>+/+</sup> ; Irf6 <sup>fl/fl</sup>	Pitx2 <sup>Cre/+</sup> ; Irf6 <sup>fl/fl</sup>
	N=22	N=15	N=19	N=20
<b>Peg shaped molars</b>	0%	0%	0%	25%
<b>Hypodontia</b>	0%	0%	0%	10%
<b>Taurodontism (2<sup>nd</sup> molars)</b>	0%	13.3%	0%	100%