SUPPORTING INFORMATION

Supplementary Methods

Probes for *in situ* hybridization. Specific probes for mouse *Adamtsl6α* and *β* were generated as follows: amplification of mouse *Adamtsl6α* (500 bp), *β* (500 bp) and the spacer domain of Adamtsl6 (500 bp) was performed by RT-PCR using the specific primers (forward: 5'-GCAGCCGACATCCACAGG-3'; reverse: 5'-CCAATGCTCTTGCACTGC-3') *Adamtsl6α*, (forward: 5'-GAGACACAAGTGCATCTGC-3'; reverse: 5'-CAATGCTCTCTCCCCCAGG-3') *Adamtsl6β*, and (forward: 5'-TTGGCTGTGACGACTTCC-3'; reverse: 5'-ATCCTTTCCCACAGGTGG-3') *Adamtsl6α* and *β*. Amplification of mouse *fibrillin-1* was performed using the specific forward primer 5'-GAACCTGGATGGCTCCTACA-3' and reverse primer 5'-ACCAAAAGGACACTCGCATC-3'. PCR products were subsequently cloned into the pCR4 TOPO blunt vector (Invitrogen Corporation, Carlsbad, CA). Generation of specific probes for mouse periostin was described previously(1). Plasmid DNAs were linearized by *NotI* (sense) or *BamHI* (antisense) digestion for *in situ* hybridization.

***In situ* hybridization.** To generate antisense and sense transcripts, digoxygenin-labeled riboprobes were prepared using T7 or SP6 RNA polymerase as described elsewhere (2). The heads of C57BL mice at embryonic (E) 13 days, E15, E17 and postnatal (P) 1 day were immediately frozen after embedding in OCT compound (Sakura Fine Technical Co., Ltd., Tokyo, Japan) and 10-μm frontal sections were prepared. The mandibles of 7-day and 35-day postnatal mice were fixed in 4% paraformaldehyde at 4°C overnight, decalcified with Morse's solution (3) for 24 hours, embedded in OCT compound, and 10μm sagittal sections were then prepared. *In situ* hybridization was carried out on these sections as previously described (4), with slight modifications. Polyvinyl alcohol was used as a buffer during the color reaction.

Histochemical analysis. Frontal sections of C57BL mouse heads at E13, E15, E17 and P1 day were prepared as described above. Fresh frozen sections of P7 and P35 mice were prepared using the Kawamoto tape method, according to the manufacturer's instructions (Leica Microsystems, Tokyo, Japan) (5) and 10 μm sagittal sections were generated. Cells were fixed with 4% paraformaldehyde and blocked with 1% BSA. The primary antibodies used were anti-Adamtsl6 (R1-1) and anti-fibrillin-1 polyclonal antibodies (pAB9543). The secondary antibodies used were Alexa 488, Alexa 555

anti-rabbit, or EnVision+system HRP (DAKO) anti-rabbit or anti-mouse IgG (Invitrogen), followed by nuclear staining with DAPI or color development using 3,3'-diaminobenzidine. followed by nuclear staining with DAPI. An anti-ADAMTSL6 polyclonal antibody was labeled with Alexa 488 by using the zenon antibody labeling kit according to the manufacturer's instructions (Invitrogen) for double immunostaining with an anti-fibrillin-1 polyclonal antibody. For visualization of oxytalan fibers, sections were oxidized for 15 min in 10% Oxone (Merck, Darmstadt, Germany) and subsequently stained with aldehyde fuchsin as described previously (6). Fluorescence images were sequentially collected using a confocal microscope featuring 403 nm, 488 nm and 543 nm laser lines (LSM510; Carl Zeiss MicroImaging, Jena, Germany).

Animals. C57BL/6 mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). MgR/mgR mice were generously provided by Dr. Francesco Ramirez (The Mount Sinai Medical Center, USA). All mouse care and handling conformed to the NIH guidelines for animal research. All experimental protocols were approved by the Tokyo University of Science Animal Care and Use Committee.

Generation of adenovirus. Recombinant adenovirus was constructed by homologous recombination between the expression cosmid cassette (pAxCAwt) and the parental virus genome in 293 cells (Riken, Tsukuba, Japan) as described previously (7) using an adenovirus construction kit (Takara, Ohtsu, Japan).

Generation of ADAMTSL6β transgenic mice. FLAG-tagged murine ADAMTSL6β(8) was subcloned into the Smi I site of pAxcwit (Takara, Tokyo, Japan). The CAG (chicken-actin) promoter, FLAG-tagged ADAMTSL6β and SV40 poly(A) signal were then amplified from this vector using KOD-Plus DNA polymerase (TOYOBO, Osaka, Japan) with the primer pair AAAGGATVVGTCGACATTGATTATTGACTAGTTATTAAT and AAAAAAAAGCGGCCGCCAGCTTGGGCCCTCGAGGGGTCGAGGGATC and subcloned into pGEM-T easy (Promega, Madison, WI). The resulting plasmid was digested with BamH1 and NotI and the insert was purified by agarose gel electrophoresis and microinjected into fertilized C57BL/6 mouse eggs (PhenixBio, Tochigi, Japan). Potential founders were analyzed by genotyping using PCR, and a total of two founder mouse lines were identified, of which the higher expressing line was expanded for immunohistochemical analyses.

Tooth replantation model. The tooth replantation experiments were performed as described previously (9). Briefly, the upper first molar from 4-week-old C57BL/6(SLC) mice was extracted under deep anesthesia. Extracted teeth were then replanted into the original cavity to allow the natural repair of the PDL. The replanted teeth were collected at 3, 7 and 14 days after transplantation and subjected to immunohistochemical analysis using the Kawamoto tape method or *in situ* hybridization as described in supplemental methods.

RNA preparation and real time RT-PCR (real time-PCR). Total RNA was isolated from cells using Isogen (Nippon Gene Co., Ltd., Tokyo, Japan) as described previously(10). cDNAs were synthesized from 1 μg aliquots of total RNA in a 20μl reaction containing 10x reaction buffer, 1 mM dNTP mixture, 1 U/μl RNase inhibitor, 0.25 U/μl reverse transcriptase (M-MLV reverse transcriptase, (Invitrogen) and 0.125 μM random 9-mers (Takara, Tokyo, Japan). The mRNA expression levels were determined using *Power* SYBR® Green PCR Master Mix (Applied Biosystems, CA, USA) and products were analyzed with an AB 7300 Real-Time PCR System (Applied Biosystems). Specific primers for *type I collagen* (forward: 5'-ACGCCATCAAGGTCTACTGC-3'; reverse: 5'-GAATCCATCGGTCATGCTCT-3'), *type XII collagen* (forward: 5'-CTATTGTGGTGCCAGGGAAT-3'; reverse: 5'- CCTTGGTCCACTTCTTGGAA-3'), *F-spondin* (forward: 5'- AGGGTAGCAGGTGATGATGG-3'; reverse: 5'-CCCAGTAGACCGTCTGCATT-3'), *tenascin N* (forward: 5'-CGCTCCATAGGAAAAGCAAG-3'; reverse: 5'-CCCAGCAATCTAGGAAGTCG-3') were used for real-time PCR. The primers for *gapdh* have been described previously (11).

SUPPLEMENTARY REFERENCES

1. Yokoi, T., Saito, M., Kiyono, T., Iseki, S., Kosaka, K., Nishida, E., Tsubakimoto, T., Harada, H., Eto, K., Noguchi, T., and Teranaka, T. (2007) *Cell Tissue Res* **327**, 301-311
2. Wilkinson, D. G. (1995) *Curr Opin Biotechnol* **6**, 20-23
3. Shibata, Y., Fujita, S., Takahashi, H., Yamaguchi, A., and Koji, T. (2000) *Histochem Cell Biol* **113**, 153-159
4. Iseki, S., Wilkie, A. O., and Morriss-Kay, G. M. (1999) *Development* **126**, 5611-5620
5. Kawamoto, T. (1990) *J Histochem Cytochem* **38**, 1805-1814.
6. Staszyk, C., and Gasse, H. (2004) *Anat Histol Embryol* **33**, 17-22.
7. Nishimura, R., Hata, K., Harris, S. E., Ikeda, F., and Yoneda, T. (2002) *Bone* **31**, 303-312
8. Tsutsui, K., Manabe, R. I., Yamada, T., Nakano, I., Oguri, Y., Keene, D. R., Sengle, G., Sakai, L. Y., and Sekiguchi, K. (2009) *J Biol Chem*
9. Hasegawa, T., Suzuki, H., Yoshie, H., and Ohshima, H. (2007) *Cell Tissue Res* **329**, 259-272
10. Handa, K., Saito, M., Tsunoda, A., Yamauchi, M., Hattori, S., Sato, S., Toyoda, M., Teranaka, T., and Narayanan, A. S. (2002) *Connect Tissue Res* **43**, 406-408
11. Nishida, E., Sasaki, T., Ishikawa, S. K., Kosaka, K., Aino, M., Noguchi, T., Ternaka, T., Shimizu, N., and Saito, M. (2007) *Gene.* **404**, 70-79. Epub 2007 Sep 2019.

Supplementary Figure legends

Supplementary Figure S1. Expression patterning of Adamtsl6α and β during early tooth germ development. *A.* Frontal sections of embryonic day (E) 13 (bud stage), 15 (cap stage), 17 (bell stage) and postnatal day (P) 1 (late bell stage) mice analyzed by immunohistochemical staining using an Adamtsl6 antibody. *B.* In situ hybridization analysis of *Adamtsl6α* and *Adamtsl6β. Adamtsl6β* is weakly expressed during the postnatal day (P) 1-late bell stage of dental follicle development in the tooth germ (arrows). However, *Adamtsl6β* was not detectable in early tooth germ development. In contrast, *Adamtsl6α* expression was observed in epithelial cells at the E15 cap stage (asterisk), but in odontoblasts during the P1-late bell stage (arrowhead). The expression of *Adamtsl6α* and *β* was confirmed using control probes that detect the conserved region of *Adamtsl6*. Bar, 100 μm.

Supplementary Figure S2. ADAMTSL6 is a component of microfibrils in the PDL. The distribution of Adamtsl6-positive microfibrils (left) was compared with the aldehyde fuchsin staining pattern (right, asterisks) in the adult PDL. Bar, 100 μm

Supplementary Figure S3. Adamtsl6β expression is induced during the PDL wound healing process. *A.* Schematic representation of the PDL injury model used for tooth replantation. *B.* Frontal section of a control side and injured PDL at 3, 7 and 14 days after replantation of the tooth analyzed by hematoxylin and eosin staining (HE) and by immunostaining with Adamtsl6 and fibrillin-1 antibodies. The right panel shows a superimposed image of immunostaining with the Adamtsl6 and fibrillin-1 antibodies. The expression of Adamtsl6 and fibrillin-1 is induced three days after replantation. *C.* In situ hybridization analysis of *periostin* expression in an injured PDL at 3, 7 and 14 days after replantation. Notably, the expression of *periostin* mRNA was found to be markedly downregulated in the PDL at 3 days after replantation as compared with its control expression. However, this expression had recovered by 7 days after replantation. Bar, 100 μm.

Supplementary Figure S4. Generation of a transgenic bioengineered tooth germ. *A.* Schematic representation of transgenic bioengineered tooth germ generated using an adenovirus expression system. Mesenchymal cells obtained from developing tooth germ were infected with adenovirus to generate transgenic reconstituted tooth germ. *B.* Confocal microscopy analysis of *GFP*-transgenic bioengineered tooth germ revealing

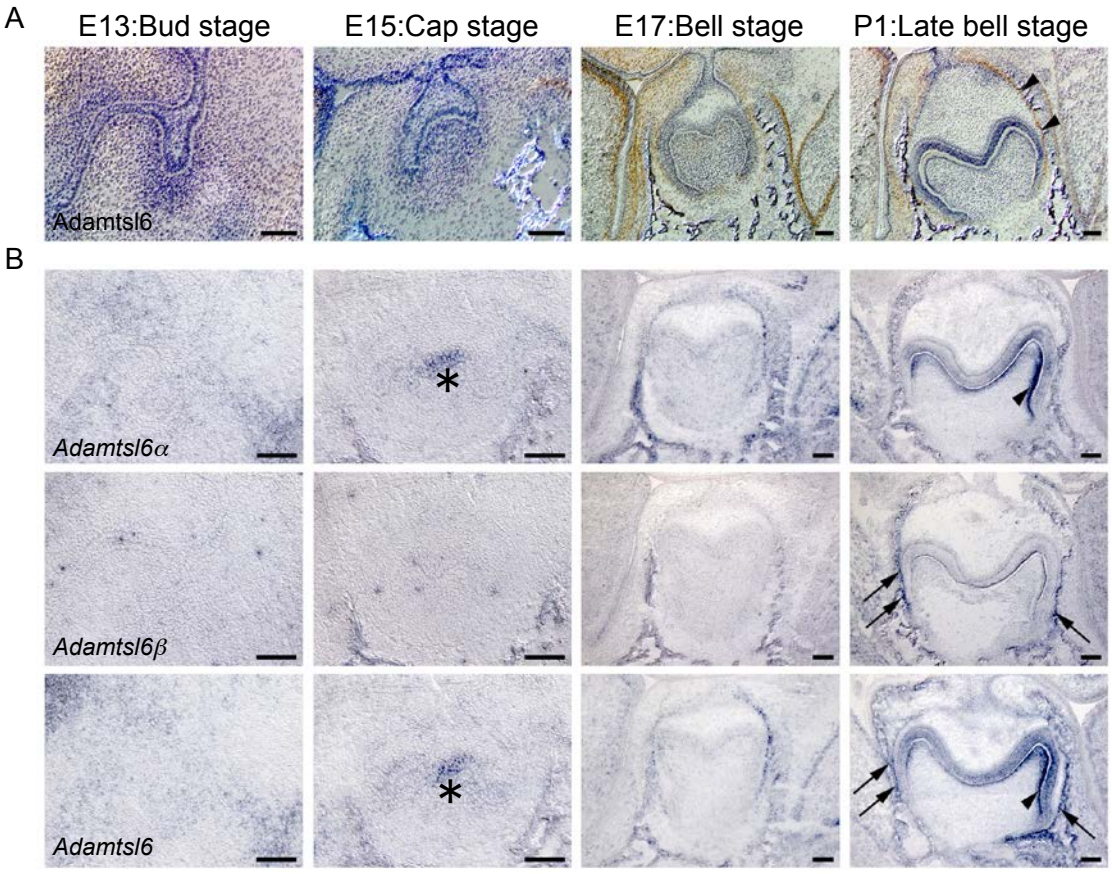
strong fluorescence in the mesenchyme. Bar, 200 μm. E, dental epithelium. *C.* Sections of *GFP*-transgenic bioengineered tooth germ analyzed by hematoxylin and eosin staining or by in situ hybridization analysis using specific probes for *periostin* and *type I collagen (ColI)*. Note that the expression patterns for *periostin* and *type I collagen* in transgenic bioengineered tooth germ are identical to those of the P1-late bell stage tooth germ. Bar, 100 μm *D*. Sections of the P1-late stage tooth germ were analyzed by hematoxylin and eosin (HE) staining or in situ hybridization analysis using specific probes for *periostin* and *type I collagen (ColI)*. The boxed area in the upper panel is shown at higher magnification in the lower panel. Strong expression of *periostin* and *type I collagen* was observed in the DF (arrow). Bar, 100 μm

Supplementary Figure S5. Effects of overexpressing *Adamtsl6β* on the genes that function in the PDL. Quantitative real-time PCR analysis of *collagen I a I chain (ColI), collagen XII a I chain (Col 12A), tenascin N (TNN), f-spondin (F-Spondin)* and *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* was performed in lacZ-(*LacZ*) and Adamtsl6β-(*Adamtsl6β*) transgenic bioengineered tooth germ. The *GAPDH* signal was used to normalize for the cDNA levels and was set at 1. The relative expression levels are shown as the means±SD and are from triplicate determinations.

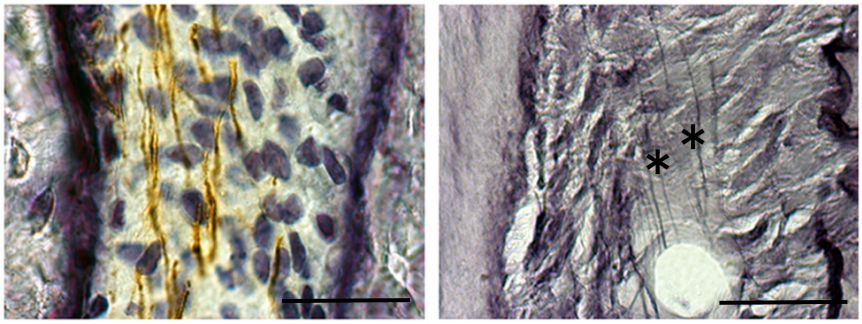
Supplementary Figure S6. Establishment of an experimental model for the local administration of recombinant Adamtsl6b into the injured PDL of mgR/mgR mice. *A*. Schematic representation of the local administration of recombinant Adamtsl6b into a PDL injury model. The first molar tooth was extracted to expose the PDL of the second molar which was then injured by dislocation. Collagen gel-containing recombinant Adamtsl6β was then injected into the injured PDL. *B.* The presence of collagen gel-containing recombinant Adamtsl6β was visualized using a fluorescence stereomicroscope after injection. *C.* Confocal microscopic analysis of collagen gels in an injured PDL 17 days after injection. The boxed areas in the left panels are shown at a higher magnification in the right panels. Note that intense fluorescence was detectable in both control collagen gel and collagen gel-containing recombinant Adamtsl6β. Bar, 50 mm. *D.* Histological analysis of an injured PDL in mgR/mgR mice after the local administration of control gel (+control) or gel containing recombinant Adamtsl6β (+Adamtsl6β) for 7 days. *E.* Immunohistochemical analysis of an injured PDL at 17 days after injection of control collagen gel or collagen gel containing recombinant Adamtsl6β using Adamtsl6 (green) and fibrillin-1 (red) antibodies. Reorganization of Adamtsl6β-positive (arrows) and fibrillin-1-positive (arrowheads) microfibrils was

found to be markedly increased upon injection with collagen gel-containing recombinant Adamtsl6β when compared with the injection of control collagen gel. Bar, 20 μm.

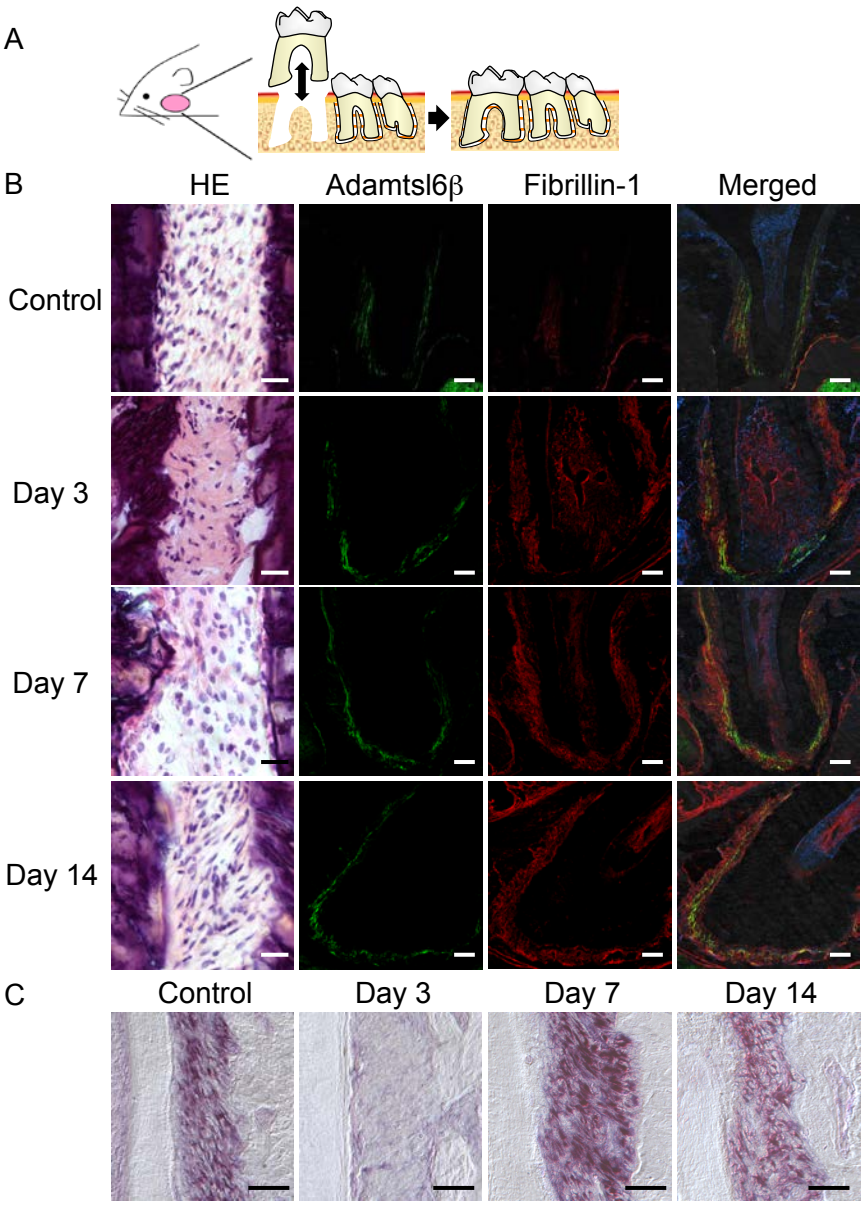
Supplementary Figure S1



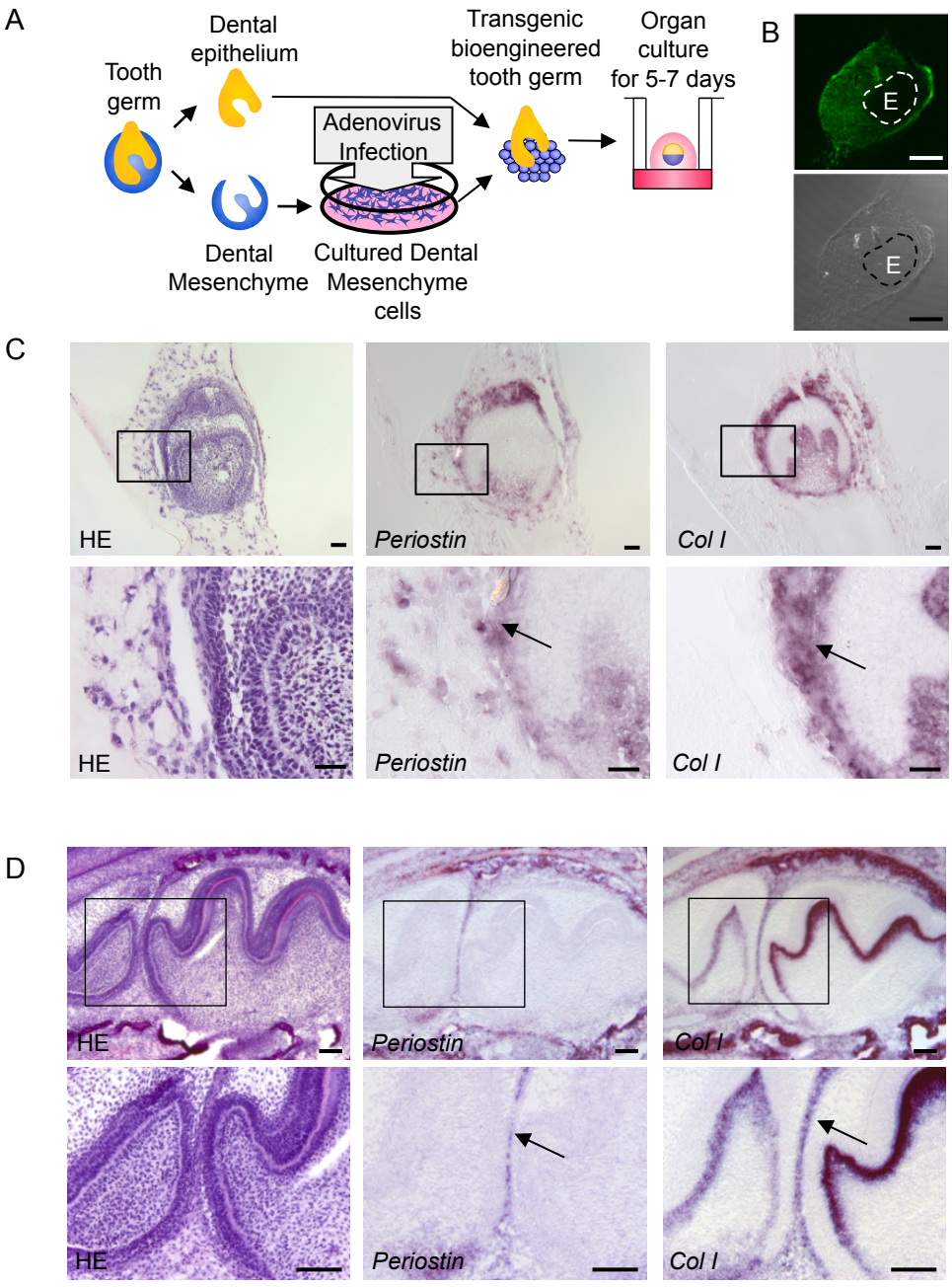
Supplementary Figure S2



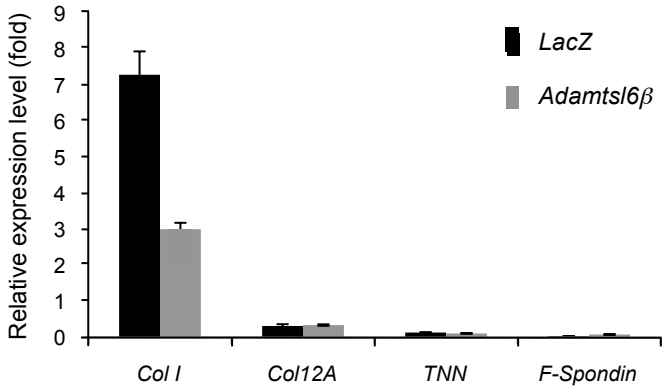
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6