***Msx2* Prevents Stratified Squamous Epithelium Formation in the Enamel Organ**

M. Nakatomi, H. Ida-Yonemochi, C. Nakatomi, K. Saito, S. Kenmotsu, R.L. Maas, and H. Ohshima

Appendix Materials and Methods

In situ hybridization

Preparation for *Krt26* (NM_001033397.5) cDNA clone was carried out as follows. Isolation of total RNA from wild-type skin at P9 and cDNA synthesis was performed for RT-PCR experiment (see below). A 482 bp fragment (nt 1718 - 2189 in 3' UTR region) was amplified using specific primers containing restriction enzyme site. Forward: CGGAATTC (EcoRI) + GCATGAGGGTCGATGGAGAG. Reverse: CGGGATCC (BamHI) + ACCCGACTCTTCTTCCGAGA. This fragment was cloned into pBluescript II SK(+) vector to generate antisense and sense RNA probes.

Immunohistochemistry

The antibodies were purchased from abcam (Cambridge, UK), BioLegend Inc. (Dedham, MA, USA), Dako (Glostrup, Denmark), Enzo Life Science Inc. (Farmingdale, NY, USA), and Santa

Cruz Biotech Inc. (Santa Cruz, CA, USA), or provided by Dr. Norio Amizuka (Hokkaido Univ., Japan) and Dr. Takashi Uchida (Hiroshima Univ., Japan). For antigen retrieval, sections were autoclaved in citric acid buffer (pH 6.0) at 121 °C for 5 min. In case of Amelogenin, K73, Notch1, and Sox2 primary antibodies, sections were treated with an anti-goat (BA5000) or anti-rabbit (BA1000) secondary antibody (Vector Laboratories, Burlingame, CA, USA), followed by the avidin-biotin peroxidase complex method (1:50 dilution, Vectastain Elite ABC kit, Vector). In other cases sections were processed for the EnVision method (Dako Japan, Tokyo, Japan). Sigmafast 3, 3'-diaminobenzidine (DAB) tablets (Sigma-Aldrich, St Louis, MO, USA) were used for the visualization of reaction products. TUNEL assay was performed with in situ apoptosis detection kits (ApopTag, Chemicon International, Temecula, CA, USA). The immunostained sections were counterstained with hematoxylin.

RT-PCR

Total RNA was extracted with using Trizol reagent (Invitrogen, Carlsbad, CA, USA), followed by oligo-dT-primed cDNA synthesis from 2 μg RNA with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Negative control experiment was performed without adding DNA polymerase. One microliter cDNA was used for each 25 μl conventional PCR as previously

described (Nakatomi et al., 2013) with the BIOTAQ DNA Polymerase (Bioline Reagents, London, UK) and 5 pmol forward and reverse primers.

TEM

The animals were fixed with 2.5% glutaraldehyde in 0.06 M cacodylate buffer (pH 7.4) containing 0.5% sucrose by perfusion and immersion fixation. Following decalcification with a 5% ethylenediaminetetraacetic acid disodium salt (EDTA-Na^2) solution for 3w, the upper jaws were cut sagittally at a thickness of about 50 μm with a freezing microtome (FX-801: Yamato Kohki Corp., Tokyo, Japan), postfixed with 1% osmium tetroxide reduced with 1.5% potassium ferrocyanide for 4 h, dehydrated through a graded series of acetone, and embedded in Epon 812 (Taab, Berkshire, UK). Semithin sections were cut at 1 μm and stained with 0.03% methylene blue. Ultrathin sections (70 nm in thickness) were double-stained with uranyl acetate and lead citrate.

Cell proliferation assay

The dissected jaws were fixed with Bouin's fixative, rinsed with PBS, dehydrated, and embedded in paraffin. Upper incisors and lower first molars were cut into 4 μm sagittal and frontal sections, respectively. Immunohistochemistry was carried out according to the manufacturer's instructions

with anti-BrdU mouse monoclonal antibody (1:200, Roche), the EnVision method (Dako Japan), and DAB tablets (Sigma-Aldrich). For molars, the frontally sectioned plane through the mesial cusps was chosen for cell counting. A 50 x 150 μm rectangle was set on the cervical, middle, and cusp areas of the enamel organ on both the lingual and buccal sides and the number of BrdU-positive cells was counted within the rectangle. The average of six serial sections (in total 18 rectangles each for the lingual and buccal sides) was calculated as a BrdU number for each individual. For incisors, a 50 x 150 μm rectangle was set on the area of the sagittally sectioned enamel organ just before ameloblasts lose cellular polarity in *Msx2*$^{-/-}$ (Fig. 1G3) and on the equivalent area in wild-type. BrdU-positive cells were counted within the rectangle and the average of six serial sections was calculated as a BrdU number for each individual.

EPMA

The upper jaws were fixed with 4% PFA, rinsed with 0.1M cacodylate buffer (pH 7.4), dehydrated with an ascending series of acetone, and embedded in Epon 812 (Taab). The samples were sagittally ground down for detecting chemical elements of calcium, magnesium, and phosphate by an EPMA (EPMA-8705, Shimadzu, Kyoto, Japan). Common voltage and electrical current settings were 15.0 kV and 0.025 μA, respectively. Other uncommon settings were as follows: spot size 15 μm and pixel matrix 477 x 477 for lower magnified incisors; spot size 5 μm

and pixel matrix 380 x 380 for higher magnified incisors; spot size 1 μm and pixel matrix 504 x 504 for molars. The obtained data of the point measurements were transferred into various colors according to the concentration of the mineral.

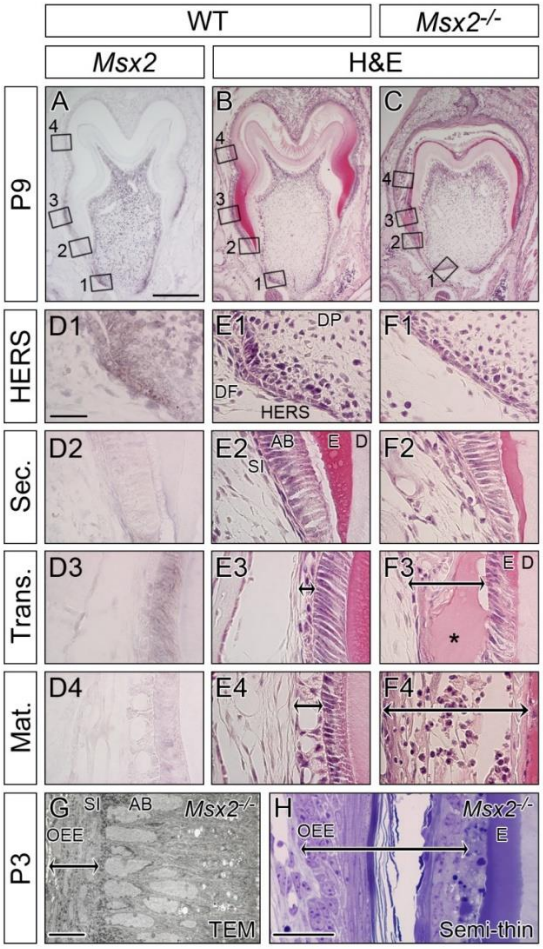
μCT

The heads were fixed with 4% PFA and rinsed with PBS. Two different magnifications were applied for with or without a contrast stain. Common CT settings were as follows: pixel matrix 512 x 512 x 256 and electrical current 101 μA. Other uncommon settings were as follows: slice thickness 33.443 μm, magnification x 2.74, and voltage 60.0 kV for simple CT, slice thickness 29.550 μm, magnification x 3.10, and voltage 55.0 kV for a contrast stain of *Msx2*$^{+/-}$, and slice thickness 30.200 μm, magnification x 3.03, and voltage 70.1 kV for a contrast stain of *Msx2*$^{-/-}$.

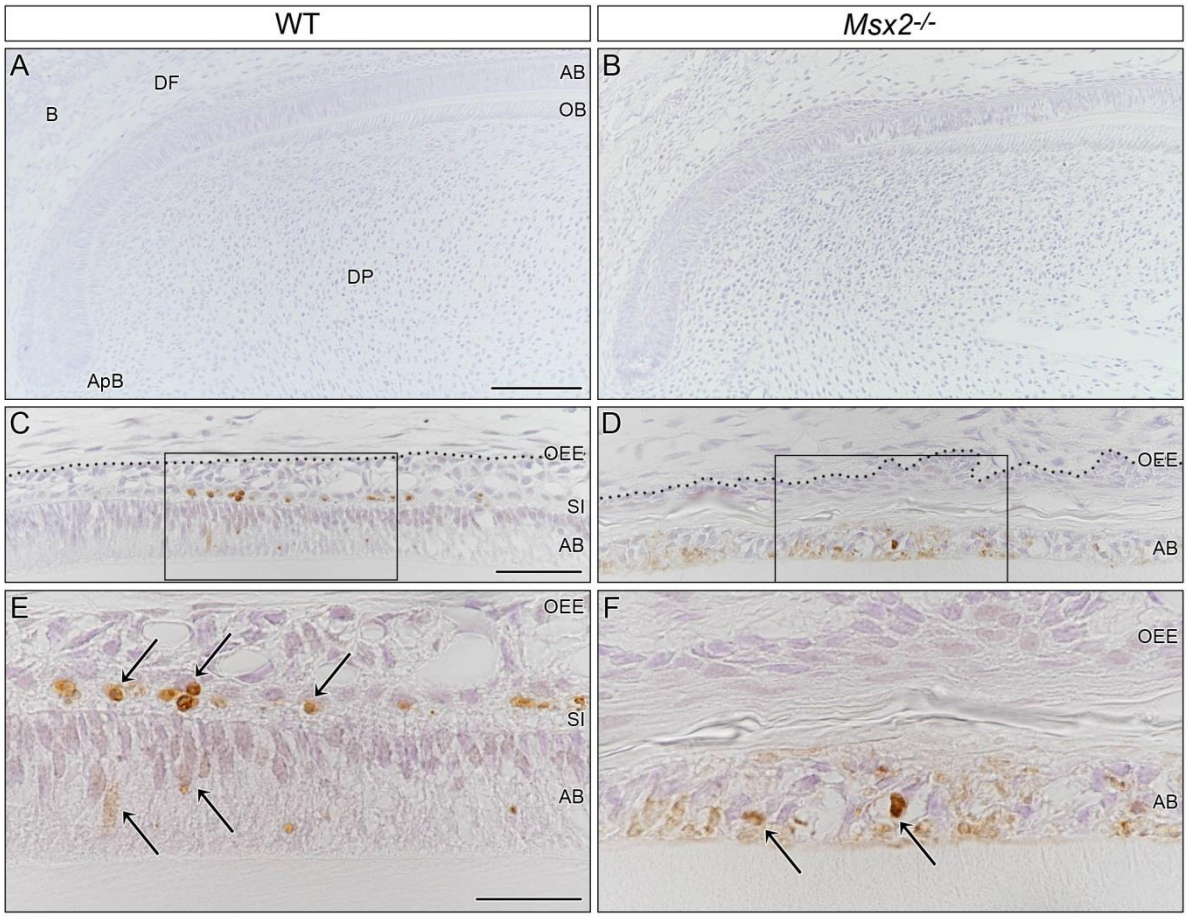
Organ culture

Dissected tooth germs were cultured at 37 °C in a modified Trowell system containing Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum, 100 μg/ml ascorbic acid (Seikagaku Kogyo, Tokyo, Japan), and 100 U/ml penicillin-streptomycin (Gibco BRL, Grand Island, NY, USA). After the cultivation period, the explants were fixed with Bouin's fixative,

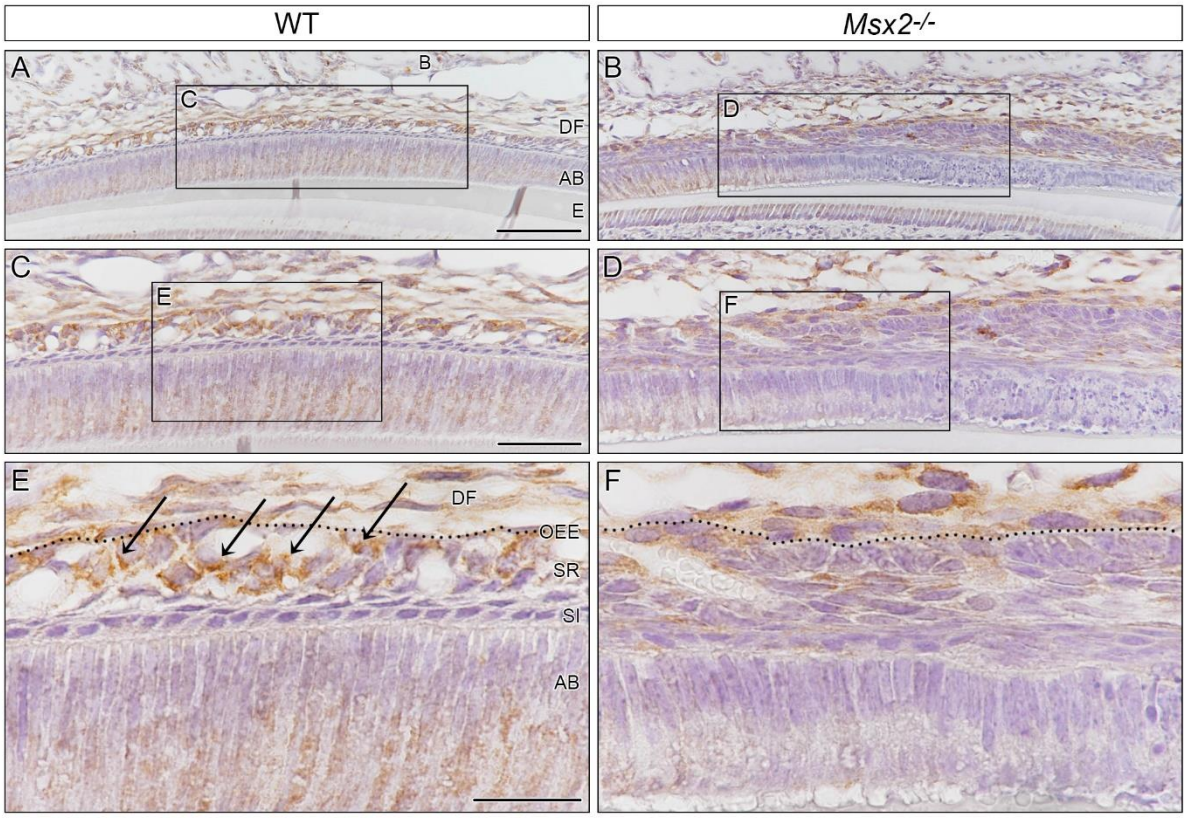
dehydrated through ethanol series, embedded in paraffin, cut into 4 μm frontal sections, and stained with H&E.



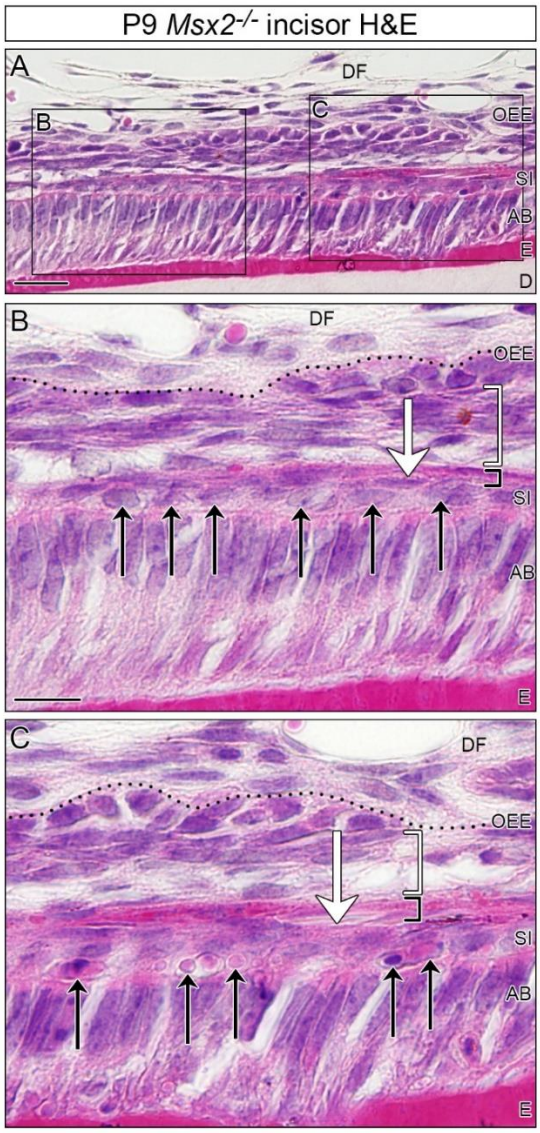
Appendix Figure 1. *Msx2* expression in WT and morphological phenotypes in mutant molars. Genotypes and stages are indicated. (A-H) Frontally sectioned lower first molars. (A-F) Section in situ hybridization of *Msx2* and H&E staining. (D-F) Magnified views of boxed areas in (A-C). *Msx2* is expressed in Hertwig's epithelial root sheath (HERS) and ameloblasts (AB) at the transition (Trans.) stage, but down-regulated at the secretory (Sec.) and maturation (Mat.) stages. Whereas mutant ameloblasts polarize at the early secretory stage (F2), they become gradually depolarized and an odontogenic cyst forms in the enamel organ (double headed arrow in F3-4). Enamel does not properly form on dentin and an ectopic amorphous structure is seen in the enamel organ (asterisk in F3). (G, H) TEM and semi-thin analyses confirm formation of a stratified squamous epithelium. D, dentin; DF, dental follicle; DP, dental pulp; E, enamel; OEE, outer enamel epithelium; SI, stratum intermedium. Bars: 250 μm in A for A-C, 25 μm in D1 for D-F, 10 μm in G, and 25 μm in H.



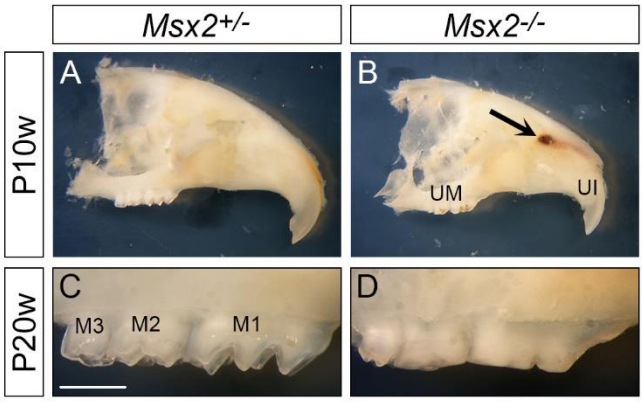
Appendix Figure 2. TUNEL assay in incisors. Genotypes are indicated. (A-F) TUNEL assay using sagittally sectioned upper incisors at P9 (incisal edge side to right). (A, B) No obvious immunoreactivity is observed in the posterior region in both genotypes, suggesting that mutant ameloblasts can be normally differentiated and survive in the initial phase of amelogenesis. (E, F) Magnified views of boxed area in (C, D). In WT, some ameloblasts and stratum intermedium (SI) cells are immunopositive at the transition stage. Immunoreaction is more intense in mutant ameloblasts where ameloblasts lose their cellular polarity. AB, ameloblast; ApB, apical bud; B, bone; DF, dental follicle; DP, dental pulp; OB, odontoblast; OEE, outer enamel epithelium. Bars: 100 μm in A, 50 μm in C, and 25 μm in E.



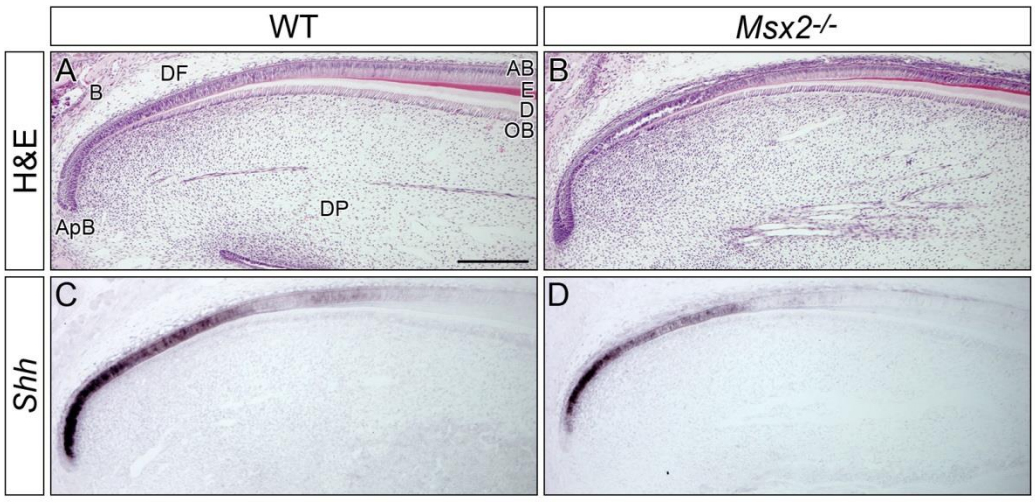
Appendix Figure 3. Stellate reticulum marker PTHrP expression in incisors. Genotypes are indicated. (A-F) Sagittally sectioned upper incisors at P9 (incisal edge side to right). Immunostaining for a stellate reticulum (SR) marker PTHrP. (C, D) and (E, F) are magnified views of boxed areas in (A, B) and (C, D), respectively. Dotted line demarcates epithelial-mesenchymal border. Intense immunoreactivity is recognized in the SR in WT (arrows in E), whereas immunoreaction is lost in an abnormally formed stratified squamous epithelium in *Msx2*$^{-/-}$, suggesting that the SR does not properly form in the mutant. AB, ameloblast; B, bone; DF, dental follicle; E, enamel; OEE, outer enamel epithelium; SI, stratum intermedium. Bars: 100 μm in A, 50 μm in C, and 25 μm in E.



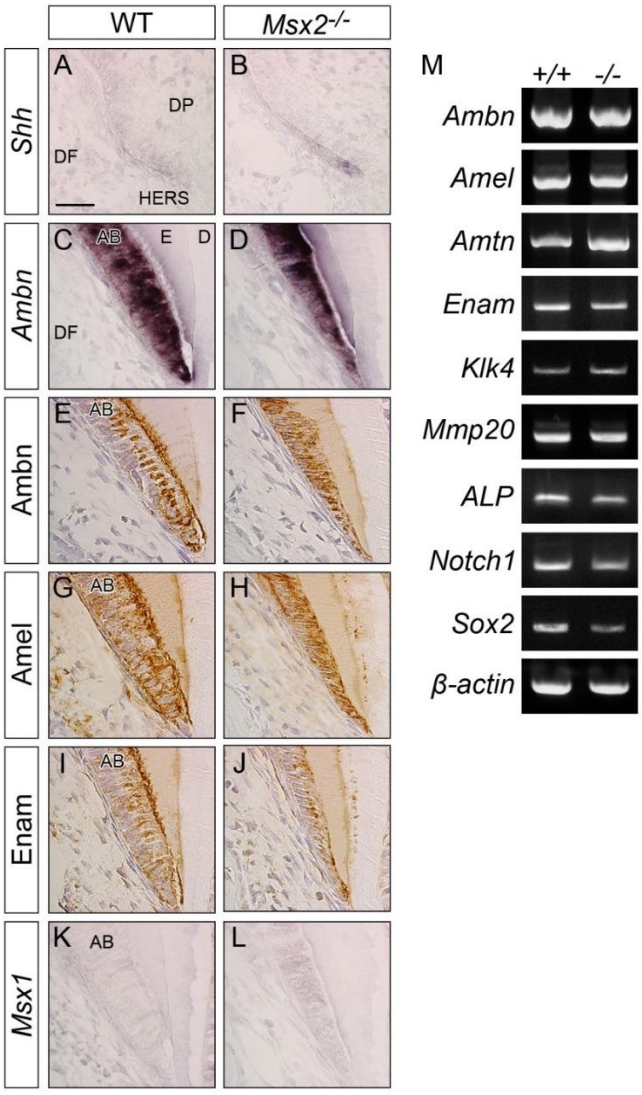
Appendix Figure 4. Keratinization progresses towards ameloblasts in the mutant enamel organ. (A-C) H&E staining of sagittally sectioned upper incisor of *Msx2*$^{-/-}$ at P9 (incisal edge side to right). (B, C) Magnified views of boxed areas in (A). Dotted lines demarcate epithelial-mesenchymal border. White and black brackets indicate less-eosinophilic squamous cells and the eosinophilic keratinized layer, respectively, suggesting that keratinization progresses from the outer enamel epithelium (OEE) towards ameloblasts (white arrow). Note that round-shaped stratum intermedium (SI) cells can be observed between the keratin layer and ameloblasts (black arrows). AB, ameloblast; D, dentin; DF, dental follicle; E, enamel. Bars: 20 μm in A and 10 μm in B for B and C.



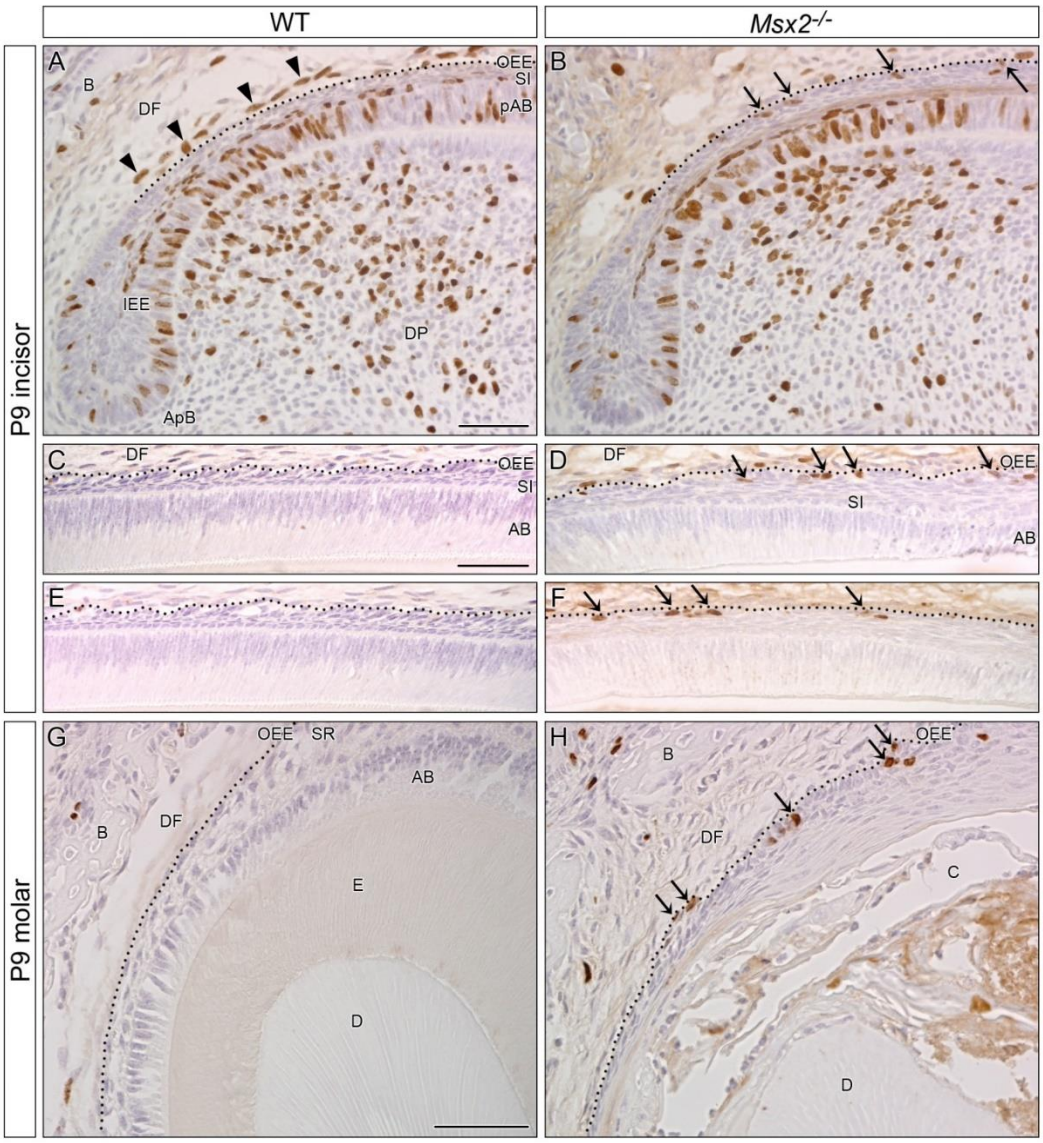
Appendix Figure 5. Morphological phenotypes of *Msx2*$^{-/-}$ in postnatal stages. Genotypes and stages are indicated. (A, B) Lateral views of the upper jaw showing blood accumulation in the cyst visible through the bone in *Msx2*$^{-/-}$ (arrow in B). (C, D) Lateral views of upper first (M1), second (M2), and third (M3) molars. Due to absence of enamel, mutant molars exhibit severe attrition. UI, upper incisor; UM, upper molar. Bar: 1 mm in C for C and D.



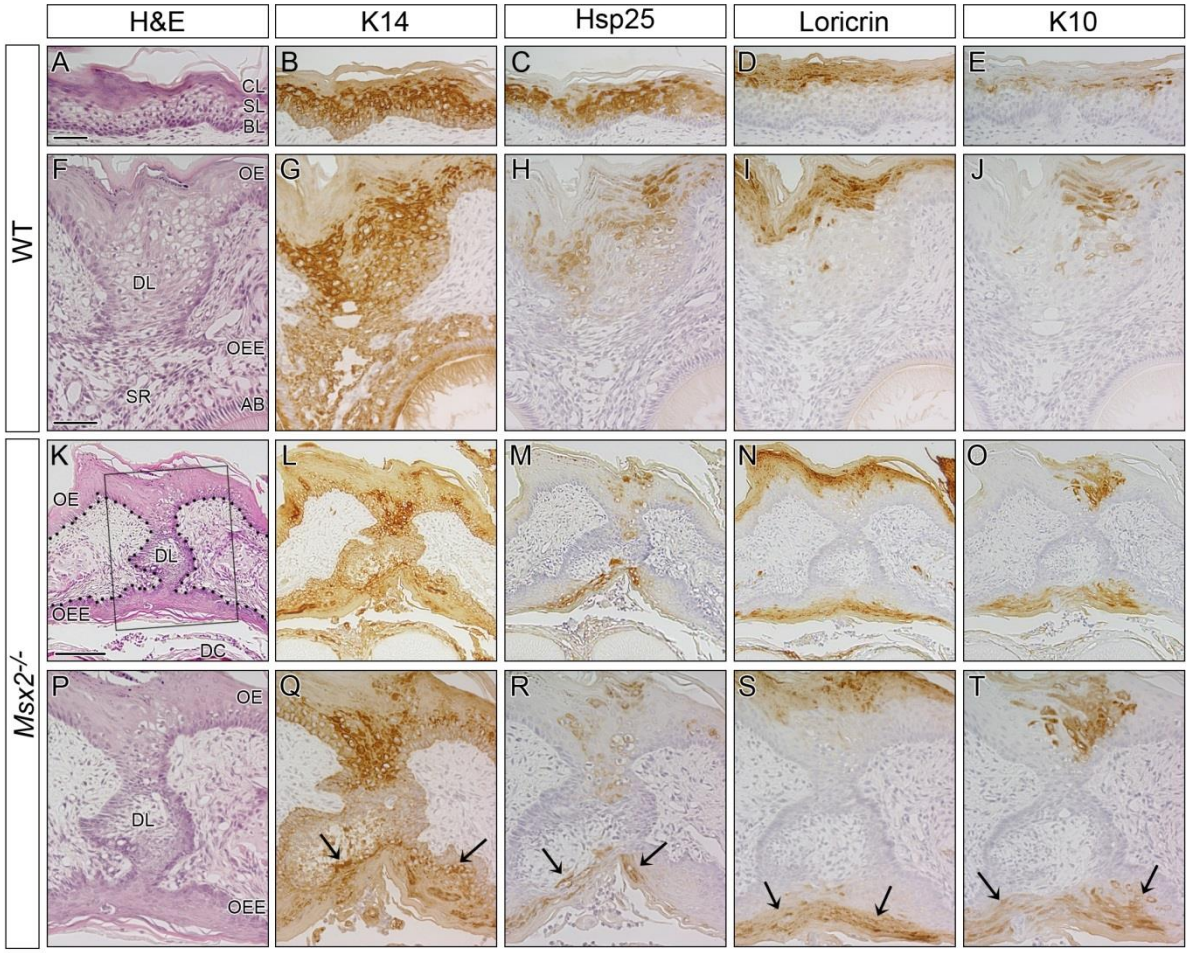
Appendix Figure 6. *Sonic hedgehog* (*Shh*) expression is unaltered in *Msx2*$^{-/-}$. Genotypes are indicated. (A-D) Sagittally sectioned upper incisors at P9 (incisal edge side to right). (C, D) In situ hybridization of *Shh*, which is identically expressed in the inner enamel epithelium and preameloblasts in both WT and *Msx2*$^{-/-}$. AB, ameloblast; ApB, apical bud; B, bone; D, dentin; DF, dental follicle; DP, dental pulp; E, enamel; OB, odontoblast. Bar: 250 µm.



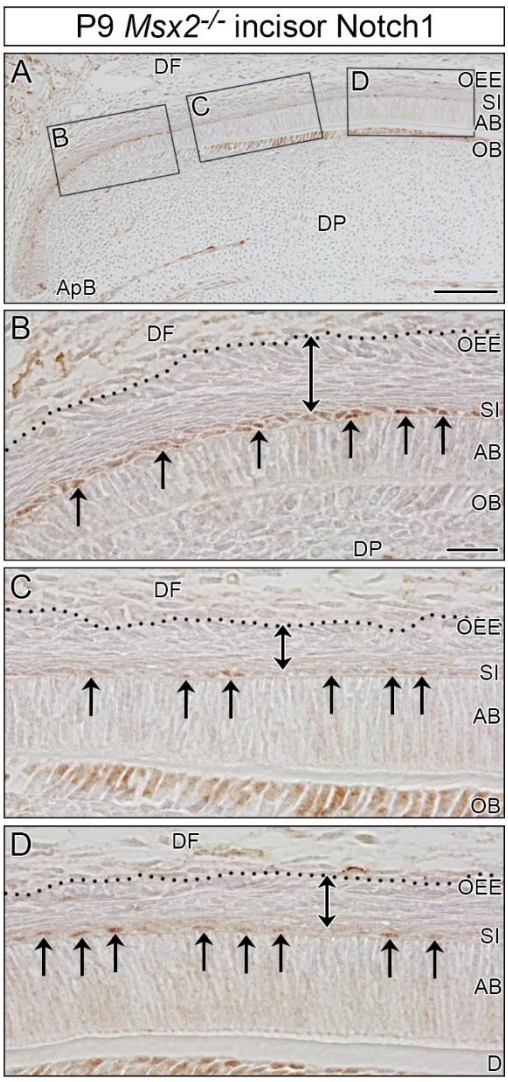
Appendix Figure 7. Expression of enamel organ genes and proteins in early amelogenesis in *Msx2*$^{-/-}$. Genotypes are indicated. (A-L) Frontally sectioned lower first molars at P9. (A-D, K-L) In situ hybridization. (E-J) Immunohistochemistry. (A-J) Hertwig's epithelial root sheath (HERS) marker *Sonic hedgehog* (*Shh*) and enamel proteins such as Ameloblastin (Ambn), Amelogenin (Amel), and Enamelin (Enam) are expressed in both genotypes. (K-L) *Msx1* is not significantly up-regulated in *Msx2*$^{-/-}$. (M) Conventional RT-PCR analysis using cDNA derived from the enamel epithelium in the lower incisor at P20w. In addition to *Ambn*, *Amel*, and *Enam*, other enamel organ related genes *Amelotin* (*Amtn*), *Kallikrein 4* (*Klk4*), *Matrix metallopeptidase 20* (*Mmp20*), *Alkaline phosphatase* (*ALP*), *Notch1*, and *Sox2* are comparably expressed in WT and *Msx2*$^{-/-}$. *β-actin* was used as an internal control. AB, ameloblast; D, dentin; DF, dental follicle; E, enamel. Bar: 25 μm.



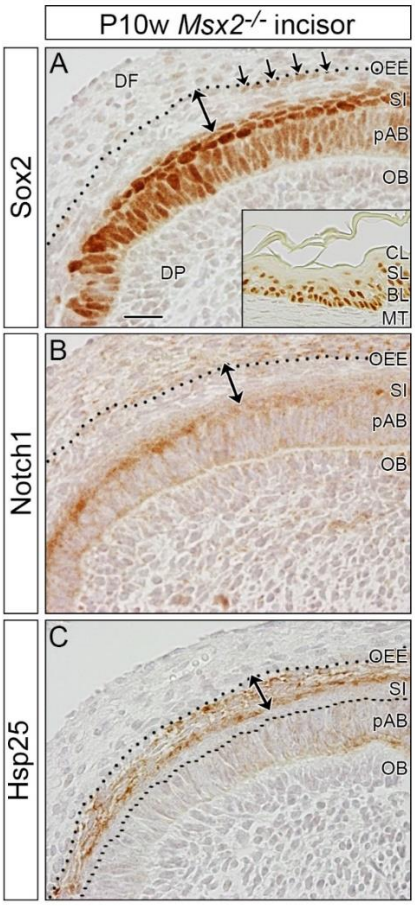
Appendix Figure 8. Magnified views of cell proliferation assay. Genotypes are indicated. Immunohistochemical cell proliferation assay using BrdU 1h labeling. Dotted line demarcates epithelial-mesenchymal border. (A-F) Sagittally sectioned upper incisors (incisal edge side to right). (A, B) Magnified views of Fig. 3A, D. (G, H) Frontally sectioned lower first molars. Cusp regions are presented. (A-H) The outermost area of the enamel organ becomes abnormally proliferative in *Msx2*$^{-/-}$ (arrows), whereas BrdU positive cells are hardly observed in the outer enamel epithelium (OEE) in WT. Note that some BrdU-positive cells are localized in the dental follicle (DF), but not within the OEE (arrowheads in A). AB, ameloblast; ApB, apical bud; B, bone; C, cyst; D, dentin; DF, dental follicle; DP, dental pulp; E, enamel; IEE, inner enamel epithelium; pAB, preameloblast; SI, stratum intermedium; SR, stellate reticulum. Bars: 50 μm.



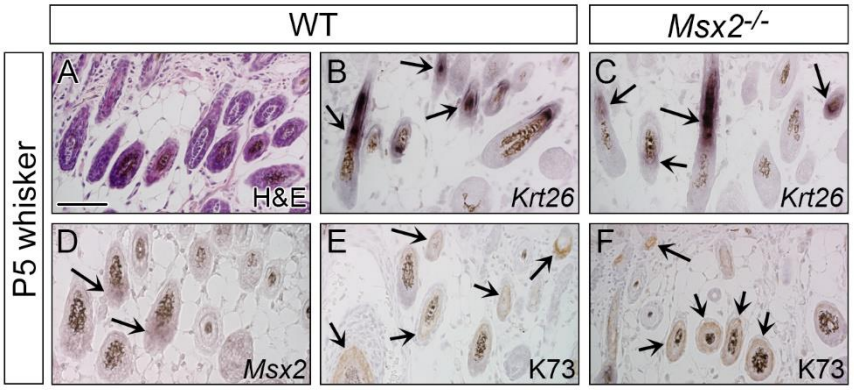
Appendix Figure 9. Differentiation markers for keratinized stratified squamous epithelia are ectopically expressed in the mutant enamel organ. Genotypes are indicated. H&E staining and immunohistochemistry for Keratin 14 (K14), Heat-shock protein 25 (Hsp25), Loricrin, and Keratin 10 (K10) at P9. (A-E) Sagittally sectioned palatal mucosa serves as a control tissue (oral side to up). K14 is recognized in almost all epithelial layers and intensely expressed in the spinous layer (SL). Hsp25 is restricted to the SL. Loricrin and K10 are markers for the cornified layer (CL). (F-T) Frontally sectioned lower first molars. (F-J, P-T) Magnified views of the junctional region between the dental lamina (DL) and the tooth germ, indicated as a boxed area in (K). Dotted lines in (K) demarcate epithelial-mesenchymal border, indicating that the basal layer (BL) of the oral epithelium (OE) directly connects with the outer enamel epithelium (OEE) in *Msx2*$^{-/-}$. Hsp25, Loricrin, and K10 are ectopically expressed in the mutant enamel organ (arrows in R-T). AB, ameloblast; DC, dental cusp; SR, stellate reticulum. 50 μm in A for A-E, 50 μm in F for F-J and P-T, and 100 μm in K for K-O.



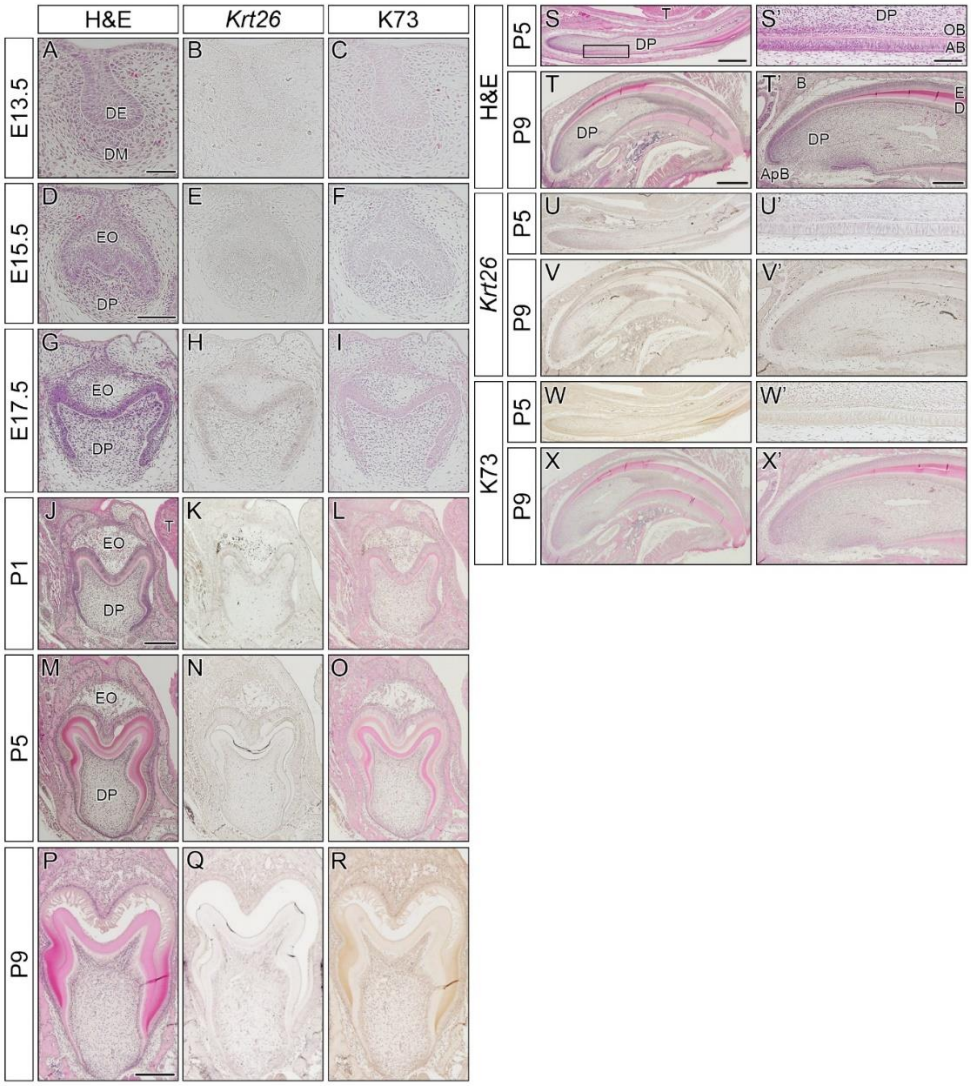
Appendix Figure 10. Stratum intermedium cells remain differentiated in the thickened mutant enamel organ at P9. (A-D) Immunohistochemistry for Notch1 of sagittally sectioned upper incisor of *Msx2*$^{-/-}$ at P9 (incisal edge side to right). (B-D) Magnified views of boxed areas in (A). Dotted lines demarcate epithelial-mesenchymal border. Stratum intermedium (SI) cell marker Notch1 can be recognized (arrows) as a cell layer adjacent to ameloblasts (AB), though a stratified squamous epithelium already forms in the enamel organ (double-headed arrow). ApB, apical bud; D, dentin; DF, dental follicle; DP, dental pulp; OB, odontoblast; OEE, outer enamel epithelium. Bars: 100 μm in A and 20 μm in B for B-D.



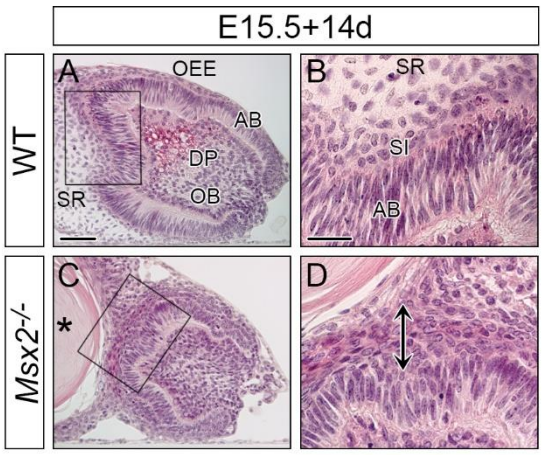
Appendix Figure 11. Stratum intermedium cells remain differentiated in the thickened mutant enamel organ at P10w. (A-C) Immunohistochemistry for Sox2, Notch1, and Hsp25 in the posterior region of sagittally sectioned upper incisor of *Msx2*$^{-/-}$ at P10w (incisal edge side to right). Dotted lines demarcate epithelial-mesenchymal border. Stratum intermedium (SI) cell marker Sox2 and Notch1 can be recognized adjacent to ameloblasts (AB), though a stratified squamous epithelium already forms in the enamel organ (double-headed arrow). Inset in (A) indicates Sox2 expression in the basal layer (BL) of palatal mucosa of WT at P9. Notably, some cells in the outer enamel epithelium (OEE) ectopically express Sox2 (arrows), suggesting that these cells have acquired a cellular character of the basal layer of stratified squamous epithelia. Dashed line in (C) demarcates the border between SI cells and preameloblasts (pAB). Stratified squamous epithelium marker Hsp25 is expressed in the thickened enamel organ, but negative in SI layer and pAB, suggesting that SI layer is not responsible for the formation of the stratified squamous epithelium. CL, cornified layer; DF, dental follicle; DP, dental pulp; MT, mesenchymal tissue; OB, odontoblast; SL, spinous layer. Bar: 20 μm.



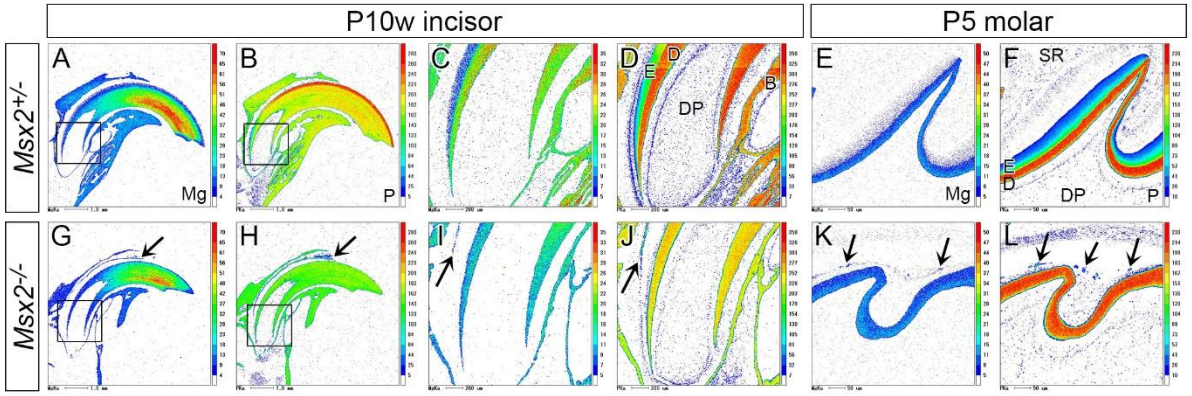
Appendix Figure 12. Co-expression of *Msx2*, *Krt26*, and K73 in whisker follicles. Genotypes are indicated. (A-F) Frontally sectioned heads at P5 containing whisker follicles. *Msx2* and *Keratin 26* (*Krt26*) mRNA and Keratin 73 (K73) protein are co-expressed in whisker follicles. Bar: 25 µm.



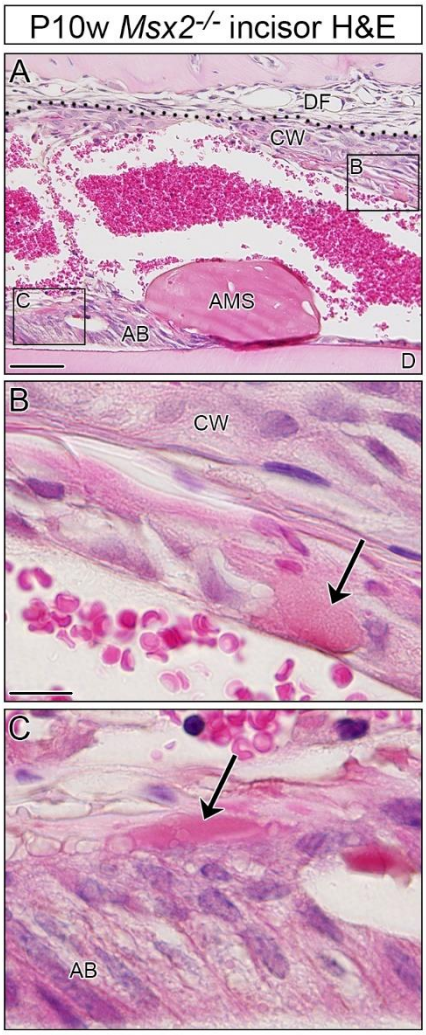
Appendix Figure 13. *Krt26* and K73 are undetectable in WT tooth germs. Stages are indicated. H&E staining, in situ hybridization of *Keratin 26* (*Krt26*), and immunohistochemistry for Keratin 73 (K73) in frontally sectioned lower first molars (A-R, lingual side to right) and sagittally sectioned lower (S, U, W) and upper (T, V, X) incisors (incisal edge side to right) of WT. (S', U', W') Magnified views of boxed area in (S). (T', V', X') Magnified views of the posterior end region of (T, V, X). *Krt26* mRNA and K73 protein are hardly detectable in developing tooth germs of WT. AB, ameloblast; ApB, apical bud; B, bone; D, dentin; DE, dental epithelium; DM, dental mesenchyme; DP, dental papilla or dental pulp; E, enamel; EO, enamel organ; OB, odontoblast; T, tongue. Bars: 50 μm in A for A-C, 100 μm in D for D-I, 200 μm in J and P for J-O and P-R, 500 μm in S and T for S, U, W and T, V, X, 100 μm in S' for S', U', W', and 200 μm in T' for T', V', X'.



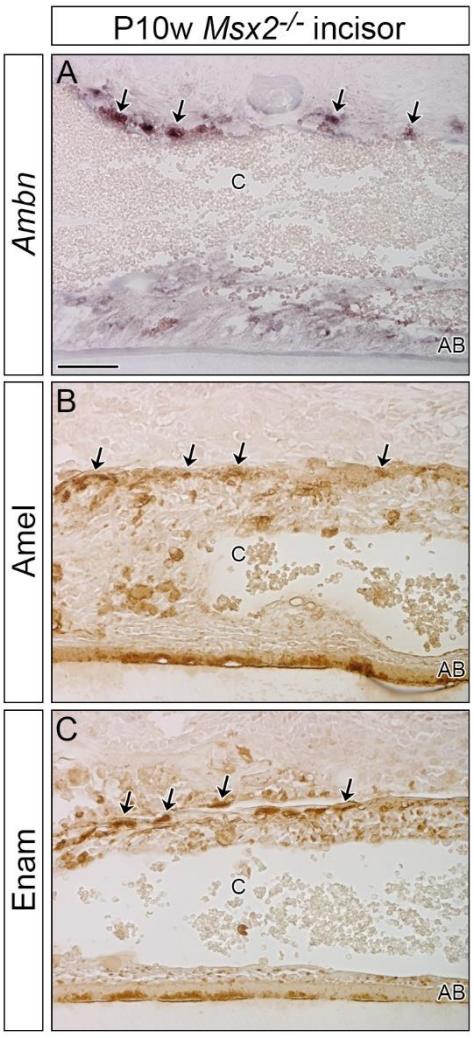
Appendix Figure 14. The transformation of the outer enamel epithelium reflects a tooth germ intrinsic defect. Genotypes are indicated. (A-D) H&E staining of the lower first molar cultured for 14 days after dissection at embryonic day (E) 15.5. (B, D) Magnified views of boxed area in (A, C). In *Msx2*$^{-/-}$, a stratified squamous epithelium ectopically forms in the area corresponding to the SR and SI (double-headed arrow in D), while ameloblasts normally polarize. Note that as tooth germs were dissected together with the oral epithelium, the original oral epithelium could form the keratin layer exteriorly during organ culture regardless of genotypes, termed the keratin pearl, and thus it is not a phenotype of the mutant (asterisk in C). AB, ameloblast; DP, dental pulp; OB, odontoblast; OEE, outer enamel epithelium; SI, stratum intermedium; SR, stellate reticulum. Bars: 50 μm in A and 25 μm in B.



Appendix Figure 15. Ectopic amorphous mineralized structures are observed in the mutant enamel organ. Genotypes and stages are indicated. Sagittal views obtained by electron probe micro analyzer (EPMA). (A, C, E, G, I, K) Magnesium (Mg) and (B, D, F, H, J, L) phosphate (P). (A-D, G-J) Upper incisors (incisal edge side to right). (C, D, I, J) Magnified views of boxed area in (A, B, G, H). Compared to control, almost no enamel is on dentin and ectopic mineralized structures are observed between the incisor and surrounding bone in *Msx2*$^{-/-}$ (arrow in G-J). (E, F, K, L) Upper first molars (distal side to right). Similarly, almost no enamel forms and ectopic mineralized structures exist within the mutant enamel organ (arrows in K, L). Note that color scale is different depending on the magnification. B, bone; D, dentin; DP, dental pulp; E, enamel; SR, stellate reticulum.



Appendix Figure 16. Ectopic amorphous mineralized structures are observed in the odontogenic cyst in *Msx2*$^{-/-}$. (A-C) H&E staining of sagittally sectioned upper incisor of *Msx2*$^{-/-}$ at P10w (incisal edge side to right). (B, C) Magnified views of boxed areas in (A). Dotted line demarcates epithelial-mesenchymal border. A huge amorphous mineralized structure (AMS) forms in the odontogenic cyst. Smaller mineralized structures also form in the cystic wall (arrow in B) and adjacent to ameloblast layer (arrow in C). AB, ameloblast; CW, cystic wall; D, dentin; DF, dental follicle; E, enamel. Bars: 50 μm in A and 10 μm in B for B and C.



Appendix Figure 17. Enamel proteins are ectopically expressed in the cystic wall in *Msx2*$^{-/-}$. (A-C) In situ hybridization of *Ameloblastin* (*Ambn*) and immunohistochemistry for Amelogenin (Amel) and Enamelin (Enam) of sagittally sectioned upper incisors of *Msx2*$^{-/-}$ at P10w (incisal edge side to right). Enamel protein-producing ameloblast-like cells localize to the cystic wall (arrows). AB, ameloblast; C, cyst. Bar: 20 µm.

Appendix Table 1. List of antibodies for immunohistochemistry.

	Primary antibody				Secondary antibody	
Name	Type (*1)	A.R. (*2)	Resource	Dilution	Type	Dilution
Ambn	R.P.	None	Dr. Uchida	1:20	Envision	1:1
Amel	G.P.	None	Dr. Uchida	1:500	Anti-goat	1:800
Desmoplakin	R.P.	Autoclave	Santa Cruz, sc33555	1:500	Envision	1:1
Enam	R.P.	None	Dr. Uchida	1:100	Envision	1:1
Hsp25	R.P.	None	Enzo, ADI-SPA-801	1:50	Envision	1:1
K10	M.M.	Autoclave	Dako, M7002	1:1000	Envision	1:1
K14	R.P.	Autoclave	BioLegend, PRB155P	1:1000	Envision	1:1
K73	G.P.	Autoclave	Santa Cruz, sc168284	1:2000	Anti-goat	1:1600
Loricrin	R.P.	Autoclave	abcam, ab24722	1:1000	Envision	1:1
Notch1	G.P.	Autoclave	Santa Cruz, sc6014	1:500	Anti-goat	1:1000
PTHrP	M.M.	None	Dr. Amizuka	1:100	Envision	1:1
Sox2	R.M.	Autoclave	abcam, ab92494	1:300	Anti-rabbit	1:400

*1: G.P., goat polyclonal; M.M., mouse monoclonal; R.M., rabbit monoclonal; R.P., rabbit polyclonal.
*2: A.R., antigen retrieval.

Appendix Table 2. List of RT-PCR primers.

Name	Forward	Reverse
ALP	5'-ACGAGATGCCACCAGAGG-3'	5'-AGTGCGGTTCCAG-3'
Ambn	5'-GAACTGTTTTGATTGGCATC-3'	5'-AAGAAGGGACCTACACTATTT-3'
Amel	5'-GCTTCAGACAGAAACTCACT-3'	5'-GAACACTACTACATGCCATTAT-3'
Amtn	5'-CCGAGTAAAGTGGAGAAG-3'	5'-AGGTCTGTCTGAAGTGGC-3'
Enam	5'-CTATGGGAGGCAAGACGA-3'	5'-AGCCACCAATACAGCAAGA-3'
Klk4	5'-ACTAAAGAATGGGAAACTGC-3'	5'-TGAAAGAGGGTATTGTGGTC-3'
Krt25	5'-CACGCTTGCCCTGAGATCAT-3'	5'-GGTGGAGGTGATGATCTGGTT-3'
Krt26	5'-GCTGGGCATCAAAACGTGTT-3'	5'-CTCTCCATCGACCCTCATGC-3'
Krt27	5'-CACCATGTCCGTTCGCTTTT-3'	5'-TGCAGAGATCTGGGTCC-3'
Krt28	5'-GCATACAGTCCGGATCCCTC-3'	5'-ACTGCAGGACTTTCATTTCCTCT-3'
Krt71	5'-TGTCTGTTCGAAGCCGAGAT-3'	5'-GCTGACGGGGGAAGAGTATT-3'
Krt72	5'-CATTTGGGAGCAGAAGCCTG-3'	5'-CCTCGTACCTCTTGCAGT-3'
Krt73	5'-TCGCCAATTCACCTGCAAAC-3'	5'-CCTCACCCTGTCCCCAGATA-3'
Mmp20	5'-AGGTGGACAAGGCAATAC-3'	5'-ACTGCATCAAAGGACGAG-3'
Notch1	5'-GAAGACCTGGAGACCAAGAAG-3'	5'-GAAGTCAGAGATGACAGCAGG-3'
Sox2	5'-GCCCAGGAGAACCCCAAGAT-3'	5'-GGGTGCCCTGCTGCGAGTA-3'
β-actin	5'-TGGAATCCTGTGGCATCCATGAAAC-3'	5'-TAAAACGCAGCTCAGTAACAGTCCG-3'

References: *ALP* (Li *et al*., *J*. *Endod*., 35:382-8, 2009); *Ambn*, *Amel*, *Amtn*, and *Enam* (Gao *et al*., *Anat*. *Rec*., 293:135-40, 2009); *Klk4* and *Mmp20* (Gao *et al*., *Anat*. *Rec*., 292:885-90, 2009); *Notch1* (Chen *et al*., *J*. *Oral Pathol*. *Med*., 40:235-42, 2011); *Sox2* (Zhang *et al*., *J*. *Cell Biochem*., 112:1009-21, 2011); *β-actin* (Ida-Yonemochi *et al*., *Dev*. *Biol*., 363:52-61, 2012).