

Fig. S1. Generation of *Plap-1-GFP-2A-CreER* knock-in mice

- A. Representative *Sparc* mRNA expression in the periodontal ligament (PDL). In contrast to *Plap1*, cells on the surface of the alveolar bone and cementum expressed *Sparc*. Arrows indicate cementoblasts. Arrowheads indicate osteoblasts. All PDL images in this paper are presented in this direction (left: cementum/dentin/root, right: alveolar bone).
- B. Representative *Plap-1* immunofluorescent staining in the PDL. Both fibroblastic and cemento/osteoblastic cells were stained.
- C. Gene targeting strategy for *Plap-1-GFP-2A-CreER* knockin mice. Structure of the mouse *Plap-1* locus (WT allele), the *Plap-1* targeting construct (Targeting vector), and the predicted mutant *Plap-1* gene (KI). Exons are represented by boxes. Exons 2 and 3 of *Plap-1* gene were replaced with the *EGFP-T2A-CreERT2-WPRE-PGK-Neo* sequence. PGK-DTA gene was attached to the 5' end of the genomic fragment for negative selection. The external homologous regions shown in the targeting allele were used as genomic probes for Southern blot analysis. Southern blot analysis for targeted clone screening was carried out using *PacI* (P) and *MunI* (M). The primer pairs for genotyping PCR are shown in the red arrow.
- D. Southern blot analysis using Neo probe. The targeted (12.3 kb) band was detected using a neo probe and the *PacI*-digested DNA from ES cells.
- E. Southern blot analysis using 5' probe. The endogenous (6.7 kb) and targeted (12.3 kb) bands were detected using a 5' probe and the *PacI*-digested DNA from ES cells.
- F. Representative genotyping PCR result. The expected size of wildtype and knockin alleles are 368 bp and 564 bp, respectively.
- G. Representative fluorescent images of 14-week-old *Plap-1-GFP-2A-CreER; Rosa26-tdTomato* mice without tamoxifen treatment. No leaky tdTomato expressions were detected (lower magnification in left panel, higher magnification in right panel).
- H. Representative tdTomato (magenta) and *Ibsp* mRNA (green) expression in the PDL 16 days after a 1/10 dose tamoxifen treatment. A limited number of PDLC were labeled and some cells in cemento-/osteoblastic zones were double-positive for tdTomato and *Ibsp*. Insets show *Ibsp* expressing *Plap-1*-lineage cells.
- D: Dentin, Ab: Alveolar bone, scale bars: 100 µm.

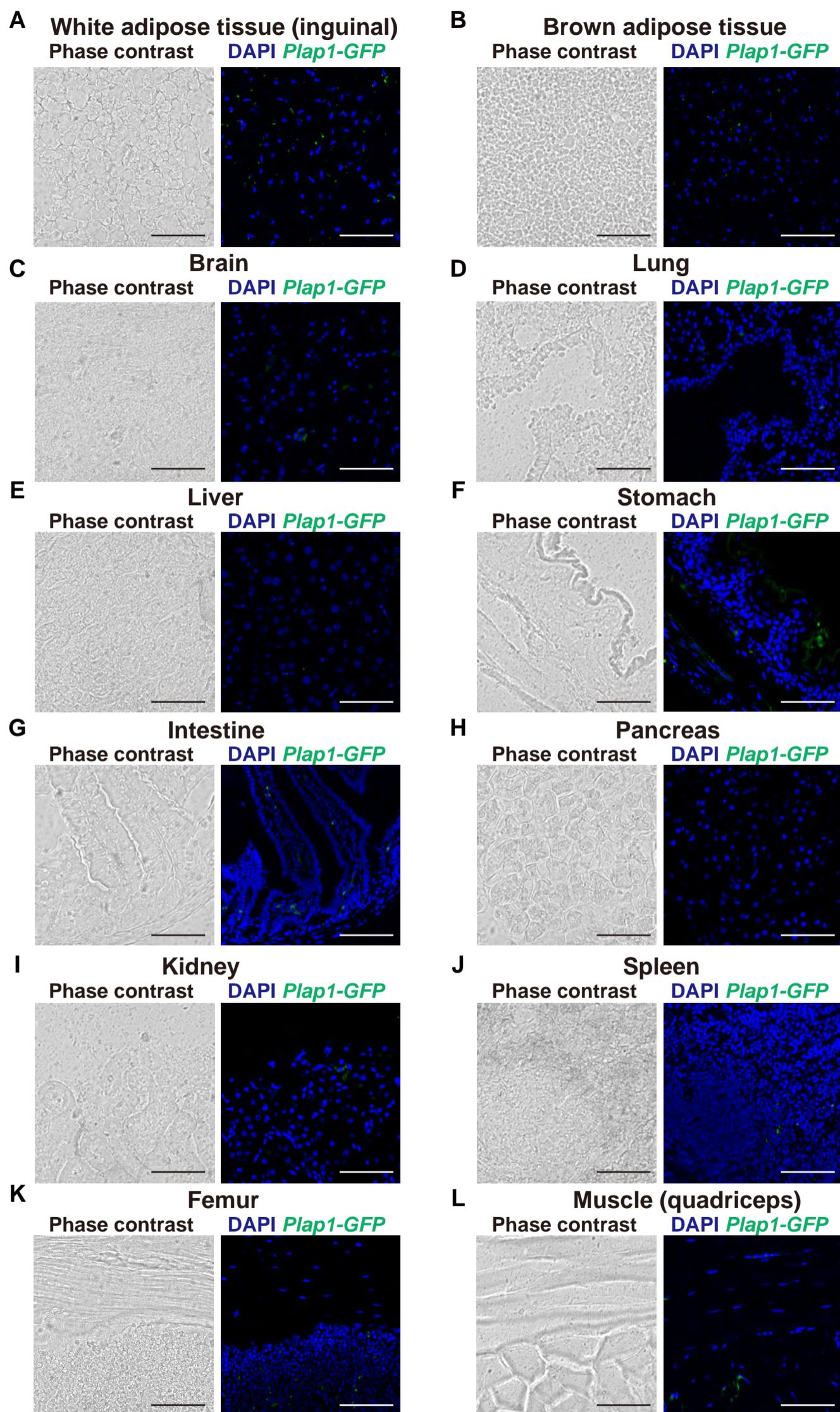


Fig. S2. Plap-1-positive cells in the tissues outside the oral cavity.

Representative phase contrast and fluorescent images of inguinal white adipose tissue (A), brown adipose tissue (B), brain (C), lung(D), liver (E), stomach (F), intestine (G), pancreas (H), kidney (I), spleen (J), femur including bone marrow (K), and skeletal muscle of quadriceps (L) of *Plap-1-GFP-2A-CreER* knockin mice. Scale bars: 100 μ m.

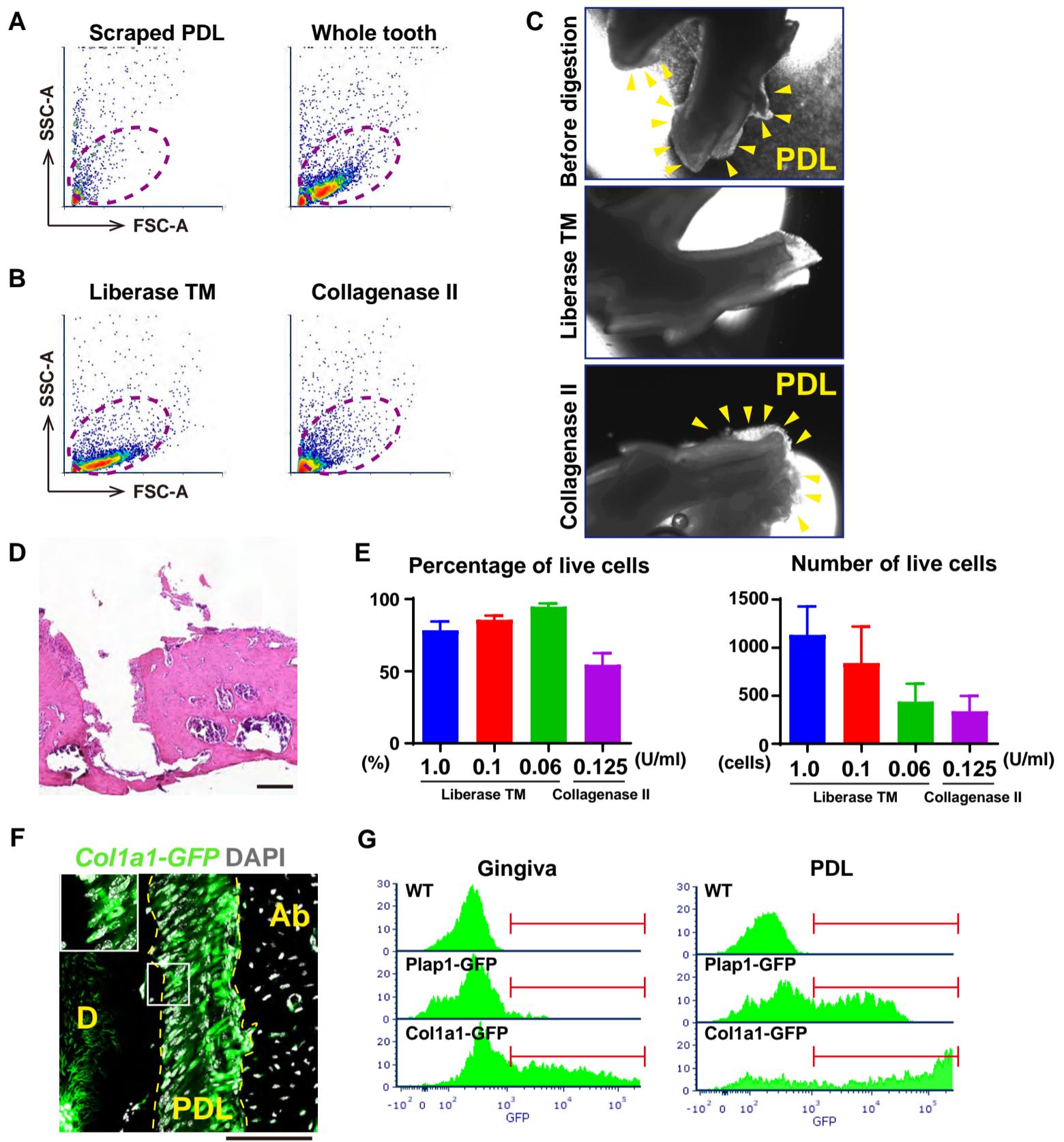


Fig. S3. Development of isolation method for PDL

- A. Representative flow cytometry FSC/SSC plot for digested PDL. As a starting material, scraped PDL and whole teeth were compared.
 - B. Representative flow cytometry FSC/SSC plot for digested PDL. Liberase TM and collagenase II were compared.
 - C. Representative Microscopic images of extracted teeth before and after enzymatic digestion. Complete digestion of PDL by Liberase TM was confirmed. On the contrary, PDL remained on the root surface in collagenase II treated teeth. The yellow arrowheads showed PDL.
 - D. Representative HE staining image of the tooth socket after the extraction. PDL tissue remained on the alveolar bone surface, but cells in the furcation area close to the alveolar crest were mostly removed.
 - E. Ratio and the absolute number of live cells obtained from Liberase TM or collagenase II treatment.
 - F. Representative epifluorescent images of *Col1a1-GFP* mouse.
 - G. Representative flow cytometry GFP histograms from gingiva and PDL of wildtype, *Plap- 1-GFP-2A-CreER*, and *Col1a1-GFP* mice.
- D: Dentin, Ab: Alveolar bone, scale bars: 100 µm.

Fig. S4

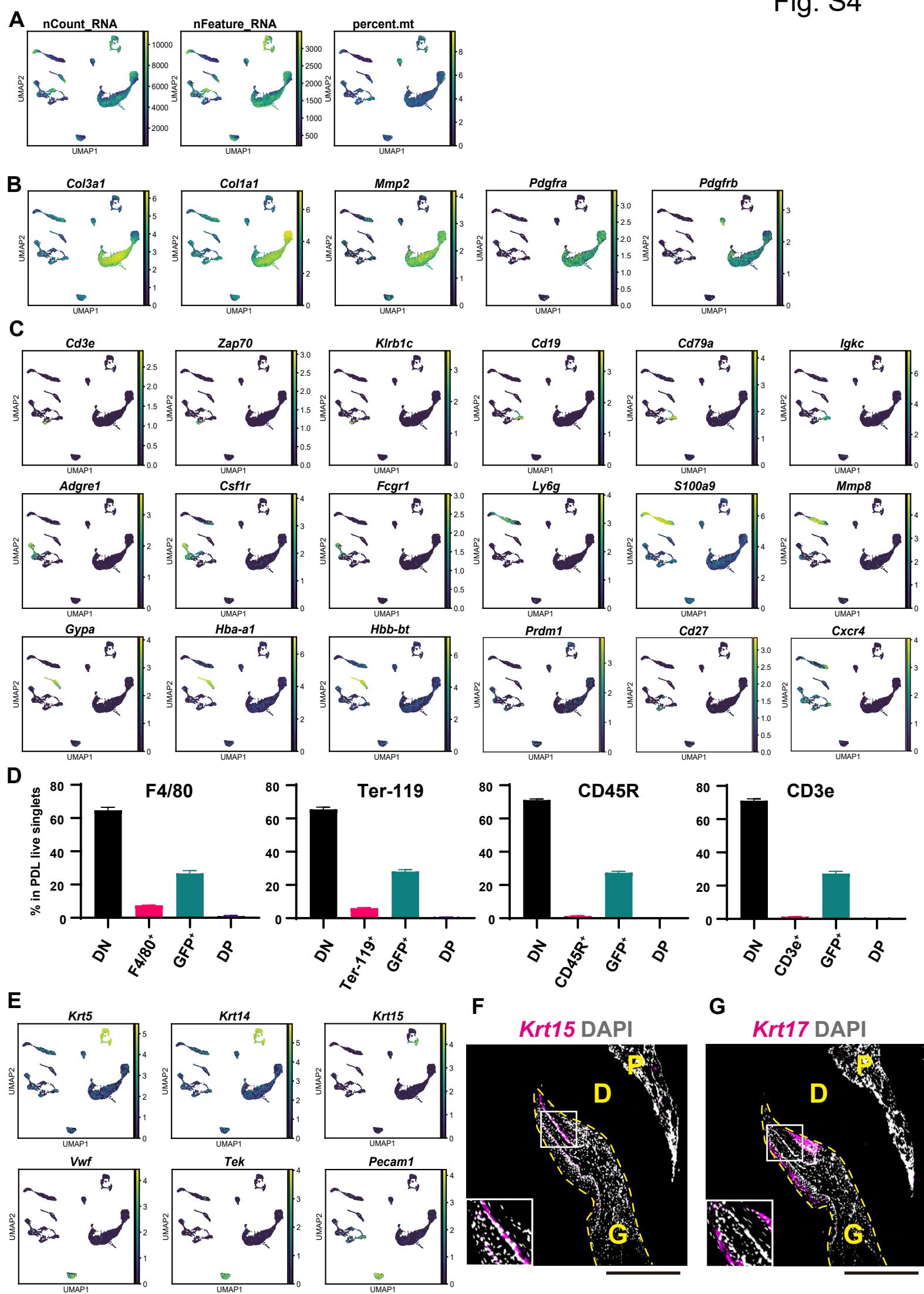


Fig. S4. Clustering analysis of non-stromal PDL-derived cells

- A. UMAP plots representing UMI counts, unique gene numbers, and percentages of mitochondrial genes.
- B. UMAP plots showing expressions of stromal cell marker genes.
- C. UMAP plots showing expressions of immune cell marker genes.
- D. Cellular composition analysis by flow cytometry. Within the PDL-enriched population, F4/80, Ter-119, CD45R, and CD3e-positive cells were quantified (n=3 mice).
- E. UMAP plots showing expressions of epithelial cell marker genes.
- F. Representative *Krt15* mRNA expression in the gingival epithelium. The expression was restricted to the basement membrane of the gingival epithelium.
- G. Representative *Krt17* mRNA expression in the gingival epithelium. The expression was restricted to the junctional epithelium and oral epithelium.
- D: Dentin, Ab: Alveolar bone, scale bars: 200 µm.

Fig. S5

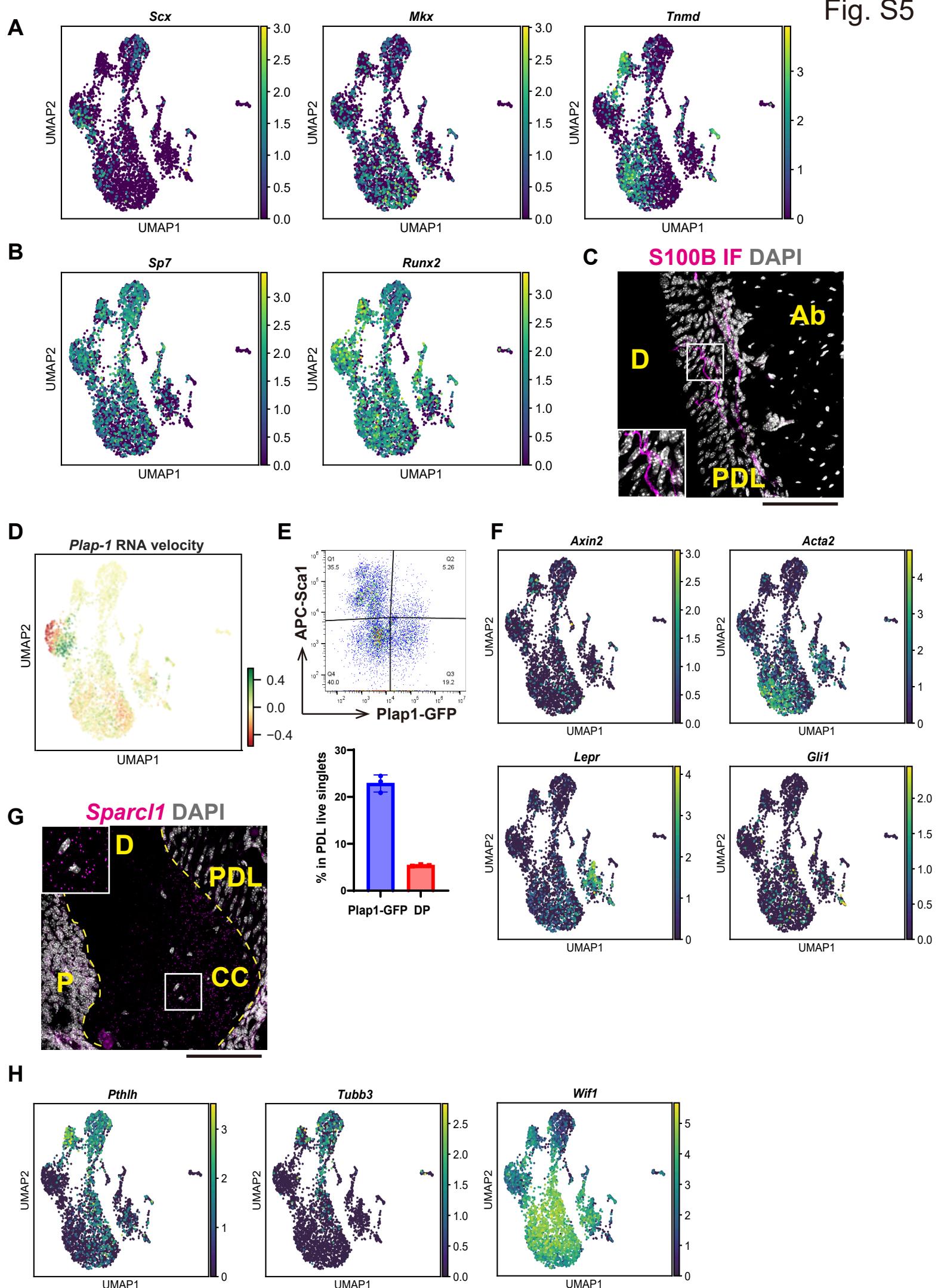


Fig. S5. Clustering analysis of stromal PDL-derived cells

- A. UMAP plots showing expressions of stromal cell marker genes.
- B. *Plap-1* and *Ibsp* expressions overlayed on UMAP plots.
- C. Representative S100B immunofluorescent staining in the PDL.
- D. *Plap-1* RNA velocity analysis overlaid on scRNA-seq UMAP plot.
- E. Representative Sca-1/Plap-1-GFP plot of PDL live singlet cells (left panel). The detailed gating strategy was described in Fig. 3C. The quantification of the Plap-1-GFP (including DP) and DP cells (right panel). DP: double positive cells for Sca-1 and Plap-1-GFP.
- F. UMAP plots showing expressions of putative stem cell marker genes.
- G. Representative *Sparc1* mRNA expression at root apex of the tooth. The expression was restricted to the cellular cementum.
- H. Expressions of potent cementoblast marker genes on UMAP plots.

D: Dentin, Ab: Alveolar bone, P: Pulp, CC: Cellular cementum, scale bars: 100 µm.

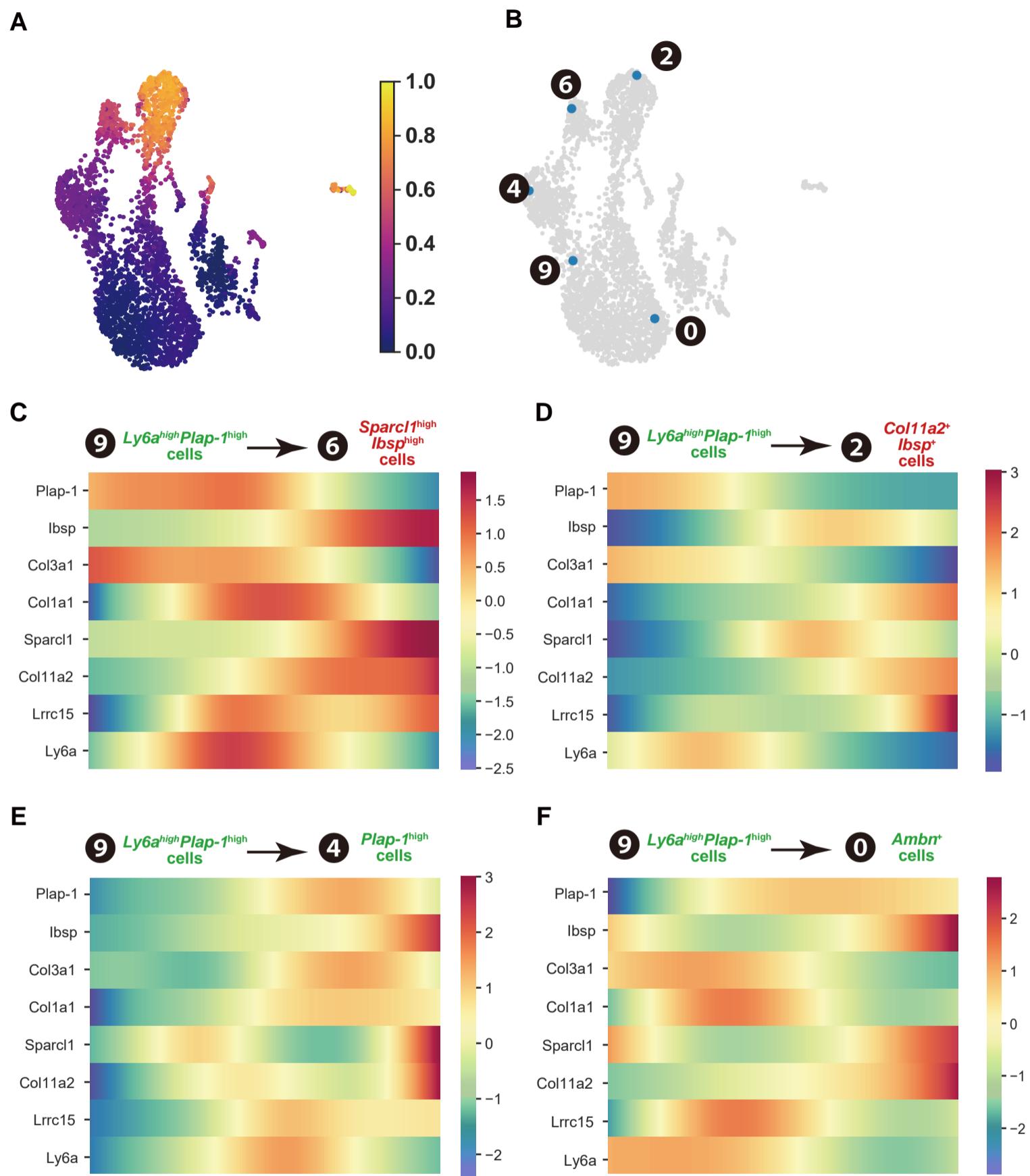


Fig. S6. Pseudotime analyses of PDLSC differentiation

- A. Pseudotime analysis of stromal cells. Each dot in the UMAP plot was colored according to its pseudotime value.
- B. Visualization of the cell used for following trajectory analysis in each cluster (labeled as blue). The cell in cluster 9 was designated as the root node, and others as the terminal nodes.
- C-F. Heatmaps of the expression dynamics of *Plap-1*, *Ibsp*, *Col3a1*, *Col1a1*, *Sparc1*, *Col11a2*, *Lrrc15*, and *Ly6a* through the trajectory.

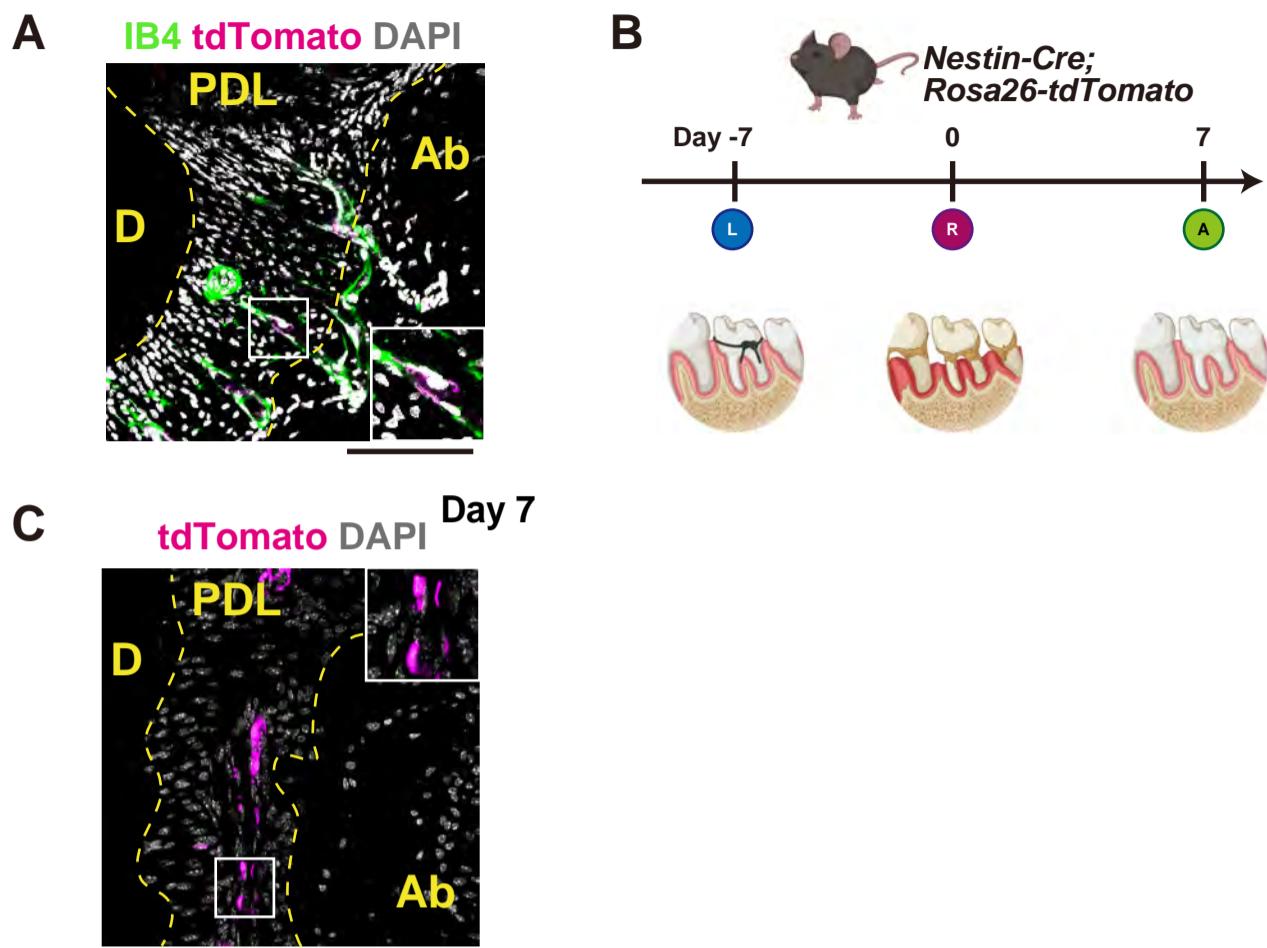


Fig. S7. Quiescent Nes⁺ mural cells in the PDL

- A. Representative Nestin-lineage tracing and GS-IB-4 staining in the PDL of 1-year-old *Nes-Cre; Rosa26-tdTomato* mice. Nes⁺ cells remained perivascular for up to a year.
- B. Overview of ligature-induced periodontitis experiments. *Nes-Cre; Rosa26-tdTomato* mice were subject to ligature placement around the second molar of the maxilla. The suture was removed 7 days after ligature placement (designated as day 0). The repair process was analyzed on days 7. A week after suture removal, periodontal tissue was repaired. L: Ligature placement. R: removal of suture. A: Analysis of the mice.
- C. Representative tdTomato expression in repaired periodontal tissue of *Nes-Cre; Rosa26-tdTomato* mice 7 days after suture removal.
- D: Dentin, Ab: Alveolar bone, scale bars: 100 µm.

Table S1. Reagents used in this study**Genotyping PCR primer sequences**

Plap-1-GFP-2A-CreER1	GAGGTCAAGCCTGCACTTGTAA
Plap-1-GFP-2A-CreER2	GGCGGACTTGAAGAACGTCGT
Plap-1-GFP-2A-CreER3	TTCCTCAGTGCTGTGTGGGA

Amplifying 472bp mutantnt allele, 298bp WT allele

Cre1	TCCAATTACTGACCGTACACCAA
Cre2	CCTGATCCTGGCAATTCCGGCTA
Cre3	CTAGGCCACAGAATTGAAAGATCT
Cre4	GTAGGTGGAATTCTAGCATCATCC

Amplifying 540bp transgene, 324bp WT II2 genome

R26tdTomato1	CTCTGCTGCCTCCTGGCTTCT
R26tdTomato2	CGAGGCGGATCACAAAGCAATA
R26tdTomato3	TCAATGGCGGGGGTCTGTT

Amplifying 322bp wildtype Rosa26, 243bp Rosa26-mTmG

GFP1	GCCACAAGTTCAGCGTGTCC
GFP2	GATGCCCTTCAGCTCGATGC

Amplifying 314bp transgene**Antibodies used in flow cytometry experiments**

Name	Company	Cat#	Isotype	Clone	Dilution
CD45	BioLegend	103105	PE-conjugated Rat IgG2b, κ	30-F11	1:100
CD31	BioLegend	102507	PE-conjugated Rat IgG2a, κ	MEC13.3	1:100
CD326	BioLegend	118205	PE-conjugated Rat IgG2a, κ	G8.8	1:100
CD51	BioLegend	104105	PE-conjugated Rat IgG1, κ	RMV-7	1:100
CD3e	BioLegend	100311	APC-conjugated Armenian Hamster IgG	145-2C11	1:100
CD45R/B220	BioLegend	103211	APC-conjugated Rat IgG2a, κ	RA3-6B2	1:100
F4/80	BioLegend	123115	APC-conjugated Rat IgG2a, κ	BM8	1:100
Ter-119	BioLegend	116211	APC-conjugated Rat IgG2b, κ	TER-119	1:100
Ly6a/Sca-1	BioLegend	108111	APC-conjugated Rat IgG2a, κ	D7	1:100
SYTOX AADvanced	Thermo	S10349			
SYTOX Blue	Thermo	S34857			

Antibodies used in immunofluorescent experiments

Name	Company	Cat#	Host	Dilution
Plap-1	Abcam	ab31303	Goat pAb	1:1,000
GFP	Thermo	A11122	Rabbit pAb	1:1,000
Laminin	Sigma-Aldrich	L9393	Rabbit pAb	1:1,000
Sca-1	BioLegend	108101	Rat pAb	1:1,000
S100B	Abcam	ab52642	Rabbit mAb	1:1,000

Probes used in RNAscope experiments

Gene Name	Cat #	Accession #	Target Region
Col1a1	319371	NM_007742.3	1686 - 4669
Plap-1/Aspn	502051	NM_025711.3	281 - 1315
Aspn-C2	502051-C2	NM_025711.3	281 - 1315
Bgn	455361	NM_007542.4	22 - 894
Ibsp	415501	NM_008318.3	235 - 1238
Spp1	435191	NM_001204201.1	2 - 1079
Col11a2	1077971	NM_001317722.1	112-1312
Sparcl1	424641	NM_010097.4	772 - 1909
Sparc	466781	NM_009242.5	337 - 1185
Krt15	319091	NM_008469.2	364 - 1703
Krt17	479911	NM_010663.3	3 - 1486