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# The Role of Discoidin Domain Receptor 2 in Tooth Development

**F.F. Mohamed[1], C. Ge[1], A. Binrayes[1,2], and R.T. Franceschi[1,3,4]**

## Abstract

Collagen signaling is critical for proper bone and tooth formation. Discoidin domain receptor 2 (DDR2) is a collagen-activated tyrosine kinase receptor shown to be essential for skeletal development. Patients with loss of function mutations in *DDR2* develop spondylo-meta-epiphyseal dysplasia (SMED), a rare, autosomal recessive disorder characterized by short stature, short limbs, and craniofacial anomalies. A similar phenotype was observed in *Ddr2*-deficient mice, which exhibit dwarfism and defective bone formation in the axial, appendicular, and cranial skeletons. However, it is not known if *Ddr2* has a role in tooth formation. We first defined the expression pattern of *Ddr2* during tooth formation using *Ddr2-LacZ* knock-in mice. *Ddr2* expression was detected in the dental follicle/sac and dental papilla mesenchyme of developing teeth and in odontoblasts and the periodontal ligament (PDL) of adults. No LacZ staining was detected in wild-type littermates. This *Ddr2* expression pattern suggests a potential role in the tooth and surrounding periodontium. To uncover the function of *Ddr2*, we used *Ddr2*[slie/slie] mice, which contain a spontaneous 150-kb deletion in the *Ddr2* locus to produce an effective null. In comparison with wild-type littermates, *Ddr2*[slie/slie] mice displayed disproportional tooth size (decreased root/crown ratio), delayed tooth root development, widened PDL space, and interradicular alveolar bone defects. *Ddr2*[slie/slie] mice also had abnormal collagen content associated with upregulation of periostin levels within the PDL. The delayed root formation and periodontal abnormalities may be related to defects in RUNX2-dependent differentiation of odontoblasts and osteoblasts; RUNX2-S319-P was reduced in PDLs from *Ddr2*[slie/slie] mice, and deletion of *Ddr2* in primary cell cultures from dental pulp and PDL inhibited differentiation of cells to odontoblasts or osteoblasts, respectively. Together, our studies demonstrate odontoblast- and PDL-specific expression of *Ddr2* in mature and immature teeth, as well as indicate that DDR2 signaling is important for normal tooth formation and maintenance of the surrounding periodontium.

**Keywords:** collagen receptor, periodontal tissues, alveolar bone, cell differentiation, root formation, collagen fiber

## Introduction

During tooth development, cell–extracellular matrix (ECM) interactions play an important role in cell adhesion, proliferation, differentiation, and formation of dental structures (Thesleff et al. 1989; Fukumoto and Yamada 2005; Chen et al. 2009). Dysregulation of proteins mediating ECM interactions is implicated in several deformities affecting tooth structure and function (Mardh et al. 2002; Umemoto et al. 2012). Type I collagen is the core structural protein in the tooth dentin and bone where collagen-mediated signaling is critical for tissue differentiation and mineralization (Thesleff and Hurmerinta 1981; Andujar et al. 1991; Nicholls et al. 1996). Type I collagen organized into fiber bundles in periodontal ligaments (PDLs) is important for tooth function during masticatory loads, proprioception, and regulation of alveolar bone volume (Andujar et al. 1991; McCulloch et al. 2000). Defects in PDL collagen fibers contribute to periodontal diseases, alveolar bone resorption, and tooth loss (Uitto and Larjava 1991). Therefore, proper collagen signaling is essential for development and maintenance of dental and periodontal tissues.

Two classes of receptors mediate cell-collagen interactions: β1-integrins and the discoidin domain receptors, DDR1 and DDR2 (Leitinger 2011). Unlike integrins, DDRs have intrinsic tyrosine kinase activity and are selectively activated by triple-helical collagens (Shrivastava et al. 1997; Vogel et al. 1997). In addition, DDR1 preferentially binds to and is activated by type IV collagen while DDR2 is strongly activated by type I and III collagens found primarily in bones and teeth (Shrivastava et al. 1997; Vogel et al. 1997). Unlike DDR1, which is found primarily in epithelia, DDR2 is exclusively expressed in mesenchyme (Alves et al. 1995). DDR2 is essential for skeletal development in humans and mice. Patients carrying loss-of-function mutations in *DDR2* develop spondylo-meta-epiphyseal dysplasia (SMED), a rare, autosomal recessive

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disorder characterized by short stature, short limbs, and craniofacial anomalies (Bargal et al. 2009; Rozovsky et al. 2011; Mansouri et al. 2016). A similar phenotype is observed in *Ddr2*-deficient mice and associated with impaired postnatal bone growth, osteoblast differentiation, chondrocyte proliferation, and maturation (Labrador et al. 2001; Zhang et al. 2011; Ge et al. 2016). While these studies uncovered a critical role for *Ddr2* in postnatal skeletal bone development, possible functions of *Ddr2* in teeth and associated structures have not been previously examined.

In this study, we define the temporospatial expression of *Ddr2* in dentoalveolar tissues and investigate the functional importance of DDR2 during postnatal development of teeth and surrounding periodontium using a *Ddr2*-deficient mouse model. These findings provide new insights into the role of cell-ECM interactions in the development of teeth and associated structures and may have important implications for understanding periodontal disease.

## Materials and Methods

### Mice

Generation and genotyping of *Ddr2*$^{slie/slie}$ mice and *Ddr2*-LacZ (*Ddr2*$^{+/LacZ}$) knock-in mice were previously described (Ge et al. 2018). *Ddr2*$^{fl/fl}$ mice in which exon 8 of the *Ddr2* gene is flanked by 2 LoxP sequences were generated from "knockout-first" ES cell clone *Ddr2*$^{tm1a(EUCOMM)Wtsi}$ (EPD0607__B01; European Mutant Mouse Repository) as described in Appendix Figure 1. All mouse experimental procedures conformed to standards for the use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Michigan. This study conforms to the Animal Research: Reporting In Vivo Experiments (ARRIVE) Guidelines.

### Detection of β-gal (Lacz) Expression

Samples dissected from heterozygous *Ddr2*-LacZ (*Ddr2*$^{+/LacZ}$) mice were processed for X-gal staining using standard procedures. Frozen sections were stained with freshly prepared X-gal solution and counterstained with Vector Nuclear Fast Red.

### Tissue Preparation and Histological Analysis

Whole skulls were fixed in 4% paraformaldehyde and processed for paraffin sections. For immunofluorescence, histological sections were incubated with rabbit antiperiostin polyclonal antibody (ab14041; Abcam) and rabbit anti–RUNX2-S319-P (generated in the project laboratory) (Ge et al. 2012) overnight at 4°C, and then with Alexa Fluor 488–conjugated donkey anti–rabbit IgG (A21206; Invitrogen). For Picro Sirius Red (PSR) staining, paraffin sections were processed as previously described (Coleman 2011) and visualized under polarizing light using an Olympus BX51-P microscope.

### Micro–Computed Tomography Analysis of Bone

After fixation in 10% formalin (Fisher), skulls were subjected to micro–computed tomography (CT) analysis using a Scanco Model 100 (Scanco Medical). Scan settings were as follows: voxel size of 12 μm, 70 kVp, 114 μA, 0.5-mm aluminum filter, and integration time of 500 ms. Tooth measurements were made on first molars unless stated otherwise. To avoid examiner bias, the genotype of mice was not specifically highlighted during quantification analysis.

### Cell Proliferation and Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling Assay

For proliferation assays, 2-wk-old mice were intraperitoneally injected with 5-ethynyl-2′-deoxyuridine (EdU; Invitrogen) and sacrificed 4 h after injection. Cells incorporating EdU were detected using the Click-iT EdU Alexa Fluor 488 Imaging Kit (C10337; Invitrogen). For terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays, sections were incubated in TUNEL reaction mixture according to the manufacturer instructions (Calbiochem).

### Cell Culture, Transfection, and In Vitro Differentiation

Periodontal ligament (PDL) and dental pulp stromal cells (DPSCs) were isolated as described (Balic and Mina 2010; see Appendix Fig. 5). The adenoviruses Ad5-CMV-LacZ (AdLacZ) and Ad5-CMV-CRE (AdCre), at a titer of $1 \times 10^{11}$ pfu/mL, were obtained from the Vector Core, University of Michigan, and used at a multiplicity of infection of 100 particles/cell. Viral transduction did not affect cell viability, cell number, or RNA yield (results not shown). Cells were stimulated for 21 d in α–minimum essential medium/10% fetal bovine serum containing 50 μg/mL ascorbic acid, 10 mM β-glycerophosphate, and 10 nM dexamethasone (DPSCs only). For gene expression analysis, total RNA (mRNA) was isolated using TRIzol (Invitrogen), complementary DNA (cDNA) was synthesized, and quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed on the following mRNAs: *Runx2, Col1a1, Ibsp, Bglap*, and *Postn*.

### Statistical Analysis

All data were analyzed using the GraphPad Prism software version 6.0e (GraphPad Software). Values were reported as mean ± SD. Student's *t* test was used for statistical comparison between the 2 experimental groups. Differences were considered significant at $P < 0.05$. A sample size of 5 to 7 mice (including both sexes) was used for experiments except where indicated. Cell culture experiments were done in triplicate and repeated at least twice.
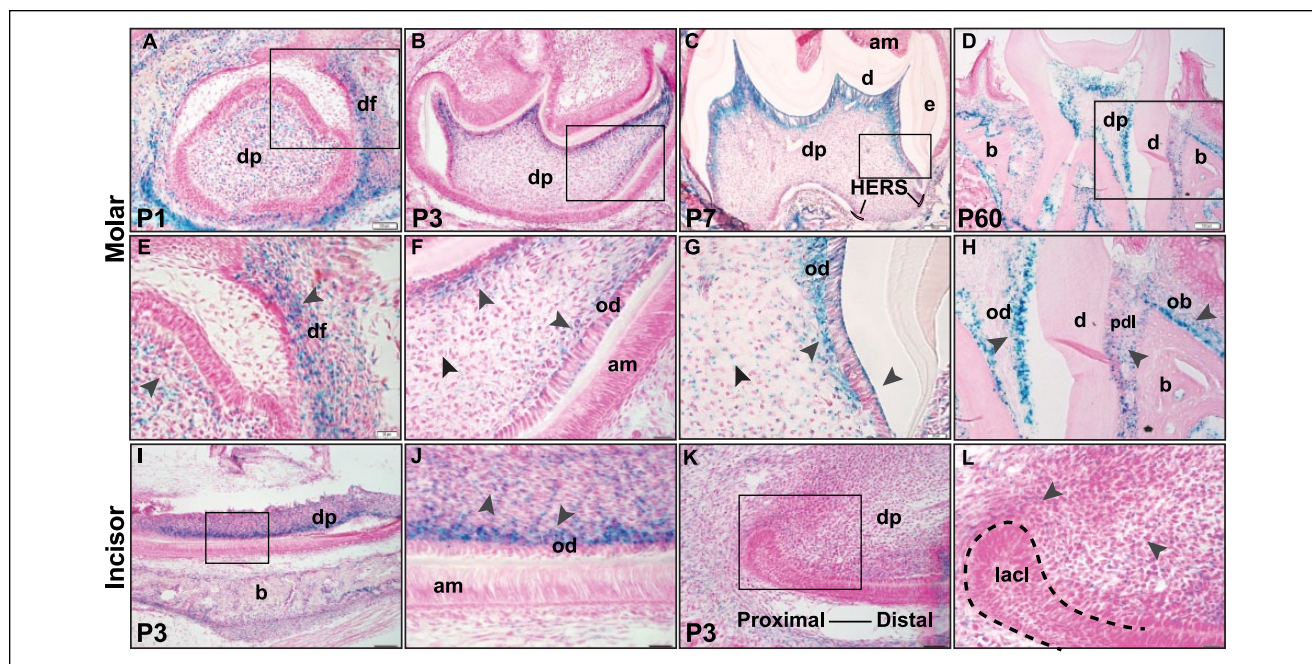
**Figure 1.** Localization and expression of *Ddr2* in dental and periodontal tissues. (**A–H**) X-gal staining of *Ddr2*^+/LacZ mice reveals *Ddr2* expression in the molars during development through adulthood. (A) At P1, *Ddr2* expression is observed in dental papilla (dp) and the surrounding dental follicle (df) in developing molars (high magnification in E). (B–D) *Ddr2*-LacZ expression is selectively high in dentin-producing odontoblasts (od) at P3, P7, and P60. (F, G) High magnification also reveals *Ddr2* expression in odontoblasts (od) and a subset of cells in dental pulp (dp). In a fully erupted molar (D, H), *Ddr2* is expressed in PDL cells (pdl) and putative alveolar bone–associated osteoblasts (ob). Black arrowheads point to the expression sites. (**I–L**) *Ddr2* expression in odontoblasts (od) of lower incisors, in dental pulp (dp), and around the labial cervical loop (lacl) at the proximal end of the incisor (dashed lines). (J, L) Higher magnification of boxed areas. No *Ddr2* expression was seen in molar (F) or incisor (J) ameloblasts (am). Scale bar: 100 μm in A–D, I, K; 20 μm in E–H, J, L.

## Results

### *Ddr2* Is Highly Expressed in Dentin-Forming Odontoblasts, PDL Fibroblasts, and Alveolar Osteoblasts

As a prerequisite to examining possible *Ddr2* functions in teeth and associated structures, we examined its spatiotemporal expression pattern using a *Ddr2*-lacZ reporter mouse line. In this mouse, the bacterial LacZ gene is regulated by the endogenous *Ddr2* locus (Ge et al. 2018). *Ddr2*-LacZ expression assessed by X-gal staining was detected in developing and mature teeth of *Ddr2*^+/LacZ mice at postnatal days 1 (P1), 3 (P3), 7 (P7), and 60 (P60) (Fig. 1). However, no X-gal staining was shown in wild-type (WT) littermates (Appendix Fig. 2). At P1, X-gal staining was predominately found in the dental papilla mesenchyme and surrounding dental follicle, which gave rise to cementum, PDL, and alveolar bone (Fig. 1A, magnified inset in Fig. 1E). As the tooth continued to form, strong X-gal staining was observed in dentin-forming odontoblasts of the coronal dental pulp and in differentiating odontoblasts of developing molar roots at P3 and P7 (Fig. 1B, C, magnified insets in Fig. 1F, G). Weak X-gal staining was found in mesenchymal cells around Hertwig's epithelial root sheath (HERS) of developing molar roots (Fig. 1C). Similar to molars, the mouse incisor also showed strong X-gal staining in dentin-forming odontoblasts along the incisor length with less evident staining in the

proximal mesenchyme around the labial cervical loop at the incisor apex (Fig. 1I, K, magnified insets in Fig. 1J, L). Consistent with the exclusive mesenchymal expression of *Ddr*2, no detectable X-gal staining was seen in dental epithelial cells such as HERS and enamel-secreting ameloblasts. In addition to odontoblast-specific expression, *Ddr2*-LacZ expression was also evident in PDL-forming cells or fibroblastic cells aligned along PDL collagen fibers and in putative alveolar bone–associated osteoblasts (Fig. 1D, H). The odontoblast- and PDL-specific pattern of *Ddr2* expression was maintained after the completion of tooth formation and eruption into the oral cavity (Fig 1D, H). From these results, we conclude that *Ddr2* is expressed throughout tooth and PDL development and suggest it may have important functions in these tissues.

### *Ddr2* Is Important for Optimal Development of the Molar Root and Periodontium

To investigate the functional role of *Ddr2* in tooth formation, we used *Ddr2*^slie/slie mice, which contain a spontaneous 150-kb deletion in the *Ddr2* locus, resulting in an effective null (Kano et al. 2008). Our results showed that molar roots in *Ddr2*^slie/slie mice were shorter than in their WT littermates (Fig. 2A–F). The mean root length (cementum-enamel junction [CEJ] to root tip) in *Ddr2*^slie/slie mice was reduced by 26% at 2 wk and by 17% at 3 mo of age, suggesting delayed root formation. In
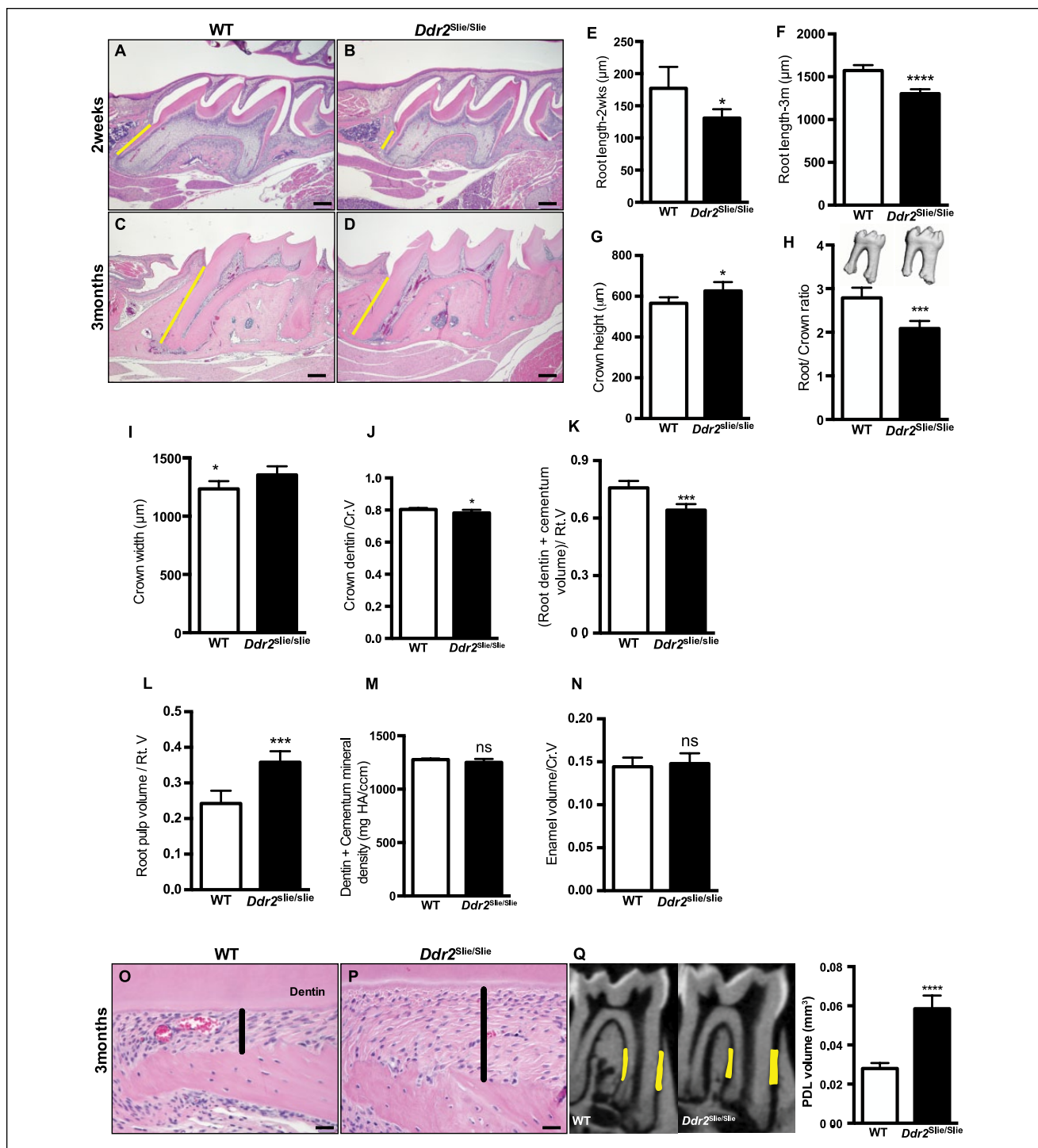
**Figure 2.** Tooth and periodontal phenotypes in *Ddr2*-deficient mice. (**A–D**) Representative hematoxylin and eosin (H&E) staining of first molars showing delayed root formation (yellow lines) in developing (A, B) and adult teeth (C, D) compared with wild-type (WT). (**E**, **F**) Quantification of the molar root length at 2 wk and 3 mo of age. (**G–I**) Quantification of the crown height, root/crown ratio, and crown width in 3-mo-old mice. (**J–N**) Quantification of the crown dentin volume/total crown volume (Cr.V), root dentin + cementum volume/total root volume (Rt.V), root pulp volume/ (Rt.V), dentin + cementum mineral density, and enamel volume/(Cr.V). (**O**, **P**) H&E staining shows wide periodontal ligament (PDL) spacing (highlighted in black lines) in *Ddr2*slie/slie mice compared with WT. (**Q**) Micro–computed tomography (CT) analysis of PDL spacing in WT and *Ddr2*slie/slie mice. Region of interest is shown in yellow. Quantitation of micro-CT data (right). *$P < 0.05$. ***$P < 0.001$. ****$P < 0.0001$. ns, not significant. $n = 5$–7 mice/group. Scale bar: 50 μm in A, B; 200 μm in C, D; 20 μm in O, P.
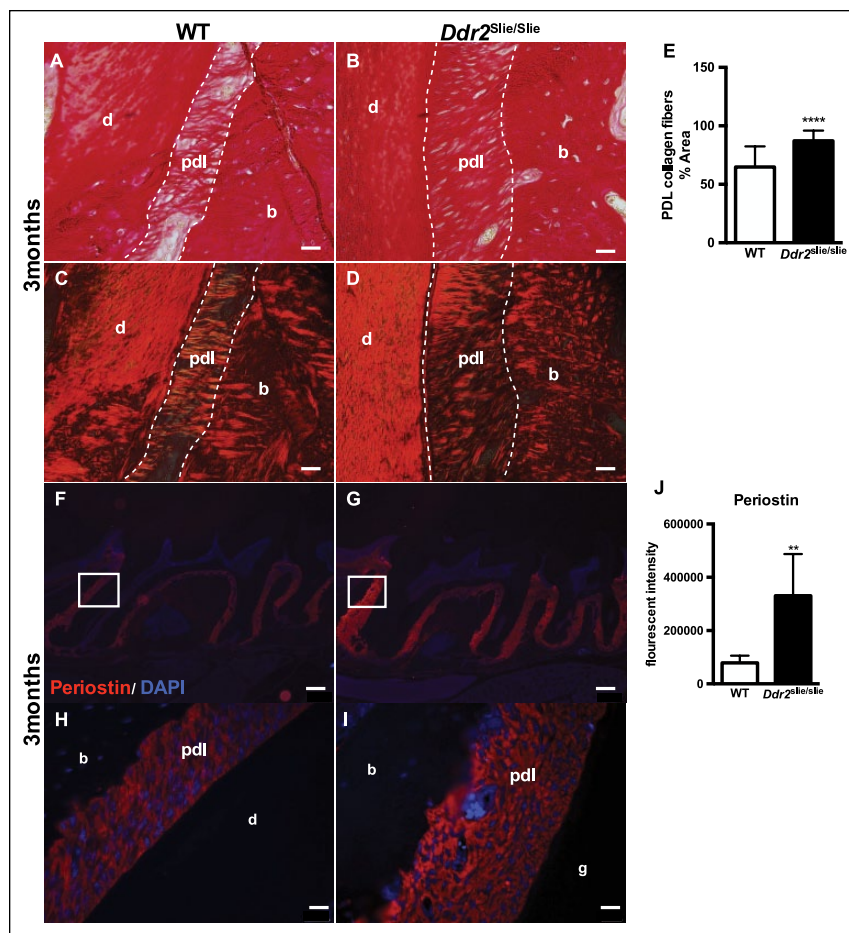
**Figure 3.** *Ddr2*^slie/slie teeth had atypical periodontal collagen fibers. (**A–D**) Picro Sirius Red (PSR) staining of periodontal ligament (PDL) collagen fibers in 3-mo-old mice under bright-field (A, B) and polarizing (C, D) microscopy. (**E**) Quantification of the PDL collagen area stained with PSR, $n = 3$ mice, 6 measurements per mouse. (**F–I**) Immunostaining of periostin (red) with inserts (H, I) showing periostin expression in the PDL space surrounding the molar roots. The cell nuclei were stained with DAPI (blue). (**J**) Quantification of the fluorescent intensity of periostin immunostaining, $n = 3$ mice, 5 measurements per mouse. **$P < 0.01$. ****$P < 0.000$. Scale bar: 200 μm in F, G; 20 μm in A–D, H, I.

addition, crown height (cusp tip to CEJ) and crown width both increased by approximately 11% in 3-mo-old *Ddr2*^slie/slie mice (Fig. 2G, I). When linear tooth measurements were normalized by expressing data as root length/crown height (root/crown ratio), an overall 25% decrease was observed in *Ddr2*^slie/slie mice (Fig. 2H).

Crown and root compositional changes were also seen in *Ddr2*^slie/slie molars. These included a 3% decrease in crown dentin/total crown volume (Fig. 2J), a 15% decrease in the mineralized portion of the root (dentin + cementum volume/total root volume, Fig. 2K), and a 46% increase in root pulp volume/total root volume (Fig. 2L). No differences were observed in dentin + cementum mineral density or enamel/total crown volume (Fig. 2M, N). While incisor teeth were shorter in *Ddr2*^slie/slie mice, no differences were observed in enamel volume and mineral density (Appendix Fig. 3). In addition to the tooth phenotypes, *Ddr2*^slie/slie teeth exhibited a widened PDL space around molar roots (Fig. 2O, P). This was quantified by

micro-CT analysis in 3-mo-old mice, which showed that the PDL space adjacent to molars of *Ddr2*^slie/slie animals was twice the width of WT controls (Fig. 2Q, right). From these studies, we conclude that DDR2 signaling is required for optimal tooth morphogenesis and periodontal structure.

## Ddr2 Is Necessary for Proper PDL Collagen Organization

In addition to the wide PDL space, collagen fiber organization was abnormal in PDLs of *Ddr2*^slie/slie teeth. For detailed analysis, we used PSR staining to visualize type I and III collagen fibers under bright-field and polarized light microscopy. This allowed us to assess collagen content, maturation, and polarization (Fig. 3A–D). PSR staining, quantified using NIH ImageJ software, revealed an increase (34%) in PDL collagen area in 3-mo-old *Ddr2*^slie/slie mice compared with WT littermates (Fig. 3E). The data are presented as a percentage of collagen area to total area assessed using the thresholding function of ImageJ. Sharpey's fiber insertions into alveolar bone were also less evident in *Ddr2*^slie/slie mice. Periostin is an abundant noncollagenous matricellular protein in the PDL matrix that binds type I collagen and is necessary for maintenance of periodontal health (Rios et al. 2008). We conducted immunofluorescence staining to determine if *Ddr2* status affected levels of this important PDL component. Periostin immunoreactivity was increased in PDLs of *Ddr2*^slie/slie mice (320% increase, Fig. 3J) and was specifically localized in the PDL space surrounding the molar roots (Fig. 3F, G and magnified insets in Fig. 3H, I). These findings suggest that DDR2 signaling may directly or indirectly regulate periostin expression and thus is important for periodontal integrity and function. Together, our data suggest that DDR2 signaling may determine the amount or integrity of collagen matrix required to maintain periodontal health.

## Ddr2 Deficiency Is Associated with Progressive Alveolar Bone Loss

To explore the role of *Ddr2* in alveolar bone, micro-CT and MicroView software (Parallax Innovations) were used to assess bone parameters such as bone volume and bone volume fraction. *Ddr2*^slie/slie mice displayed a 10% reduction in interradicular alveolar bone volume at 3 mo that progressively increased as mice aged (Fig. 4A–D, K). With age, *Ddr2*^slie/slie

mice showed a progressive bone loss of about 26% after 5 mo and 30% after 10 mo (Fig. 4K). Transverse micro-CT views showed a widened PDL space and severe bone defects around the molar roots (Fig. 4E–H, arrows). However, no sign of spontaneous tooth loss was observed at any of the ages examined. Three-dimensional reconstruction of the lower jaw of 10-mo-old animals clearly showed severe alveolar bone loss in *Ddr2^slie/slie^* mice compared with WT (Fig. 4I, J). Given the significant loss of alveolar bone volume, we performed tartrate-resistant acid phosphatase (TRAP) staining to assess whether *Ddr2* knockout affected osteoclast activity in the supporting alveolar bone. Our results revealed a significant increase in osteoclast numbers in 10-mo-old *Ddr2^slie/slie^* mice (Fig. 4L–P), suggesting that increased osteoclast activity contributes to age-dependent alveolar bone resorption in knockout mice.

## Ddr2 Signaling Regulates Differentiation Potential of Osteogenic and Odontoblastic Lineages of PDL and Dental Pulp Cells, Respectively

To further understand the function of *Ddr2*, we sought to elucidate its effects on cellular activities involved in root formation such as proliferation, differentiation, and apoptosis. To measure proliferation, we administered EdU to 2-wk-old *Ddr2^slie/slie^* mice, and the number of EdU+ cells was counted and reported as a percentage of EdU+ cells to the total cells (stained blue with DAPI). Our analysis of the first molars revealed positive EdU staining in PDL and mesenchymal cells surrounding the HERS of developing molar roots in *Ddr2^slie/slie^* and WT mice (Fig. 5A–C). However, there was no significant difference in the percentage of EdU+ cells, suggesting the proliferation potential of dental cells in *Ddr2^slie/slie^* and WT teeth was not affected. In addition, no change in the number of apoptotic cells was seen between *Ddr2^slie/slie^* and WT, as measured by TUNEL staining along the root dentin odontoblast and PDL regions (Appendix Fig. 4).

To assess the influence of *Ddr2* on the differentiation potential of relevant tooth-associated cells, we examined phosphorylation of RUNX2 at Serine 319, which at least in part mediates the effect of DDR2 on osteogenesis (Ge et al. 2016). Immunostaining of p-RUNX2 showed nuclear staining mostly
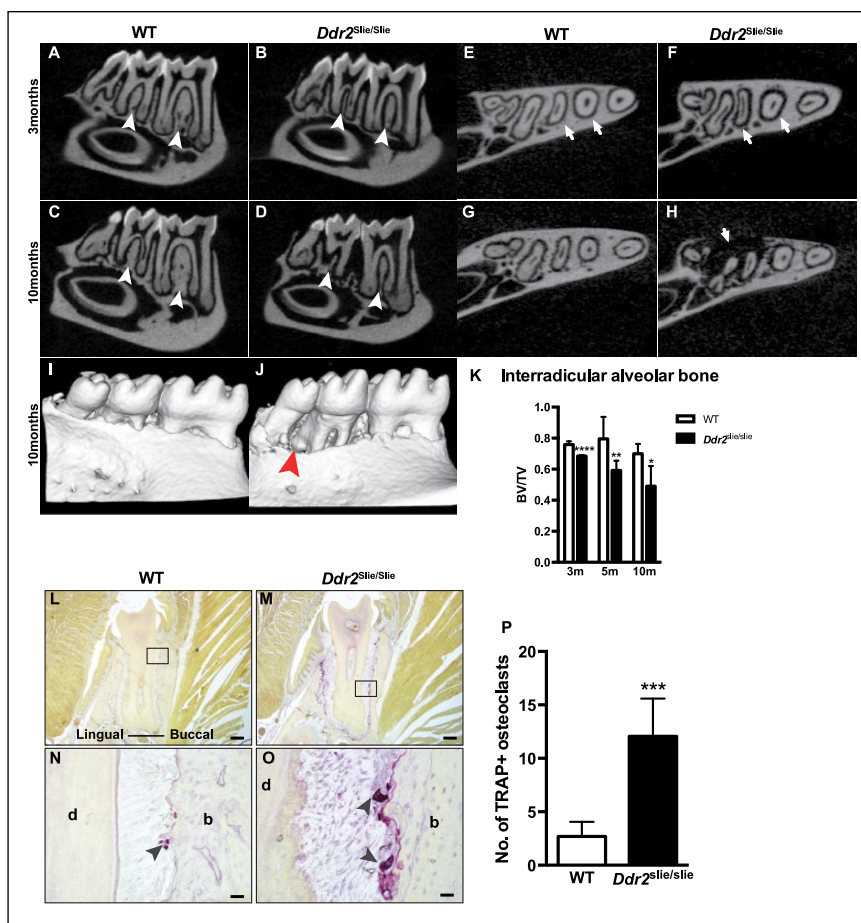


**Figure 4.** Progressive alveolar bone loss in *Ddr2* knockout. (**A–H**) Representative micro–computed tomography (CT) images showing thin interradicular alveolar bone (white arrowheads) and wide periodontal ligament (PDL) spacing (white arrows) in *Ddr2^slie/slie^* mice at 3 mo (B, F) and 10 mo (D, H) of age compared with wild-type (WT). (**I, J**) Three-dimensional reconstruction of the lower jaw section showing severe bone defects (J, red arrowhead) around lower teeth at 10 mo of age. (**K**) Micro-CT analysis of bone volume/total volume of interradicular alveolar bone of first and second molars (*n* = 5–7 mice/group). (**L–O**) Representative tartrate-resistant acid phosphatase (TRAP) staining images showing osteoclasts (purple color, arrowheads in N–O) along the alveolar bone surface at 10 mo of age. (**P**) Shows quantification of osteoclast numbers on frontal sections of the second molar alveolar bone (*n* = 4 mice). *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. ****$P < 0.0001$. Scale bar: 200 μm in L, M; 20 μm in N, O.

in PDLs and mesenchymal cells around HERS at the apex of the developing roots (Fig. 5D, E). *Ddr2^slie/slie^* teeth showed reduced levels of RUNX2 phosphorylation compared with WT controls (Fig. 5D–F), suggesting that RUNX2 activity mediates DDR2 regulation of root development. We also generated cultures of incisor PDL cells (PDLs) and DPSCs from 3-mo-old *Ddr2^flox/flox^* mice and examined differentiation after treatment with either AdLacZ or AdCre (Appendix Fig. 5A). PCR analysis of DNA from cultured PDLs and DPSCs treated with AdCre confirmed the deletion of the floxed *Ddr2* allele with generation of an approximately 0.7-kb PCR product (Appendix Fig. 5B). In addition, *Ddr2* mRNA levels were reduced by >80%. Control cultures of both cell types mineralized and expressed typical osteoblast/odontoblast/PDL cell markers such as *Runx2, Col1a1, Bglap, Ibsp*, and *Postn*. For both cell
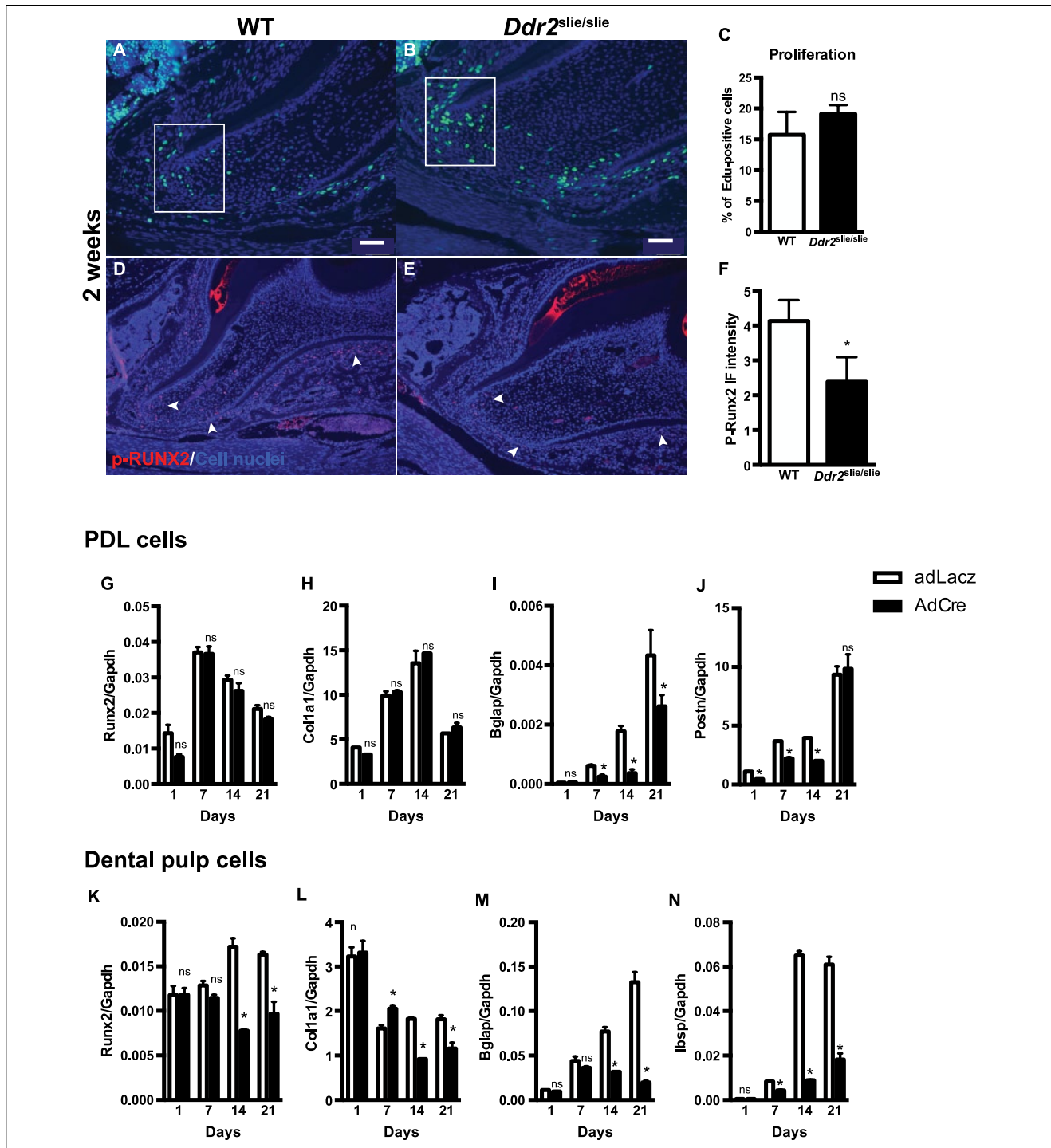
**Figure 5.** *Ddr2* knockout exhibits decreased osteogenic and odontogenic differentiation of periodontal ligament (PDL) and dental pulp cells, respectively. (**A**, **B**) Representative images of EdU staining for labeling proliferating cells (green) and DAPI staining for cell nuclei (blue). (**C**) Quantification of the percentage of EdU-positive cells. (**D**, **E**) Representative images of p-RUNX2 immunostaining (red) in the nuclei of PDL cells and at the apex of the developing root. (**F**) Quantification of the fluorescent intensity of p-RUNX2 immunostaining, $n = 3$ mice. (**G–J**) Quantification of differentiation markers for PDL cells. (**K–N**) Quantification of differentiation markers for dental pulp stromal cells after 21 d. *$P < 0.05$. ns, not significant. $n = 3$ cell cultures/group. Scale bar: 50 μm in A, B; 100 μm in D, E.

types, *Ddr2* loss dramatically reduced differentiation as measured by either expression of the differentiation markers, *Bglap, Ibsp,* and *Postn* (Fig. 5I, M, N) or mineralization (von Kossa staining) (Appendix Fig. 5C). Partial reduction of *Runx2* and *Col1a1* expression was also noted in dental pulp cultures at later culture times (Fig. 5K, L). Taken together, these results

show a clear requirement for *Ddr2* in the differentiation of PDL and dental pulp cells that may explain observed tooth and alveolar bone defects.

## Discussion

Type I collagen, the core structural protein in tooth dentin, PDL, and alveolar bone, plays an important role during tissue differentiation and mineralization (Thesleff and Hurmerinta 1981; Andujar et al. 1991; Nicholls et al. 1996). Previous studies largely focused on collagen-binding integrins as mediators of the response to this ECM component. However, bone-selective knockout of individual integrins resulted in relatively mild phenotypes (Gardner et al. 1996; Holtkotter et al. 2002; Bengtsson et al. 2005), suggesting the involvement of other collagen receptors. DDR2, a nonintegrin collagen-activated receptor tyrosine kinase, is critical for bone growth and development. The aim of this study was to examine the function of *Ddr2* in development of the tooth and associated periodontium.

Using LacZ staining, we localized *Ddr2* expression in developing and adult fully erupted teeth. *Ddr2* was expressed in undifferentiated cells in the dental papilla mesenchyme and subsequently in the dentin-forming odontoblasts and dental pulp. *Ddr2* was also highly expressed in the dental follicles during tooth development and in PDL and alveolar bone of adult teeth. This *Ddr2* distribution suggests potential functions in the formation of teeth and periodontium. However, the identity of *Ddr2*-expressing cells in the PDL needs to be determined.

Our analysis of *Ddr2*-deficient mice indicates that proper DDR2 signaling is required for optimal tooth morphogenesis and periodontal integrity. *Ddr2*$^{slie/slie}$ mice had short tooth roots and decreased root/crown ratio, resulting in disproportionate tooth size. BMP/TGF-β and Wnt/β-catenin pathways play a critical role in tooth root formation, and their disruption could cause a short root phenotype (Wang and Feng 2017). However, we do not know if either of these pathways is involved in DDR2 signaling. Mechanistically, our analysis suggests that RUNX2 phosphorylation mediates *Ddr2* regulation of root development as it does in bone (Zhang et al. 2011; Ge et al. 2016). In addition to the tooth phenotype, *Ddr2*-knockout mice exhibited a progressive alveolar bone loss first seen in the interradicular bone of 3-mo-old mice and most dramatic after 10 mo. This was accompanied by a dramatic increase in osteoclast numbers. This result was somewhat surprising in view of our previous work, which failed to detect any changes in osteoclast differentiation, tibial osteoclast surface, or serum resorption markers in *Ddr2*-deficient mice (Ge et al. 2016). These discrepancies may be explained by the different bone regions being examined. For example, it is possible that the altered PDL structure seen with *Ddr2* deficiency (Fig. 3) may lead to changes in mechanical stability of the tooth accompanied by increased osteoclast-mediated bone resorption. Changes in periodontal structure could be a primary effect of *Ddr2* deficiency or be secondary to defects in alveolar bone formation due to impaired osteogenic differentiation of *Dd2*-deficient

PDL cells. This would be consistent with the requirement for *Ddr2* in osteoblast differentiation of bone progenitors previously reported (Zhang et al. 2011; Ge et al. 2016). A direct demonstration that *Ddr2* specifically functions in PDL or alveolar bone in vivo will require cell-specific conditional knockout studies.

We also observed a widening of the PDL space in *Ddr2*-deficient mice; however, there was no evidence of PDL widening before tooth eruption (data not shown), suggesting that the PDL changes may require posteruption occlusal loading. One interpretation of this observation is that loss of *Ddr2* may compromise PDL structure, which then fails when exposed to occlusal loads, leading to PDL widening. Interestingly, there are anecdotal reports of tooth abnormalities in some SMED patients, although it is not known if the specific changes in tooth or PDL structure that we described in mice were present (Bargal et al. 2009).

Consistent with its effect on the periodontium, *Ddr2*$^{slie/slie}$ teeth also had atypical PDL collagen fibers associated with a significant increase in periostin immunostaining. However, periostin mRNA levels were significantly reduced in *Ddr2*-deficient PDL cells. This inverse correlation between periostin mRNA and protein is not understood. Periostin, a collagen-binding protein, is required to maintain PDL integrity and for proper collagen fibril formation and maturation (Rios et al. 2008). Aberrant PDL collagen fibers in *Ddr2* knockout could be due to impaired collagen fiber formation, crosslinking, and/or maturation. A possible role of *Ddr2* in collagen crosslinking was previously demonstrated; these studies showed that *Ddr2* activation with type I collagen induces lysyl oxidase, the enzyme required for ECM crosslinking (Khosravi et al. 2014). Abnormalities in collagen deposition and/or crosslinking have been reported in *Ddr2*-deficient heart and postnatal testicular tissues (Cowling et al. 2014; Zhu et al. 2015). Further studies will be required to resolve if *Ddr2* also controls collagen fibrillogenesis in dental structures.

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