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SUPPLEMENTARY MATERIAL

corresponding to:

The expression and function of thymosin beta 10 in tooth germ development

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Supplementary Materials and Methods

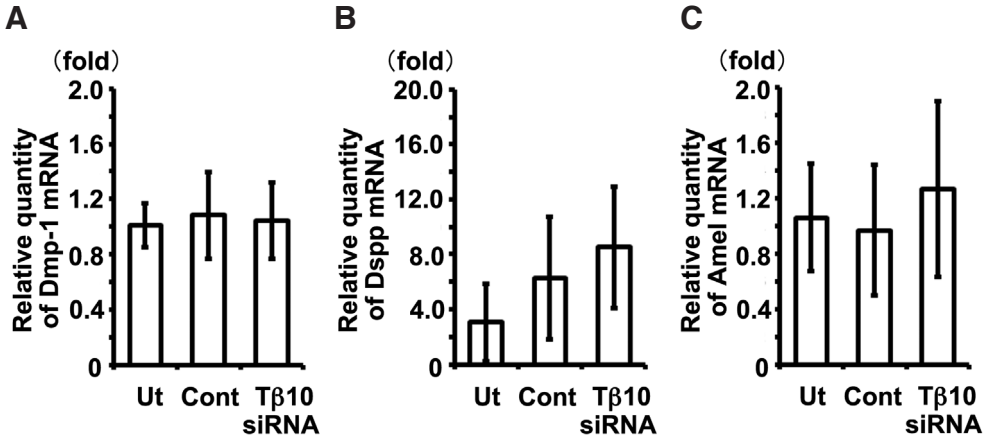
Semi-quantitative real-time PCR for the effects of* Tβ10-*siRNA treatment on the differentiation of tooth germ-derived cells

The expressions of dentin matrix protein-1 (Dmp-1) and dentin sialophosphoprotein (Dspp), differentiation markers, were examined in the mDP cells treated with *Tβ10*-siRNA using real-time PCR. The expression of amelogenin (Amel) was also analyzed in the mDE6 cells treated with *Tβ10*-siRNA. The *Tβ10*-siRNA treatment for the cultured cells and real-time PCR procedures were carried out according to the protocol described in the "Materials and Methods" section. After the treatment with *Tβ10*-siRNA for 48h, total RNA was isolated from the mDP and mDE6 cells. The cells were incubated in DMEM/F-12 with 1% serum when the cells were treated with *Tβ10*-siRNA for 48h. mDP cells were simultaneously treated with exogenous BMP-2. The GAPDH gene was used as an endogenous control. The gene-specific primers for Dmp-1, Dspp and Amel were as follows: *Dmp-1*, 5'-AAA GAC CTT GGG AGC CAG AGA-3' and 5'-AGT CTT CAT ATT GGG ATG CGA TTC-3'; *Dspp*, 5'-CTC GGA GGC TTT GAA GAC ATT GA-3' and 5'-GCT GCA GTT CCT GGA TGT GTT AGA-3'; *Amel*, 5'-AGC ATC CCT GAG CTT CAG ACA GA-3' and 5'-AAC CAG GGC TTC CAG GAT GAG-3'.

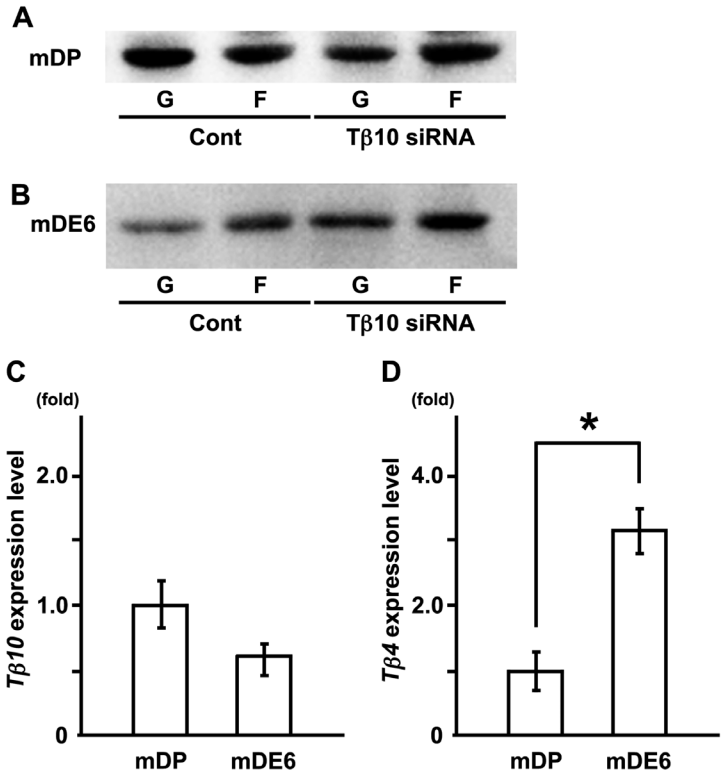
Immunoblotting for measurement of G-Actin/F-Actin Ratio

In order to evaluate the effects of *Tβ10*-siRNA on the ratio of intracellular G-actin and F-actin, immunoblotting was performed. After the treatment with *Tβ10*-siRNA for 48h, intracellular G-actin and F-actin were separately prepared from the mDP and mDE6 cells using F-actin/G-actin *in vivo* assay (Cytoskeleton, Denver, CO).

G-actin and F-actin were separation by 12% SDS-PAGE, and transferred to an Immun-Blot PVDF Membrane (Bio-Rad, Hercules, CA, USA). After blocking, the membranes were incubated with the anti-actin rabbit polyclonal antibody according to the manufacturer's instructions. Bound antibodies were reacted with HRP-conjugated secondary antibodies, and visualized using the enhanced chemiluminescence (ECL) Prime detection system (GE Healthcare Life Sciences, Piscataway, NJ, USA). The "ImageJ" densitometric analysis software program was used in the semi-quantitative analyses of the bands.



Supplementary Fig. S1 Effects of *Tβ10*-siRNA on the differentiation of tooth germ-derived cells. *Tooth germ-derived cells, mDP* **(A,B)** *and mDE6* **(C)** *cells were cultivated with or without* Tβ10-*siRNA treatment.* **(A,B)** Tβ10-*siRNA (Tβ10 siRNA) demonstrated no significant effects on the expression of dentin matrix protein-1 (Dmp-1) or dentin sialophosphoprotein (Dspp), differentiation markers, in the mDP cells in comparison to that observed in the untreated cells (Ut) and cells treated with universal negative control siRNA (Cont) (A:* P*=0.84 by one-way ANOVA, B:* P*=0.28 by one-way ANOVA).* **(C)** *In the mDE6 cells,* Tβ10 *siRNA showed no significant effects on the expression of amelogenin (Amel). (C:* P*=0.42 by one-way ANOVA).*



Supplementary Fig. S2. *Tβ10* expression was associated with the ratio of G-actin/F-actin observed in mDP cells. *The levels of F- and G-actin in the mDP and mDE6 cells treated with* Tβ10 *siRNA for 48 h were examined using an immunoblot analysis. The level of F-actin in the mDP cells was increased following the* Tβ10*-siRNA treatment* **(A)**, *while such treatment only modestly affected the ratio of G-actin/F-actin in the mDE6 cells* **(B)**. Tβ10 *expression level in the mDP cells was higher than that observed in the mDE6 cells; however, the difference was not significant* **(C)**. *On the other hand, the* Tβ4 *expression level in the mDP cells was significantly lower than that observed in the mDE6 cells* **(D)** *($p<0.05$). GAPDH was used as an internal control.*