Supplementary figures

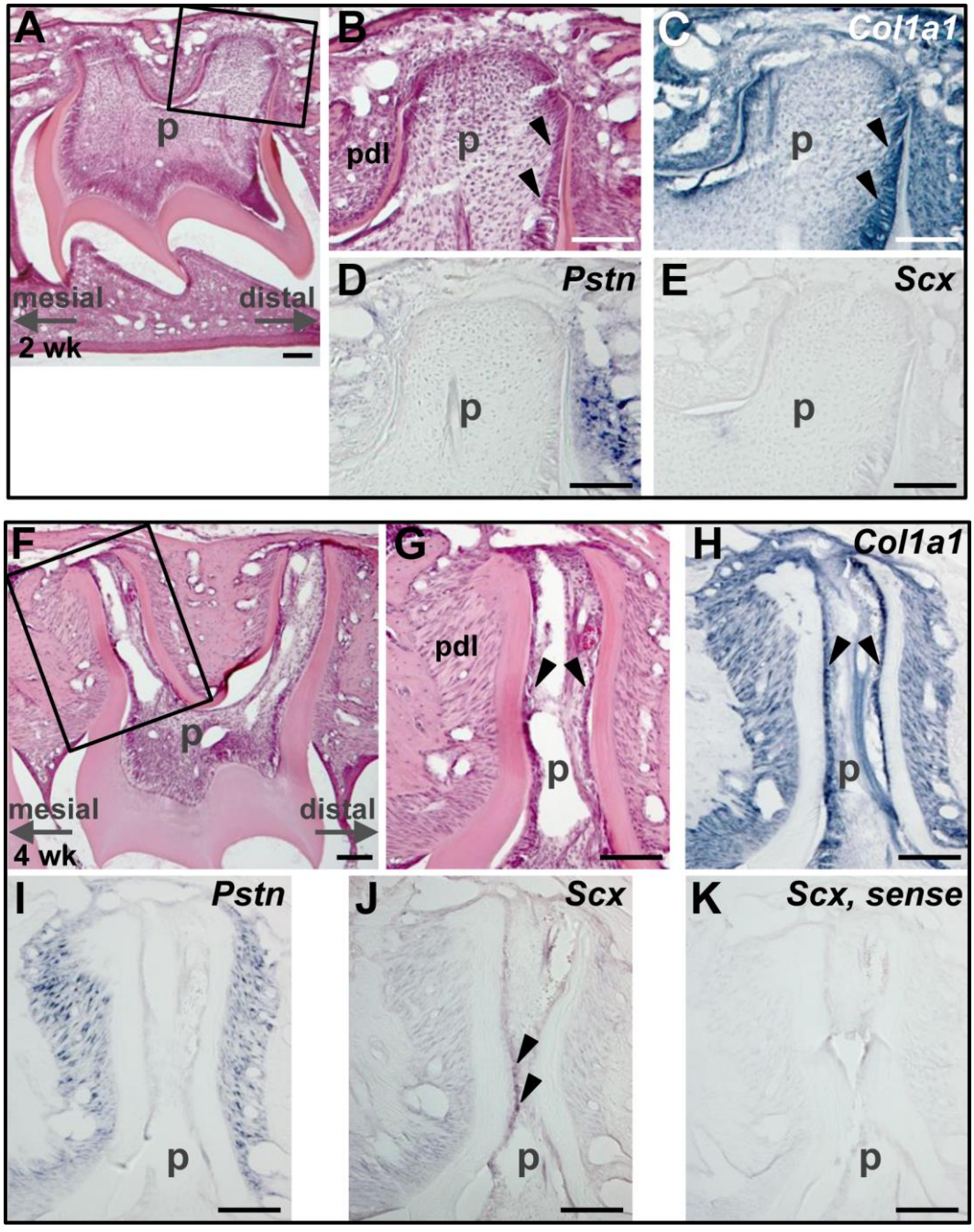

Fig. S1. *Scx* gene expression in the PDL and odontoblast-predentin layer.

(A-K) Frozen sections from the maxillary second molars of 2- (A-E) and 4-week-old (F-K) wild-type mice were processed for HE staining (A, B, F, G) or *in*

situ hybridization (C-E, H-K). The sections hybridized with anti-sense probes for *Col1a1* (C, H), *Pstn* (D, I), and *Scx* (E, J), and a sense probe for *Scx* (K) are shown. Magnified images corresponding to the boxed regions in A and F are shown in B-E and G-K, respectively. Arrowheads in B, C, G, H, J indicate the odontoblasts in the pulp. p, pulp; pdl, periodontal ligament. Scale bars: 100 μm.

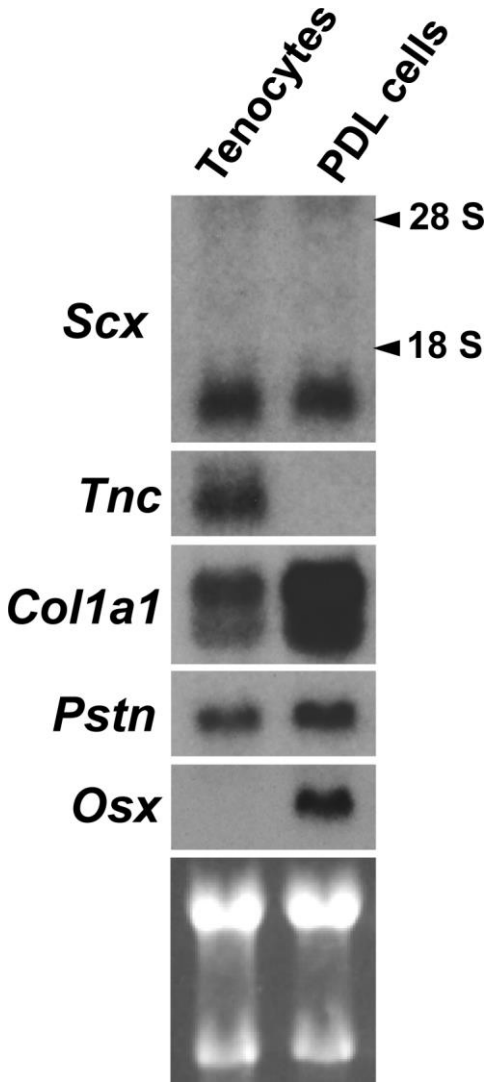

Fig. S2. Expression of *Scx*, *Tnc*, *Col1a1*, *Pstn*, and *Osx* in tenocytes and PDL cells.

Total RNA was extracted from confluent cultures of tenocytes and PDL cells. Expression of *Scx*, *Tnc*, *Col1a1*, *Pstn*, and *Osx* mRNAs was examined by northern blot analysis. Fifteen micrograms of total RNA were loaded in each lane and equal loading was verified by ethidium bromide staining.

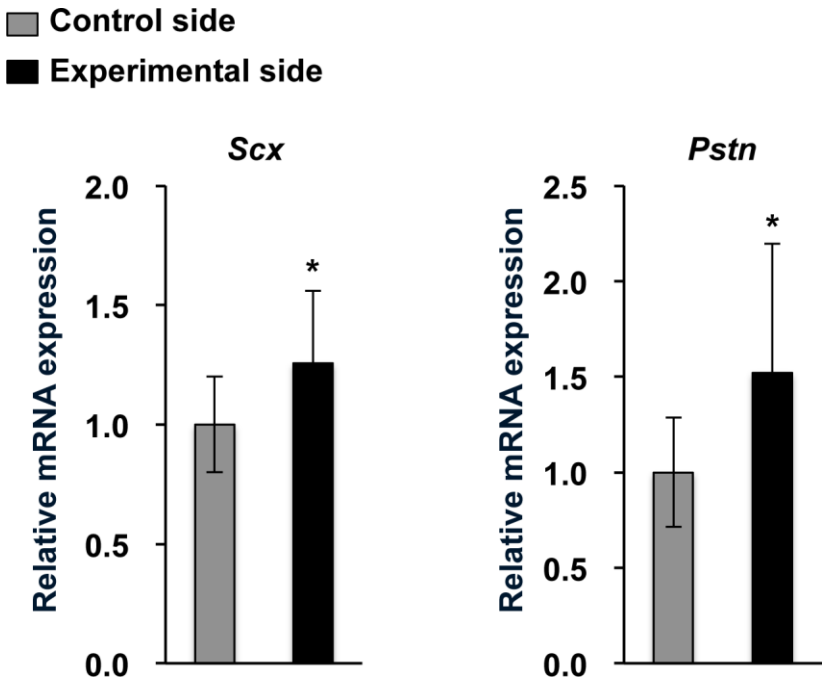

Fig. S3. Upregulation of *Scx* and *Pstn* in periodontal tissues in response to the tensile force exerted by experimental tooth movement.

Experimental tooth movement was performed using 9-week-old male ICR mice. A piece of an elastic band was inserted interproximally between the upper left first and second molars. The right side served as control. At 48 h after insertion of the elastic band, total RNA was extracted from the control and tensioned regions. Relative expression levels of *Scx* and *Pstn* were examined by RT-qPCR. The data represent the average of 5 mice calculated using the $2^{-\Delta\Delta CT}$ method after normalization with *18S rRNA*. Specific primers for RT-qPCR are listed in Table S2. The relative expression of each gene is normalized to the Control side and reported as mean ± s.d. **P* < 0.05 vs. Control side.

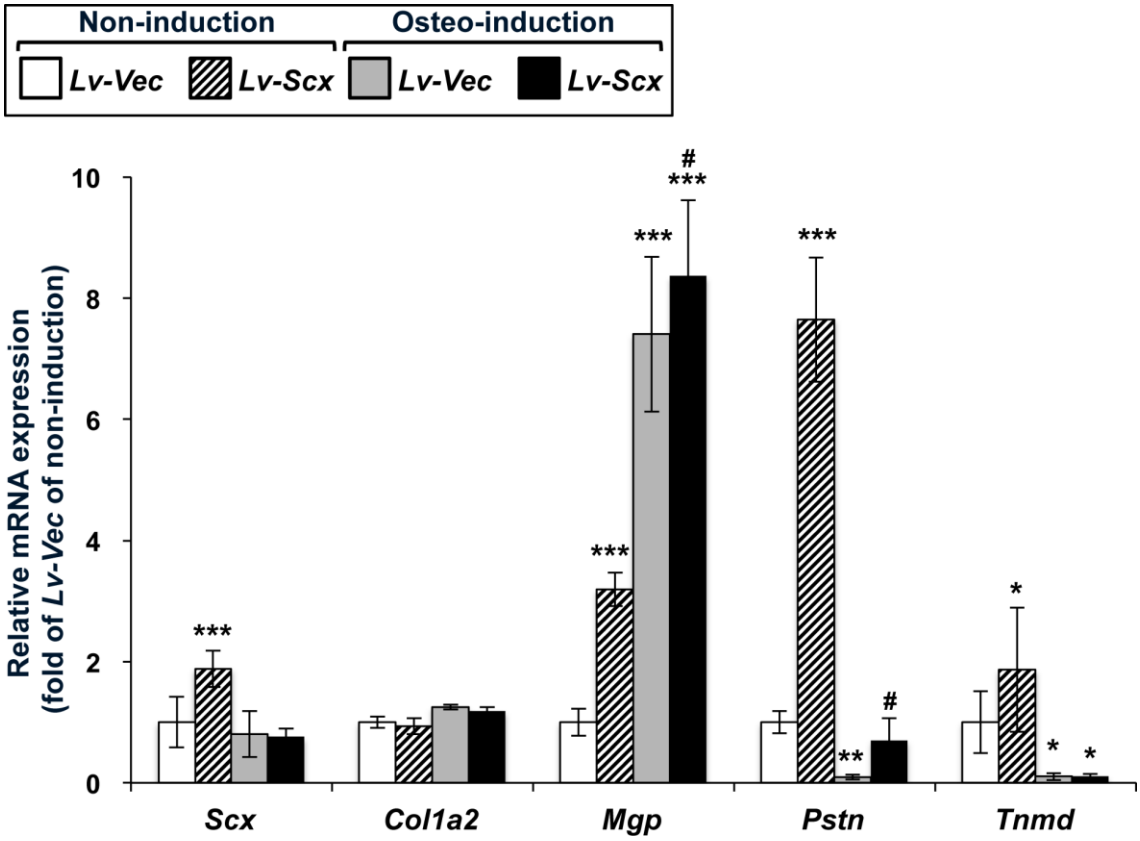

Fig. S4. Upregulation of *Mgp* and *Pstn* by *Scx*-overexpression in PDL cells cultured under the osteoinductive conditions.

Cells isolated from the PDL of molars of Wistar rats were seeded at a density of 4×10^4 cells/well in a 12-well plate. At 24 h after this inoculation, the cells were infected with *Lv-Vec* or *Lv-Scx*. The cells were grown in α-MEM containing 10% FBS and reached confluence on day 2. For osteogenic induction (Osteo-induction), the cultures on day 4 were switched to an induction medium containing rhBMP6, maintained for 3 days, and further maintained in induction medium without rhBMP6 for another 3 days. Non-induction cultures were maintained in α-MEM containing 10% FBS throughout the culture period. Total

RNA was extracted from the cultures on day 10. Relative expression levels of *Scx*, *Col1a2*, *Mgp*, *Pstn*, and *Tnmd* were examined by RT-qPCR. The primer set for *Scx* was targeted to the 3'-untranslated sequence of rat *Scx* cDNA to detect endogenous expression. The data represent the average of 3 independent experiments calculated using the $2^{-\Delta\Delta CT}$ method after normalization with *18S rRNA.* The relative expression of each gene is normalized to *Lv-Vec* of Non-induction and reported as mean ± s.d. **P* < 0.05 vs. *Lv-Vec* of Non-induction, ***P* < 0.01 vs. *Lv-Vec* of Non-induction, ****P* < 0.001 vs. *Lv-Vec* of Non-induction, $^{\#}P$ < 0.05 vs. *Lv-Vec* of Osteo-induction.

Supplementary materials and methods

In situ hybridization

Mice were anesthetized with sodium pentobarbital and perfused with 4% paraformaldehyde dissolved in PBS (PFA/PBS), and their upper jaws were dissected. The specimens were fixed in 4% PFA/PBS for 16 h, decalcified using Morse's solution (Shibata et al., 2000) for 7 days, infiltrated with 18% sucrose/PBS, embedded in Tissue-Tek O.C.T. compound (Sakura Finetek Japan, Tokyo, Japan), and frozen in liquid nitrogen. For RNA probes, the cDNAs for *type I collagen* (*Col1a1*), *periostin* (*Pstn*), and *scleraxis* (*Scx*) were amplified by reverse transcription-polymerase chain reaction (RT-PCR) based on its sequence information in GenBank (*Col1a1*, NM007742; *Pstn*, NM015784; *Scx*, S78079). The RNA probes were transcribed from the linearized plasmids with a digoxigenin (DIG) RNA labeling kit (Roche). For *in situ* hybridization, the specimens were sectioned at 8 μm with a Low Profile Microtome Blade (Leica Microsystems). The sections were fixed with 4% PFA/PBS for 10 min, treated with 10 μg/mL Proteinase K (Life Technologies) for 15 min, carbethoxylated twice in 0.1% DEPC/PBS, and hybridized with DIG-labeled RNA probes diluted in 50% formaldehyde/5 x SSC containing 40 μg/mL salmon sperm DNA at 55˚C for 16 h. To detect DIG-labeled RNA probes, immunological detection was performed with Anti-DIG-AP Fab fragment (Roche) and BM purple (Roche).

Cell culture

Tenocytes were isolated from limb tendons of 7-day-old Wistar rats. Minced tendons were incubated with 0.1% EDTA at 37ºC for 20 min and digested with

0.05% trypsin/0.53 mM EDTA (Life Technologies) at 37ºC for 5 min followed by digestion with 0.1% collagenase (Roche) at 37ºC for 10 min. Tenocytes were grown in minimum essential medium Eagle alpha modification (□-MEM) (Sigma) supplemented with 10% fetal bovine serum (FBS) and 50 μg/mL kanamycin (Sigma) on type I collagen (Koken, Tokyo, Japan) -coated dishes.

Northern blot analysis

Total RNA (15 μg) was denatured with 6% formaldehyde, fractionated by 1% agarose gel electrophoresis, and transferred onto Nytran membranes with a TurboBlotter (Schleicher and Schuell, Dassel, Germany). Hybridization was performed overnight at 42°C with an appropriate cDNA probe labeled with [α-32P] dCTP from Amersham Biosciences in a solution containing 50% formamide, 6 × SSPE, 0.1% bovine serum albumin, 0.1% Ficoll 400, 0.1% polyvinylpyrrolidone, 0.5% SDS and 100 μg/mL denatured salmon sperm DNA. The probe for *Pstn* was obtained from the same cDNA that was used as a template to generate the RNA probe mentioned above. cDNAs for *Scx*, *tenascin c* (*Tnc*), *Col1a1*, and *osterix* (*Osx*) were amplified by RT-PCR based on the published sequences in GenBank (*Scx*, NM001130508; *Tnc*, D90343; *Col1a1*, Z78279; *Osx*, NM001037632). For hybridization, specific cDNA probes were labeled with [α-^{32}P] dCTP (PerkinElmer).

RNA extraction from the periodontal tissues with the experimental tooth movement

To extract total RNA from the tensioned regions of periodontal tissues, the upper

jaw was collected in RNA*later* solution (Life Technologies). The periodontal ligament (PDL) and surrounding alveolar bone were dissected from the upper jaw using a stereomicroscope (Taddei et al., 2012), and total RNA was extracted using an RNeasy Plus Mini Kit (QIAGEN). In the present study, the distal regions of the PDL and surrounding alveolar bone neighboring the mesial and palatal roots of the left maxillary first molar were collected as the tensioned regions following experimental tooth movement. The corresponding regions of the right side were collected as control.

Supplementary references

Shibata, Y., Fujita, S., Takahashi, H., Yamaguchi, A. and Koji, T. (2000). Assessment of decalcifying protocols for detection of specific RNA by non-radioactive in situ hybridization in calcified tissues. *Histochemistry and cell biology* **113**, 153-159.

Taddei, S. R., Moura, A. P., Andrade, I., Jr., Garlet, G. P., Garlet, T. P., Teixeira, M. M. and da Silva, T. A. (2012). Experimental model of tooth movement in mice: a standardized protocol for studying bone remodeling under compression and tensile strains. *Journal of biomechanics* **45**, 2729-2735.

Table S1. Number and proportion of Scx^{high} cells in the PDL

Mouse No.	Control side			Experimental side		
	Scx^{high}	Scx^{+}	% of Scx^{high}	Scx^{high}	Scx^{+}	% of Scx^{high}
1	14	59	23.73	45	78	57.69
2	16	60	26.67	30	70	42.86
3	26	77	33.77	45	92	48.91
4	15	58	25.86	38	84	45.24
Mean			27.51			48.68

Table S2. Primers for RT-qPCR

Gene		Sequence (5’ - 3’)
Pstn (Mouse)	Forward	GAACGAATCATTACAGGTCC
	Reverse	GGAGACCTCTTTTTGCAAGA
Scx (Mouse)	Forward	CCTTCTGCCTCAGCAACCAG
	Reverse	GGTCCAAAGTGGGGCTCTCCGTGACT
18S rRNA (Mouse)	Forward	TTCTGGCCAACGGTCTAGACAAC
	Reverse	CCAGTGGTCTTGGTGTGCTGA
Col1a2 (Rat)	Forward	ACTCAGCCACCCAGAGTGGAA
	Reverse	TTGACAGGTTGGGCCTGGA
Mgp (Rat)	Forward	AGGCAGACTCACAGGACACC
	Reverse	CATTTCTCCGGTTGGTGAAG
Pstn (Rat)	Forward	CGTGGCAGCACCTTCAAAGA
	Reverse	GGCTGAAGACTGCCTTGAATGAC
Tnmd (Rat)	Forward	ATGGGTGGTCCCACAAGTGAA
	Reverse	CTCTCATCCAGCATGGGATCAA