

Supporting Information

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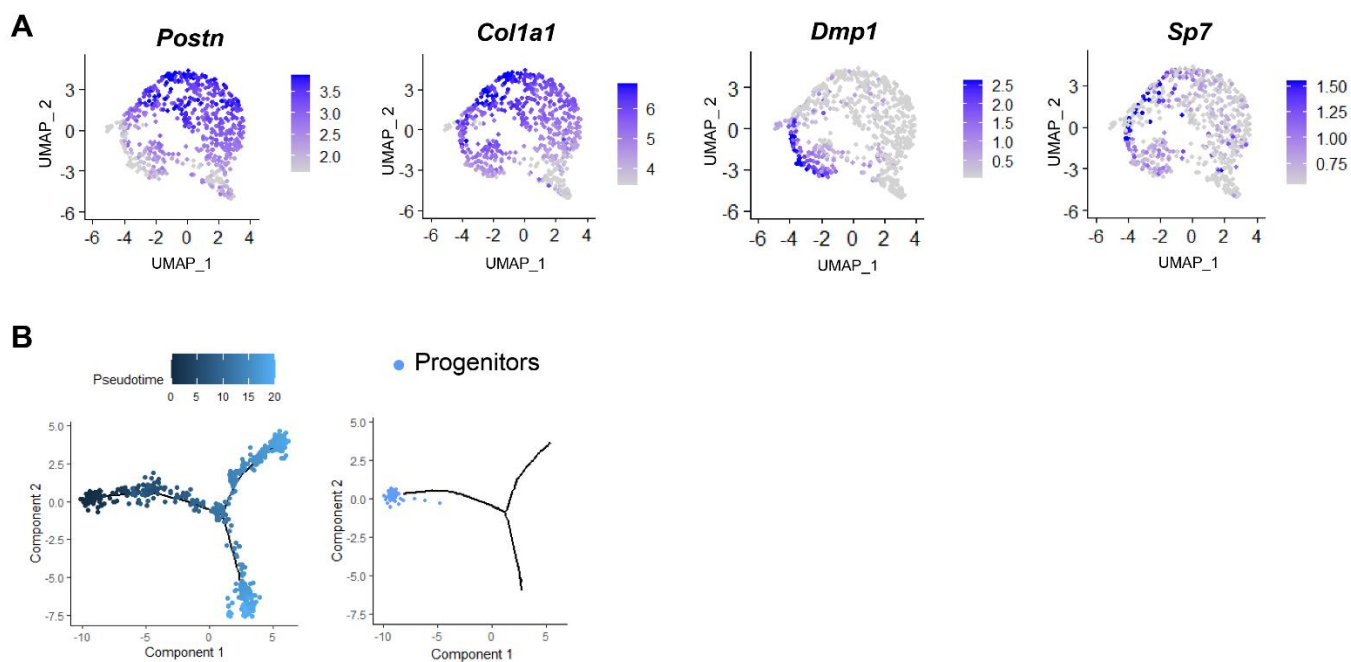
Lepr-Expressing PDLSCs Contribute to Periodontal Homeostasis and Respond to Mechanical Force by Piezo1

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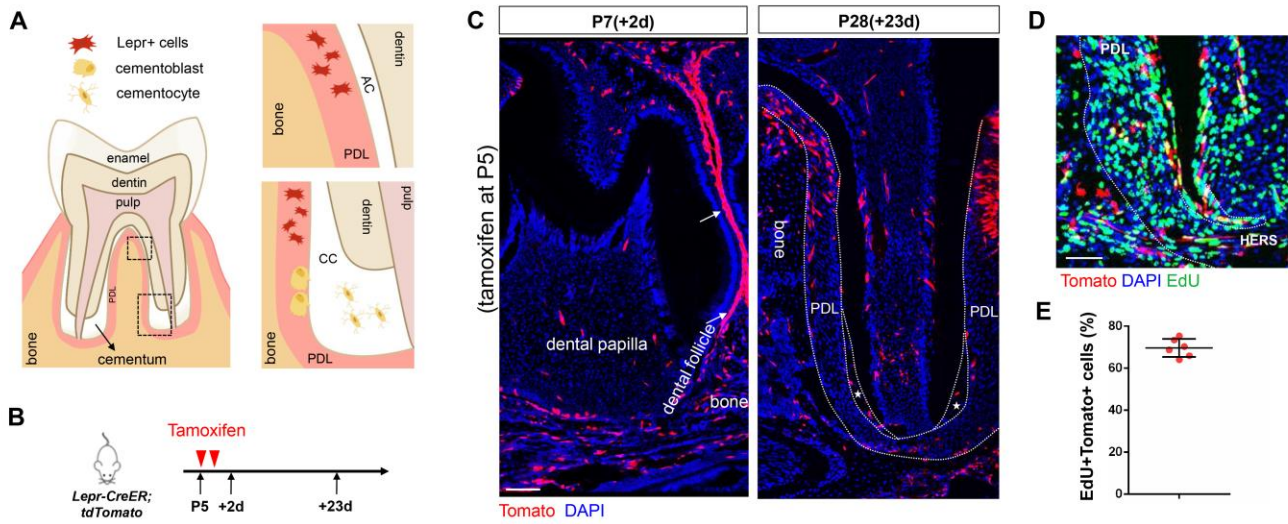
Lepr-expressing PDLSCs contribute to periodontal homeostasis and respond to mechanical force by *Piezo1*

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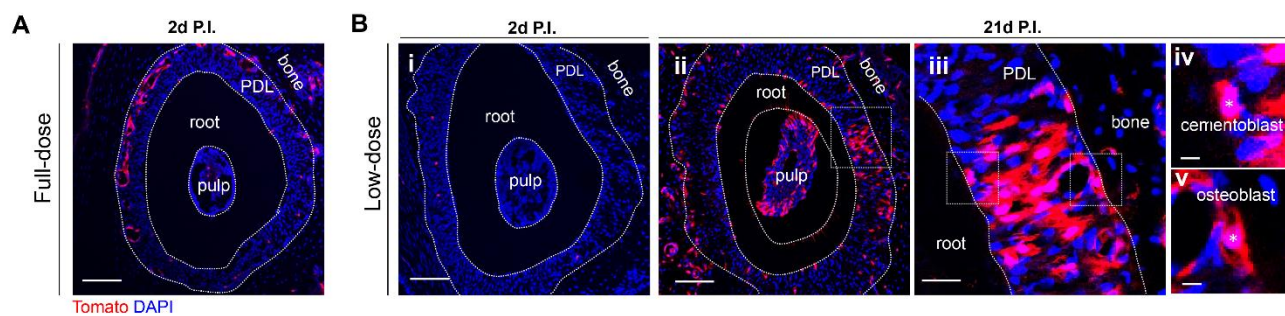
Supplementary Figure 1. Single-cell analysis of PDL stromal cells. Related to Figure 1.

- A. The expression of marker genes projected onto UMAP atlas.
- B. Left: The pseudotime developmental tree of PDL stromal cells. Right: Distribution of progenitors on the tree.



Supplementary Figure 2. Anatomy diagram and lineage tracing of Lepr⁺ cells from P5. Related to Figure 2.

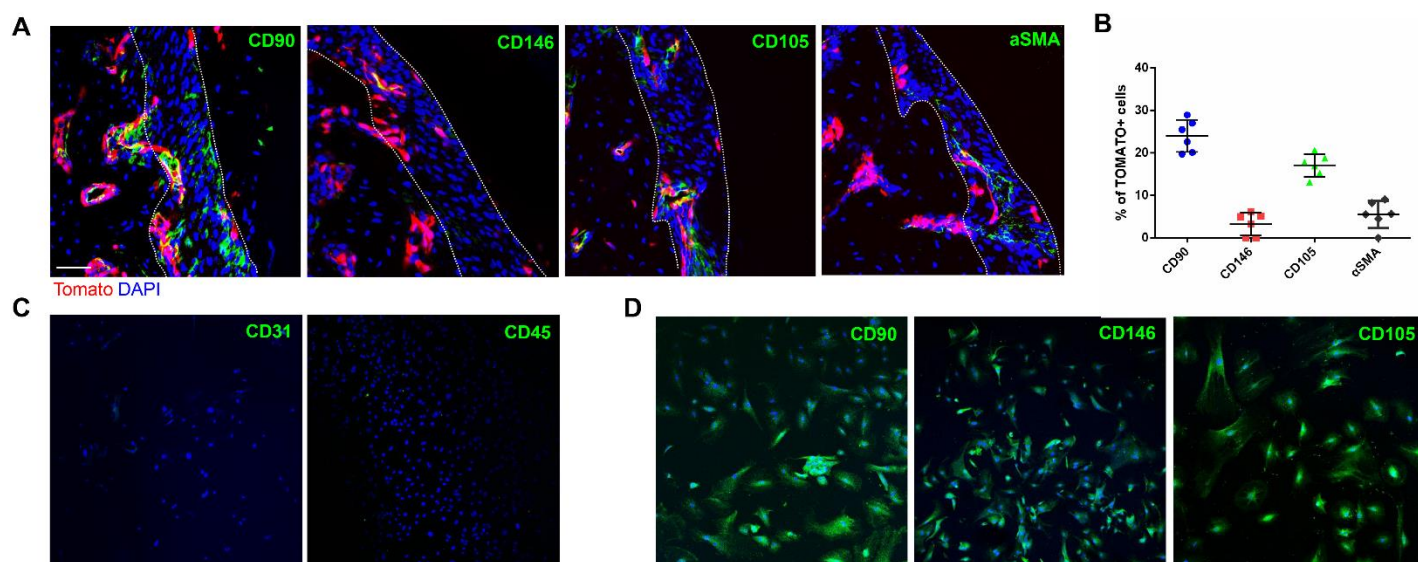
- Anatomy diagram of periodontal tissues. AC: Acellular cementum; CC: Cellular cementum.
- Schematics of tamoxifen induction.
- Representative confocal images of dental follicle and PDL from P5-induced *Lepr-CreER;tdTomato* mice. Scale bar: 100 μ m.
- D-E. Representative EdU administration images and percentage of EdU⁺ Tomato⁺ cells/ Tomato⁺ cells from P5-induced *Lepr-CreER;tdTomato* mice at P14 (n=6). Scale bar: 50 μ m.



Supplementary Figure 3. *Lepr*⁺ cells clonal tracing *in vivo*. Related to Figure 3. *Lepr-CreER;tdTomato* mice were induced with various tamoxifen dose at the age of P21. (A) Regular full-dose (0.05 mg/g) for 2 days. (B) Single low(1/30)-dose (1.67 μg/g). Images were shown on horizontal sections of the root.

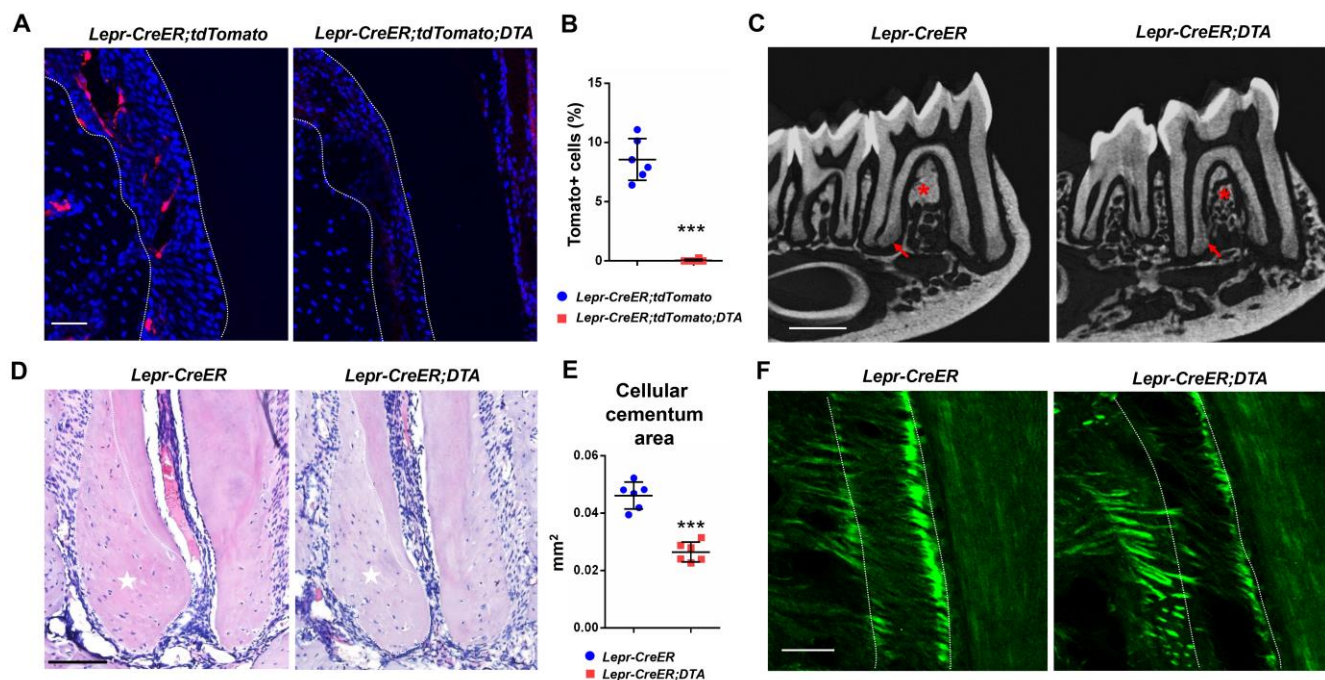
A. Representative image of full-dose tamoxifen administration on *Lepr-CreER;tdTomato* mice which induced 2 days from P21. P.I., post-induction. Scale bar: 100 μm.

B. Representative images of single low-dose tamoxifen administration on *Lepr-CreER;tdTomato* mice at the age of P21. Mice were sacrificed 2 days (i) or 21 days (ii-v) after induction. P.I., post-induction. Boxed regions in (iii) were enlarged to show Tomato⁺ osteoblast (iv) lining the alveolar bone or cementoblast (v) on the root surface. (i,ii) Scale bar: 100 μm. (iii) Scale bar: 25 μm. (iv,v) Scale bar: 5 μm.



Supplementary Figure 4. Immunofluorescent staining *in vivo* and *in vitro*. Related to Figure 3.

- A. Representative confocal images of immunofluorescent stem cell markers staining on adult *Lepr-CreER;tdTomato* mice. Scale bar: 50μm.
- B. Percentages of positively stained cells/Tomato+ cells ($n = 6$).
- C. CD31 and CD45 staining of PDLSCs *in vitro*.
- D. CD90, CD146 and CD105 staining of PDLSCs *in vitro*.



Supplementary Figure 5. Ablation of *Lepr*⁺ PDLSCs on adults disrupts periodontium homeostasis. Related to Figure 4.

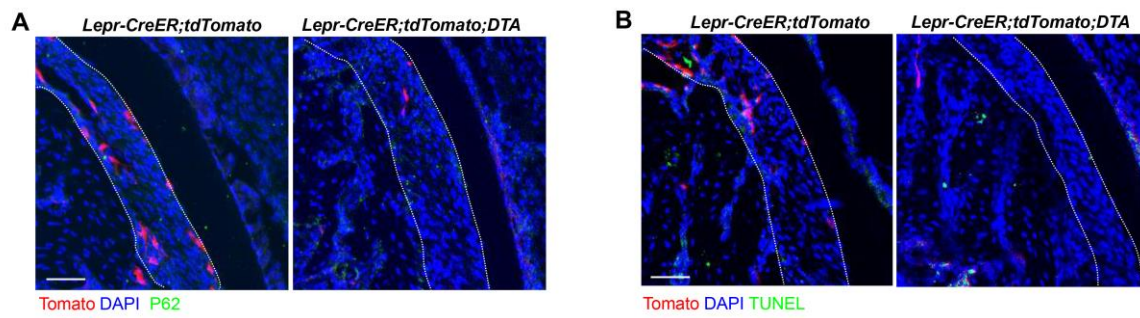
A-B. Representative images and percentage of Tomato+ cells in PDL from adult *Lepr-CreER;tdTomato;DTA* and control mice ($n = 6$). Scale bar: 50 μ m.

C. Representative μ CT images of mandible in *Lepr-CreER;DTA* and control mice. Scale bar: 500 μ m. Arrows indicate the cellular cementum. Asterisks indicate the alveolar bone.

D-E. H&E staining and statistical analysis of the cellular cementum in *Lepr-CreER;DTA* and control mice ($n = 6$). Scale bar: 100 μ m. Dotted lines outline ROI of the cellular cementum.

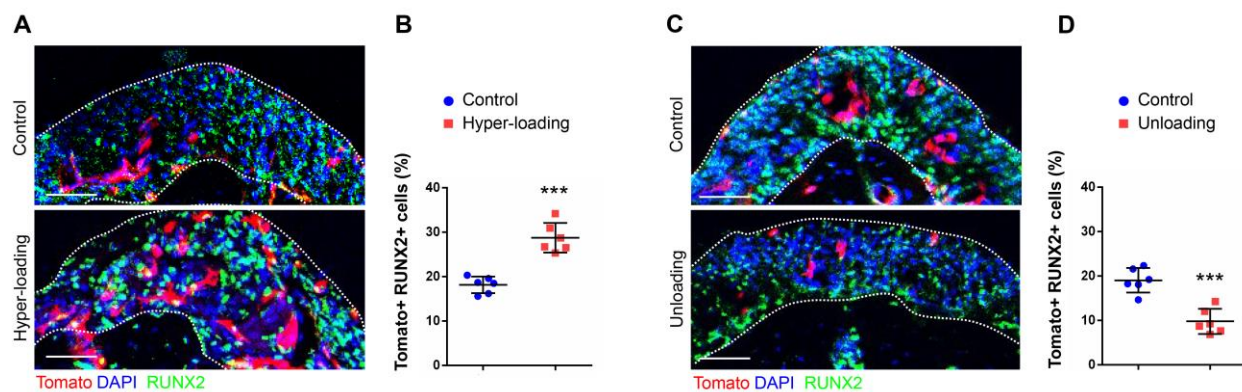
F. SHG microscopy of PDL fibrils in adult *Lepr-CreER;DTA* and control mice. Scale bar: 50 μ m.

Data are presented as mean \pm standard deviation. *** $P < 0.001$ as determined by two-tailed Student t tests.



Supplementary Figure 6. Apoptosis and autophagy are not responsible for the disruption of PDL homeostasis after *Lepr*⁺ cells ablation. Related to Figure 4.

- A. Representative images of P62 staining on *Lepr-CreER;tdTomato;DTA* and control mice. Scale bar: 50µm.
- B. Representative images of TUNEL staining on *Lepr-CreER;tdTomato;DTA* and control mice. Scale bar: 50µm.



Supplementary Figure 7. Occlusal forces impact on osteogenic differentiation of Lepr+ PDLSCs. Related to Figure 5.

A-B. RUNX2 staining and percentage of Tomato+ RUNX2+/ Tomato+ cells on hyper-loading and control nice. ($n = 6$). Scale bar: 50 μ m.

C-D. RUNX2 staining and percentage of Tomato+ RUNX2+/ Tomato+ cells on unloading and control nice. ($n = 6$). Scale bar: 50 μ m.

Data are presented as mean \pm standard deviation. *** $P < 0.001$ as determined by two-tailed Student t tests.