Supplementary Materials for

LGR5 regulates sequential tooth development: evidence from single-cell transcriptomics and a gene inactivation model

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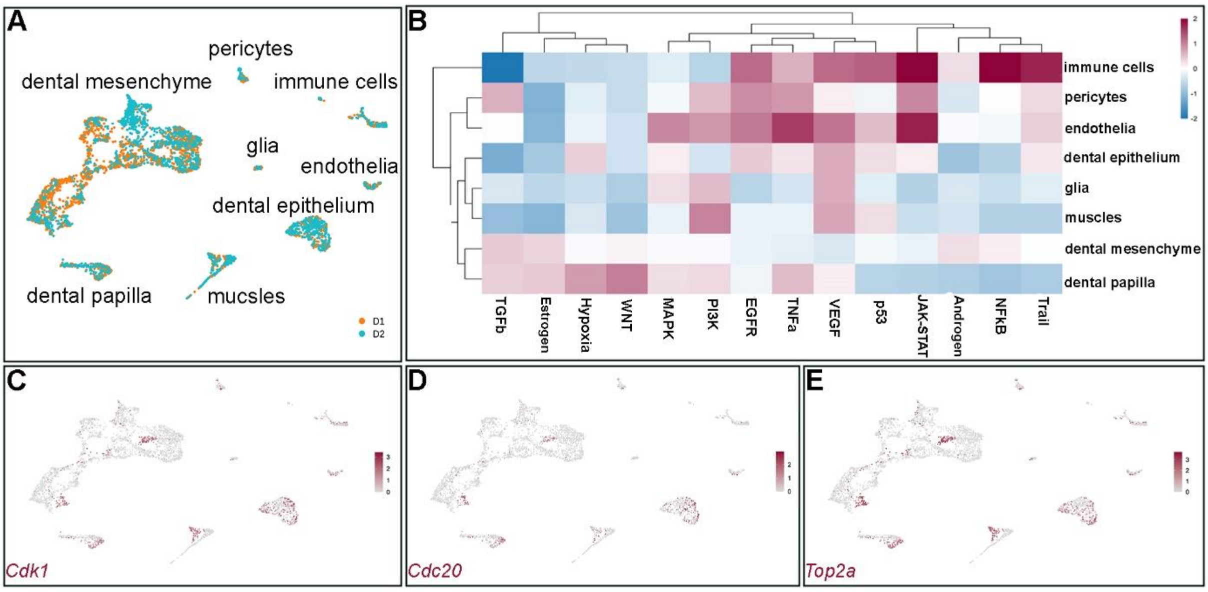
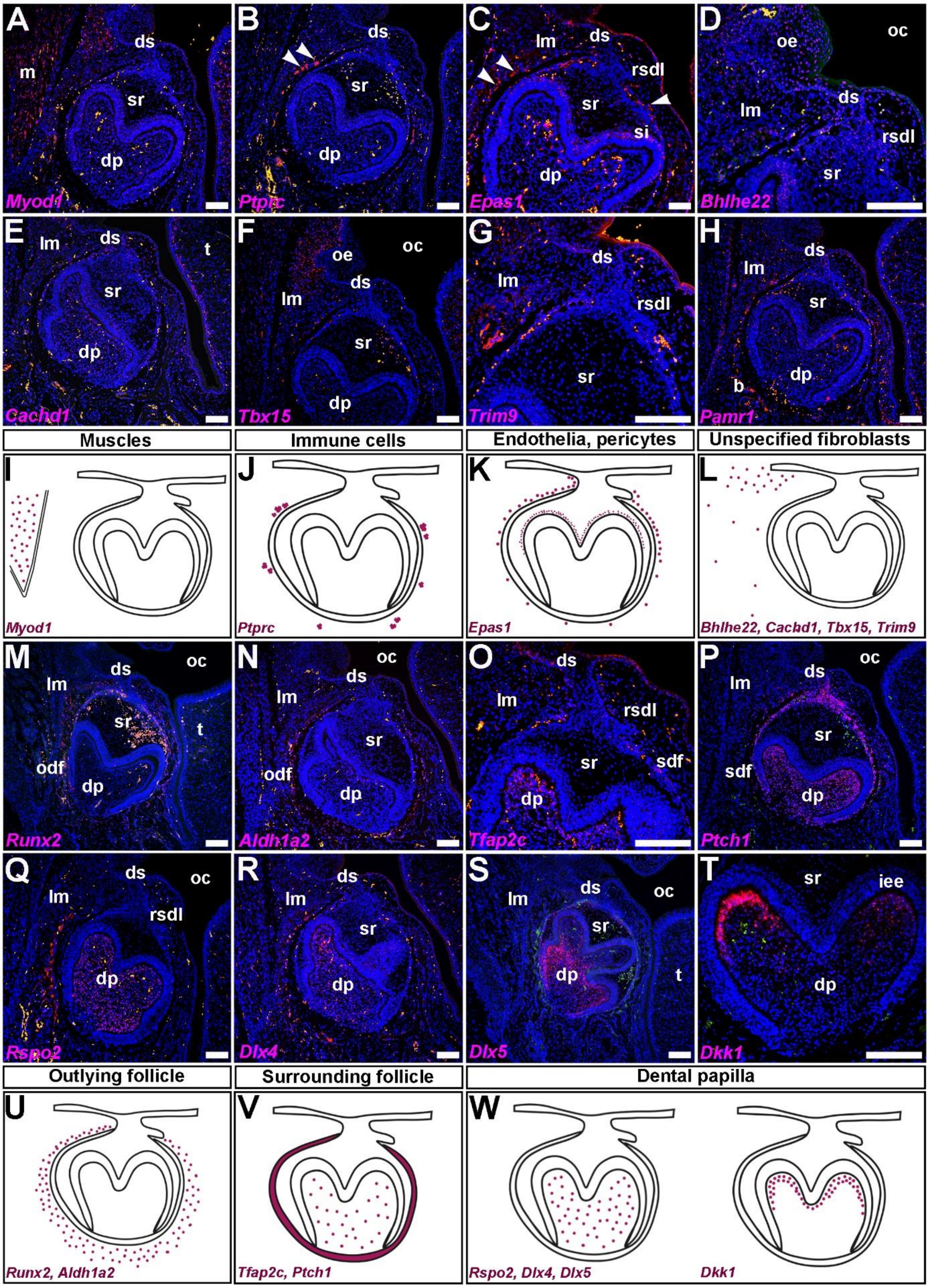


Supplementary Fig. 1 Bioinformatic analysis of single-cell RNA (scRNA-seq) sequencing data from the mouse molar region

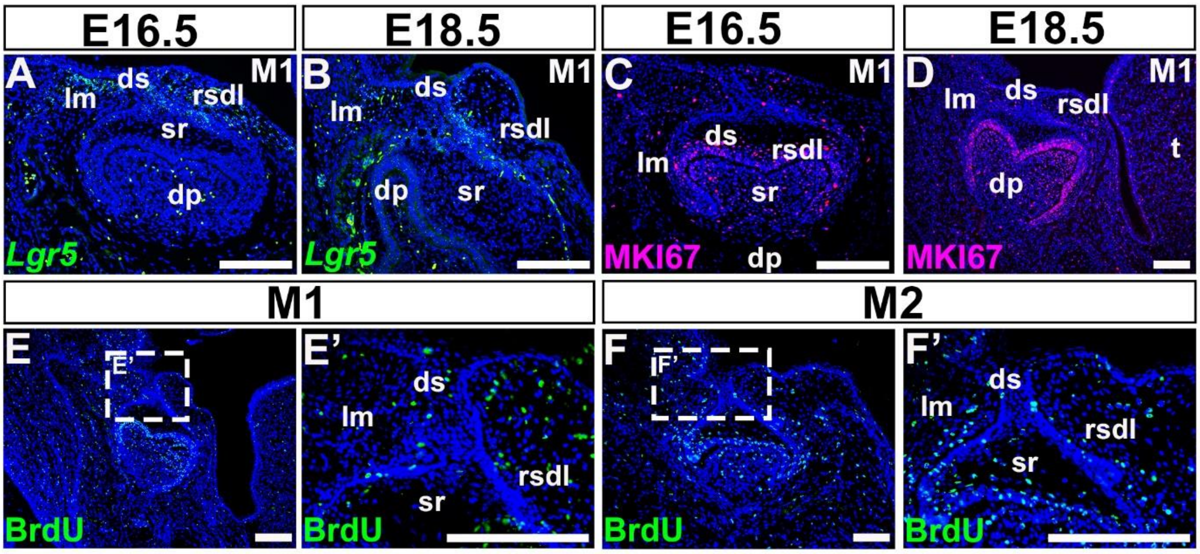
To visualize the overlap in cell populations, two independent scRNA-seq datasets were integrated: Dataset 1 (D1, orange) is enriched for cells from the dental follicle and undifferentiated osteoblast clusters, while Dataset 2 (D2, blue) is enriched for cells from the dental epithelium cluster **(A)**. The heatmap displays differences in the expression of genes involved in major developmental signaling pathways (columns) across individual clusters (rows) **(B)**. Uniform Manifold Approximation and Projection (UMAP) visualizations show the expression patterns of *Cdk1* **(C)**, *Cdc20* **(D)**, and *Top2a* **(E)**.


A
m
ds
sr
dp
Myod1
B
ds
sr
dp
Ptprc
C
lm
ds
rsdl
sr
si
dp
Epas1
D
oe
oc
lm
ds
rsdl
sr
Bhlhe22
E
lm
ds
t
sr
dp
Cachd1
F
oe
oc
lm
ds
sr
dp
Tbx15
G
lm
ds
rsdl
sr
Trim9
H
lm
ds
sr
b
dp
Pamr1
Muscles
Immune cells
Endothelia, pericytes
Unspecified fibroblasts
I
Myod1
J
Ptprc
K
Epas1
L
Bhlhe22, Cachd1, Tbx15, Trim9
M
lm
ds
oc
sr
t
odf
dp
Runx2
N
oc
lm
ds
sr
odf
dp
Aldh1a2
O
ds
lm
rsdl
sr
sdf
dp
Tfap2c
P
lm
ds
oc
sr
sdf
dp
Ptch1
Q
lm
ds
oc
rsdl
dp
Rspo2
R
lm
ds
sr
dp
Dlx4
S
lm
ds
oc
sr
dp
t
Dlx5
T
sr
iee
dp
Dkk1
Outlying follicle
Surrounding follicle
Dental papilla
U
Runx2, Aldh1a2
V
Tfap2c, Ptch1
W
Rspo2, Dlx4, Dlx5
Dkk1

Supplementary Fig. 2 Validation of mesenchymal cell clusters by spatial distribution of marker gene expression

Low-magnification RNA in situ hybridization images illustrate the spatial distribution of marker genes used to define distinct mesenchymal clusters. *Myod1* marks the muscle compartment adjacent to the developing tooth **(A)**, while Ptprc labels immune cell populations **(B)**. *Epas1* expression is enriched in endothelial cells and pericytes **(C)**. The expression of *Bhlhe22*, *Cachd1*, *Trim9*, and *Tbx15* (low magnification shown) delineates the cluster of unspecified oral fibroblasts **(D–G)**. *Pamr1* marks a population of undifferentiated osteoblasts **(H)** Schematic diagrams summarize the spatial localization of each mesenchymal cluster, including muscles **(I)**, immune cells **(J)**, endothelial cells/pericytes **(K)**, and unspecified fibroblasts **(L)**. *Runx2* and *Aldh1a2* are expressed in the outlying dental follicle **(M, N)**, as illustrated in panel **(U)**. The surrounding dental follicle cluster is validated by *Tfap2c* and *Ptch1* expression **(O, P)**, with schematic localization shown in **(V)**. The dental papilla is marked by the expression of *Rspo2, Dlx4, Dlx5*, and *Dkk1* **(Q–T)**, and their mRNA distribution is illustrated in **(W)**.

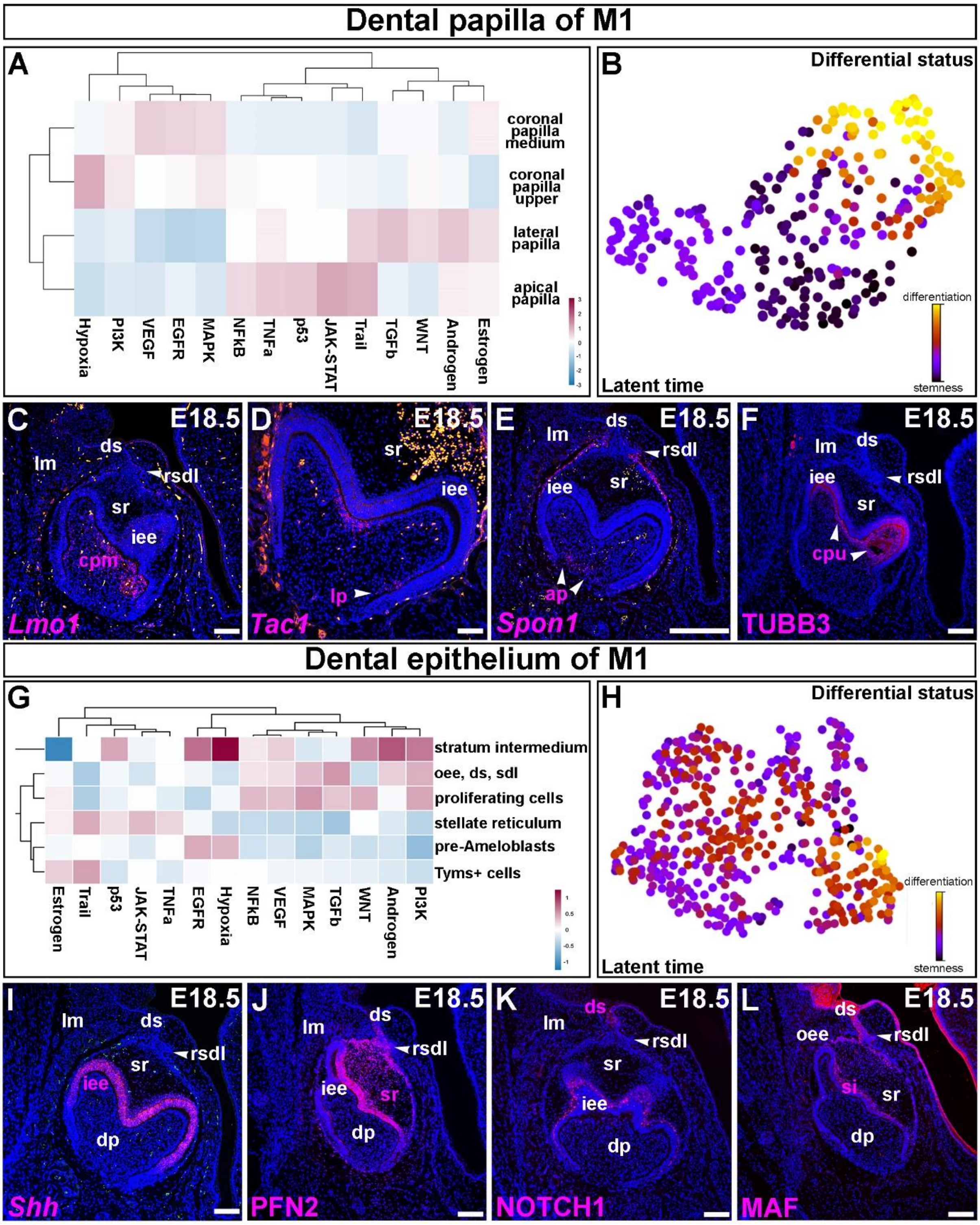
Symbols: b, bone; dp, dental papilla; ds, dental stalk; iee, inner enamel epithelium; lm, labial mesenchyme; m, muscles; oc, oral cavity; odf, outlying dental follicle; oe, oral epithelium; sdf, surrounding dental follicle; si, stratum intermedium; sdl, successional dental lamina; si, stratum intermedium; sr, stellate reticulum; t, tongue; scale bars: 100 μm.



Supplementary Fig. 3 Analysis of Lgr5 expression and cell proliferation in developing mouse molars at embryonic stages E16.5 and E18.5

RNA in situ hybridization reveals *Lgr5* expression in the lingual epithelium of the dental stalk (DS) and in the labial mesenchyme of the first molar (M1) at E16.5 **(A)** and E18.5 **(B)**. Immunohistochemical detection of Ki67 marks proliferating cells in M1 at E16.5 **(C)** and E18.5 **(D)**. A short-term 5-Bromo-2′-Deoxyuridine (BrdU) incorporation assay indicates minimal BrdU uptake in the *Lgr5*-positive regions of the DS and the rudimental successional dental lamina (RSDL) in M1 **(E, E')**. In contrast, robust BrdU labeling is observed in the DS and RSDL of the second molar (M2) **(F, F')**, suggesting higher proliferative activity in M2 compared to M1 during late embryonic development.

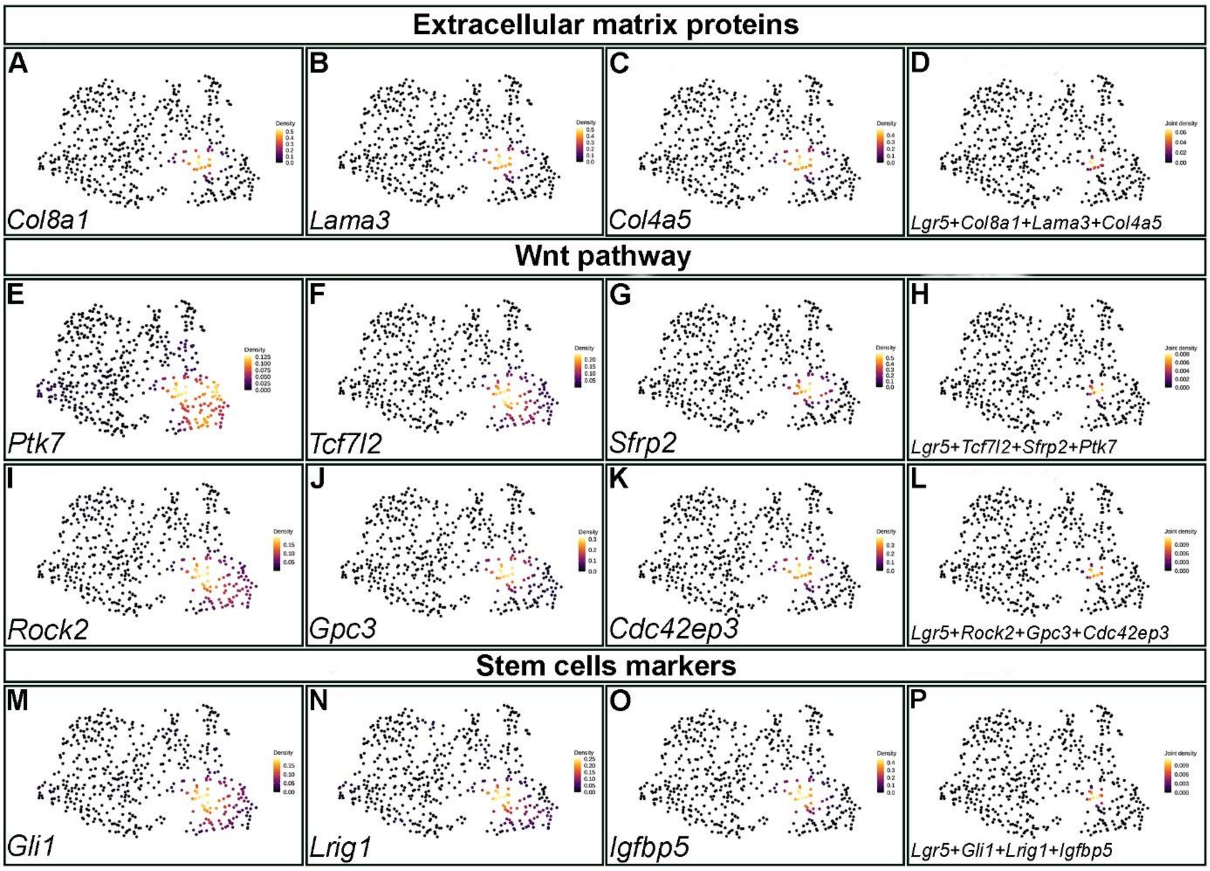
Symbols: dp, dental papilla; ds, dental stalk; lm, labial mesenchyme; rsdl, rudimental successional dental lamina; sr, stellate reticulum; t, tongue; scale bars: 100 μm.


Dental papilla of M1
A
coronal papilla medium
coronal papilla upper
lateral papilla
apical papilla
Hypoxia
PI3K
VEGF
EGFR
MAPK
NFkB
TNFa
p53
JAK-STAT
Trail
TGFb
WNT
Androgen
Estrogen
3
2
1
0
-1
-2
-3
B
Differential status
differentiation
stemness
Latent time
C
E18.5
lm
ds
rsdl
sr
iee
cpm
Lmo1
D
E18.5
sr
iee
lp
Tac1
E
E18.5
lm
ds
rsdl
iee
sr
ap
Spon1
F
E18.5
lm
ds
rsdl
iee
sr
cpu
TUBB3
Dental epithelium of M1
G
stratum intermedium
oee, ds, sdl
proliferating cells
stellate reticulum
pre-Ameloblasts
Tyms+ cells
Estrogen
Trail
p53
JAK-STAT
TNFa
EGFR
Hypoxia
NFkB
VEGF
MAPK
TGFb
WNT
Androgen
PI3K
1
0.5
0
-0.5
-1
H
Differential status
differentiation
stemness
Latent time
I
E18.5
lm
ds
rsdl
sr
iee
dp
Shh
J
E18.5
lm
ds
rsdl
iee
sr
dp
PFN2
K
E18.5
lm
ds
rsdl
sr
iee
dp
NOTCH1
L
E18.5
ds
oee
rsdl
si
sr
dp
MAF

Supplementary Fig. 4 Detailed sub-clustering of the dental papilla and dental epithelium in mouse molars at E18.5

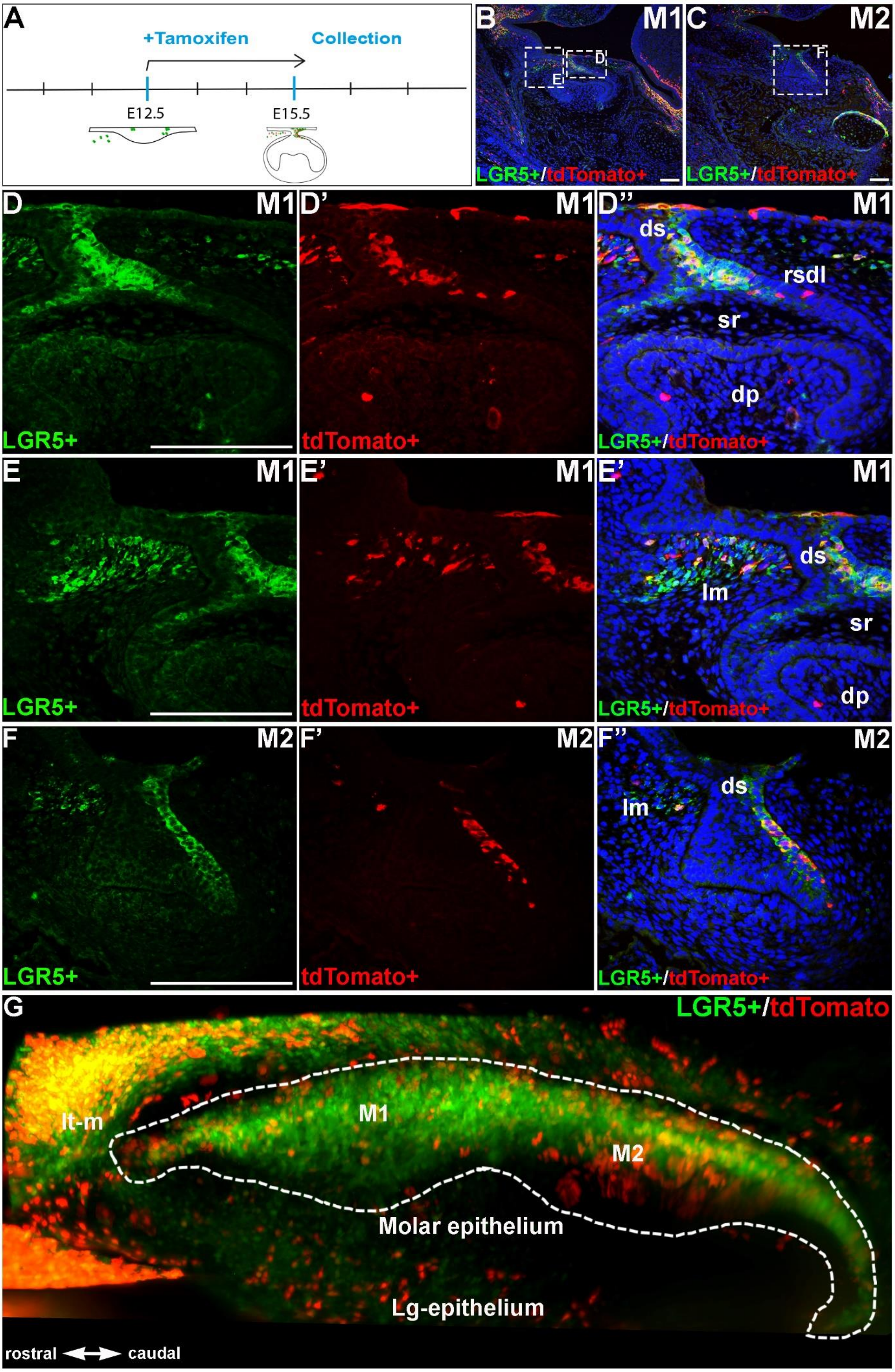
Heatmap showing the expression of the genes linked to indicated signaling pathways (columns) across four transcriptionally defined sub-clusters of the dental papilla **(A)**. Latent time analysis reveals that cells in the coronal papilla (purple) are less differentiated, whereas those in the apical and lateral papilla (yellow) are more differentiated **(B)**. **(C–F)** Validation of dental papilla sub-clusters using marker gene expression. RNA in situ hybridization confirms *Lmo1* expression in the coronal papilla (medium) **(C)**, *Tac1* in the lateral papilla **(D)**, and *Spon1* in the apical papilla **(E)**. Immunohistochemistry for TUBB3 marks the coronal papilla (upper) sub-cluster **(F)**. Heatmap showing the expression of genes linked to key signaling pathways (columns) across six sub-clusters of the dental epithelium **(G)**. Latent time analysis of the dental epithelium highlights a gradient of differentiation from less differentiated (purple) to more differentiated (yellow) cells **(H)**. **(I–L)** Marker gene expression confirms epithelial sub-cluster identities. *Shh* marks the inner enamel epithelium (pre-ameloblasts) **(I)**, PFN2 expression identifies the stellate reticulum sub-cluster **(J)**, NOTCH1 marks a population comprising the DS, RSDL outer enamel epithelium (OEE), and stratum intermedium **(K)**, and MAF is enriched in the stratum intermedium, with additional expression in the DS, RSDL, and OEE **(L)**.

Symbols: ap, apical papilla; cpm, coronal papilla medium; cpu, coronal papilla upper; dp, dental papilla; ds, dental stalk; iee, inner enamel epithelium; lm, labial mesenchyme; lp, lateral papilla; oee, outer enamel epithelium; rsdl, rudimental successional dental lamina; si, stratum intermedium; sr, stellate reticulum; scale bars: 100 μm.



Supplementary Fig. 5 Co-expression of *Lgr5* with genes involved in extracellular matrix organization, Wnt signaling, and stem cell identity in the dental epithelium

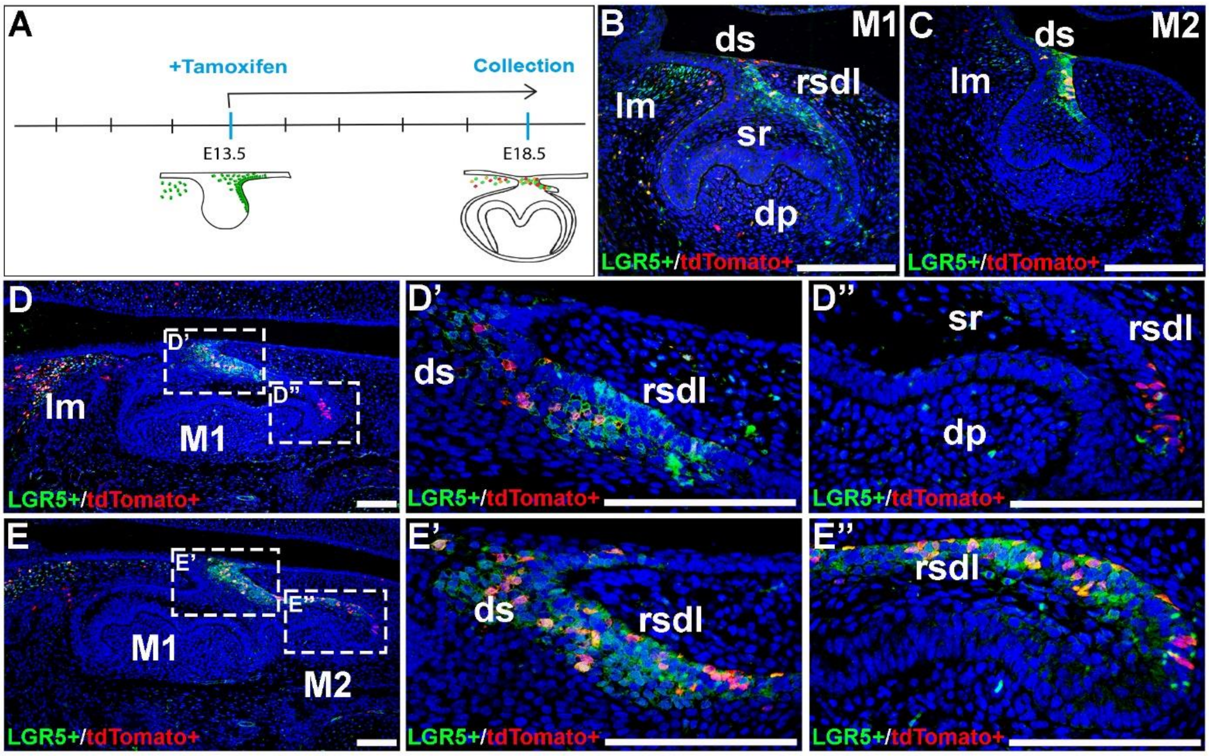
UMAP plots show the spatial expression density of selected genes co-expressed with ***Lgr5*** in the dental epithelium. **Extracellular matrix-associated genes:** Individual expression patterns of *Col8a1* **(A)**, *Lama3* **(B)**, and *Col4a5* **(C)** are visualized, along with their combined co-expression with *Lgr5* **(D)**. **Wnt signaling pathway genes:** UMAPs display the expression of canonical and non-canonical Wnt-associated genes, including *Ptk7* **(E)**, *Tcf7l2* **(F)**, *Sfrp2* **(G)**, *Rock2* **(I)**, *Gpc3* **(J)**, and *Cdc42ep3* **(K)**. Their co-expression with *Lgr5* is shown in panels **(H)** and (L), respectively. **Stem cell-associated markers:** Expression of *Gli1* **(M)**, *Lrig1* **(N)**, and *Igfbp5* **(O)** is shown, with their overlap with *Lgr5* indicated in panel **(P)**. Color scale bars indicate the expression density or joint density (in co-expression plots).


A
+Tamoxifen
Collection
E12.5
E15.5
B
M1
D
E
LGR5+/tdTomato+
C
M2
F
LGR5+/tdTomato+
D
M1
LGR5+
D'
M1
tdTomato+
D''
M1
ds
rsdl
sr
dp
LGR5+/tdTomato+
E
M1
LGR5+
E'
M1
tdTomato+
E'
M1
ds
lm
sr
dp
LGR5+/tdTomato+
F
M2
LGR5+
F'
M2
tdTomato+
F''
M2
ds
lm
LGR5+/tdTomato+
G
LGR5+/tdTomato
lt-m
M1
M2
Molar epithelium
Lg-epithelium
rostral
caudal

Supplementary Fig. 6 Lineage tracing of LGR5$^+$ cells in the developing mouse molars

Schematic timeline showing tamoxifen administration at E12.5 and sample collection at E15.5 in *Lgr5-EGFP-IRES-CreERT2 x Rosa26-tdTomato* reporter mice **(A)**. Transverse sections of M1 and M2 molars at E15.5 showing LGR5$^+$ cells (green, EGFP) and lineage-labeled LGR5-derived (LGR5-D) cells (red, tdTomato) **(B–C)**. **(D–F)** EGFP fluorescence reveals LGR5 expression in the lingual epithelium of the DS and RSDL **(D)**, the labial mesenchyme adjacent to the DS **(E)**, and in both regions of M2 **(F)**. **(D′–F′)** tdTomato$^+$ LGR5-D cells mark the descendants of LGR5$^+$ cells induced at E12.5. These are detected in the RSDL, labial mesenchyme, and DS epithelium of M1 **(D′, E′)** and in the corresponding domains of M2 **(F′)**. **(D″–F″)** Overlay images demonstrating partial co-localization of LGR5$^+$ (EGFP$^+$) and LGR5-D (tdTomato$^+$) cells in the molar region. Whole-mount view of the mandibular molar epithelium stained for EGFP and tdTomato shows the distribution of LGR5$^+$ and LGR5-D cells from M1 to M2, including the lateral and lingual epithelial regions **(G)**.

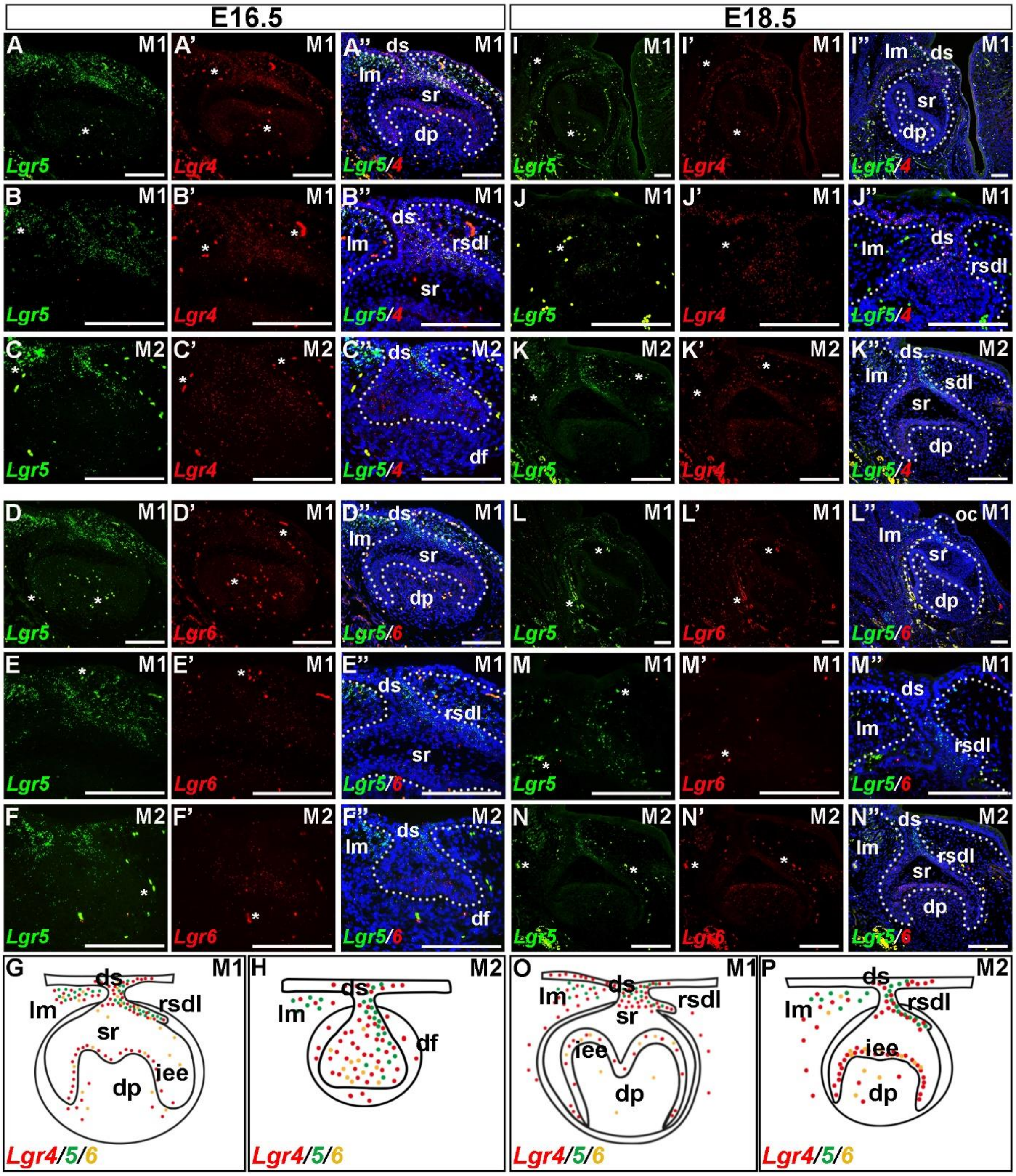
Symbols: dp, dental papilla; ds, dental stalk; Lg-epithelium, lingual epithelium; lm, labial mesenchyme; lt-m, lateral mesenchyme; rsdl, rudimental successional dental lamina; sr, stellate reticulum; scale bars: 100 μm.



Supplementary Fig. 7 Sagittal view of mouse molars following lineage tracing of LGR5$^+$ cells from E13.5 to E18.5

Schematic timeline illustrating the experimental design: tamoxifen was administered at E13.5, and tissues were collected at E18.5 for analysis in *Lgr5-EGFP-IRES-CreERT2 x Rosa26-tdTomato* mice **(A)**. Transverse sections of M1 and M2 at E18.5 show green LGR5$^+$ (EGFP$^+$) cells and red LGR5-derived (tdTomato$^+$) cells. EGFP$^+$ cells indicate active Lgr5 expression at the time of collection, while tdTomato$^+$ cells reflect descendants of LGR5$^+$ cells labeled during the tracing window (E13.5–E18.5) **(B–C)**. Sagittal section of M1 showing EGFP$^+$ cells in the DS epithelium, at the origin of the RSDL, and in the adjacent labial mesenchyme. LGR5-derived (tdTomato$^+$) cells are detected in overlapping domains, including the full length of the RSDL and the labial mesenchyme **(D–D″)**. Additional sagittal sections of M1 and M2 confirm the presence of LGR5$^+$ cells in the DS, RSDL, and labial mesenchyme. Notably, M2 exhibits a higher number of LGR5$^+$ cells within the RSDL compared to M1. LGR5-derived cells are also found in these regions, but in lower numbers, with distribution extending toward the cervical loop of M2 **(E–E″)**.

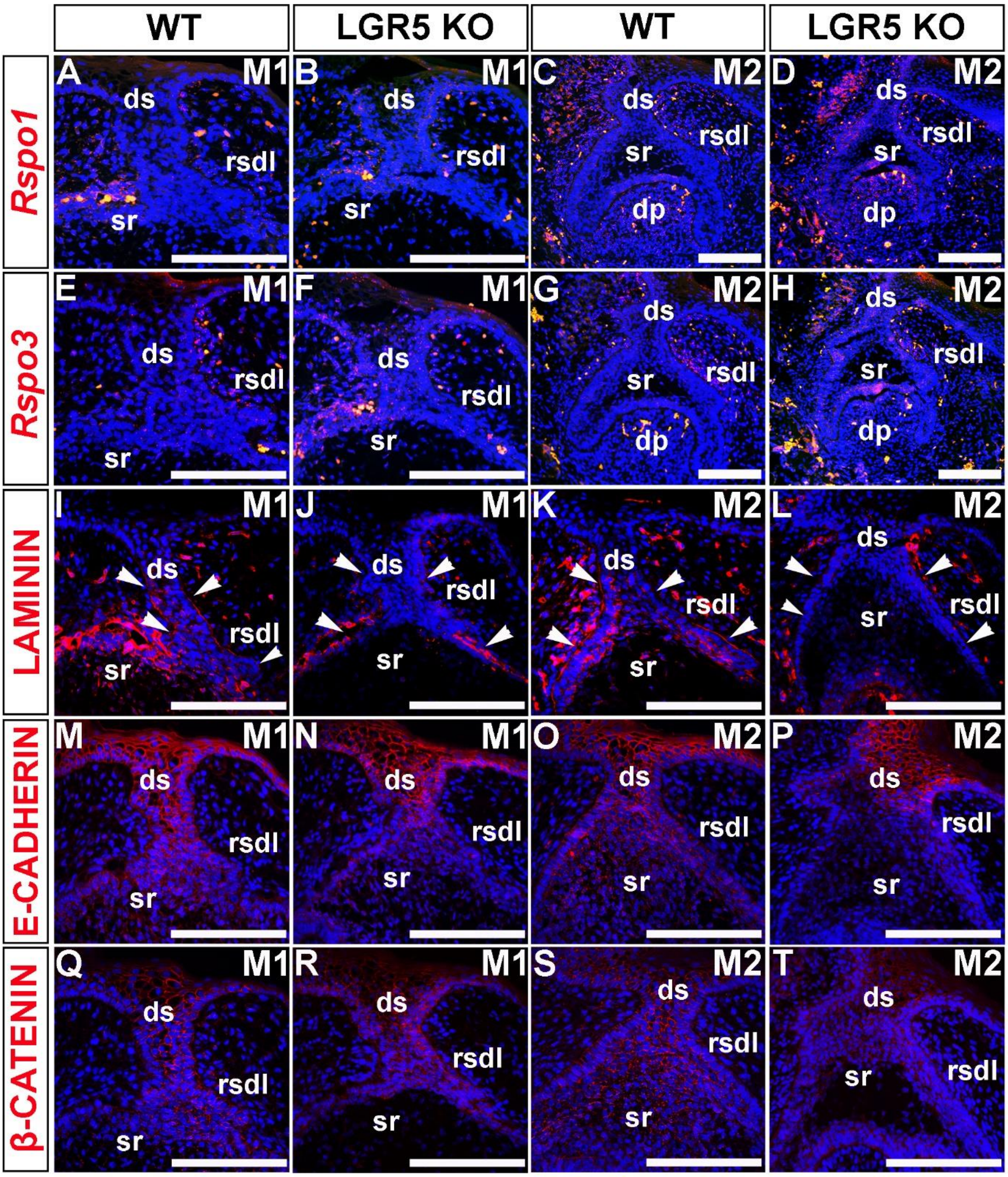
Symbols: dp, dental papilla; ds, dental stalk; lm, labial mesenchyme; rsdl, rudimental successional dental lamina; sr, stellate reticulum; scale bars: 100 µm.


E16.5
E18.5
A M1 Lgr5
A' M1 Lgr4
A'' M1 ds lm sr dp Lgr5/4
I M1 Lgr5
I' M1 Lgr4
I'' M1 lm ds sr dp Lgr5/4
B M1 Lgr5
B' M1 Lgr4
B'' M1 ds lm rsdl sr Lgr5/4
J M1 Lgr5
J' M1 Lgr4
J'' M1 lm ds rsdl Lgr5/4
C M2 Lgr5
C' M2 Lgr4
C'' M2 ds df Lgr5/4
K M2 Lgr5
K' M2 Lgr4
K'' M2 ds lm sdl sr dp Lgr5/4
D M1 Lgr5
D' M1 Lgr6
D'' M1 ds lm sr dp Lgr5/6
L M1 Lgr5
L' M1 Lgr6
L'' oc M1 lm sr dp Lgr5/6
E M1 Lgr5
E' M1 Lgr6
E'' M1 ds rsdl sr Lgr5/6
M M1 Lgr5
M' M1 Lgr6
M'' M1 ds lm rsdl Lgr5/6
F M2 Lgr5
F' M2 Lgr6
F'' M2 ds lm df Lgr5/6
N M2 Lgr5
N' M2 Lgr6
N'' ds M2 lm rsdl sr dp Lgr5/6
G M1 ds lm rsdl sr iee dp Lgr4/5/6
H M2 ds lm df Lgr4/5/6
O M1 lm ds rsdl sr iee dp Lgr4/5/6
P M2 ds lm rsdl iee dp Lgr4/5/6

Supplementary Fig. 8 RNA expression levels of *Lgr4*, *Lgr5*, and *Lgr6* in M1 and M2 at E16.5 and E18.5

Transverse sections of developing molars were analyzed at E16.5 and E18.5 to determine the distributions of *Lgr4*, *Lgr5*, and *Lgr6* transcripts. At E16.5, *Lgr5* expression was predominantly observed on the lingual side of the DS, RSDL, and in the labial mesenchyme of M1 **(A, A″, B, B″, D, D″, E, E″)**. A similar pattern was observed at E18.5 **(I, I″, J, J″, L, L″)**. *Lgr4* displayed a more dispersed expression across the DS and surrounding mesenchyme of M1 at both E16.5 **(A′, A″, B′, B″)** and E18.5 **(I′, I″, J′, J″)**. Co-expression of *Lgr5* and *Lgr4* was evident in the DS and RSDL of M1 at E16.5 **(A″, B″, G)** and E18.5 **(I″, J″, O)**. In M2, *Lgr5* was enriched in the lingual DS and labial mesenchyme at E16.5 **(C, C″, F, F″)** and E18.5 **(K, K″, N, N″)**. *Lgr4* was expressed in both the dental epithelium and mesenchyme of M2 at E16.5 **(C′, C″)** and E18.5 **(K′, K″)**. Overlapping expression of *Lgr5* and *Lgr4* was noted in the DS of M2 at E16.5 **(C″, H)** and E18.5 **(K″, P)**. Multiple *Lgr6*-positive cells were detected in the DS and RSDL of M1 at E16.5 **(D′, D″, E′, E″)** and E18.5 **(L′, L″, M′, M″)**. No co-expression of *Lgr5* and *Lgr6* was found in M1 at either E16.5 **(D″, E″, G)** or E18.5 **(L″, M″, O)**. In M2, *Lgr6* showed only weak expression in the dental epithelium at E16.5 **(F′, F″)** and was sparsely detected in the inner enamel epithelium (IEE), OEE, and dental papilla at E18.5 **(N′, N″)**. No co-expression with *Lgr5* was observed in M2 at either E16.5 **(F″, H)** or E18.5 **(N″, P)**. Asterisks indicate erythrocytes.

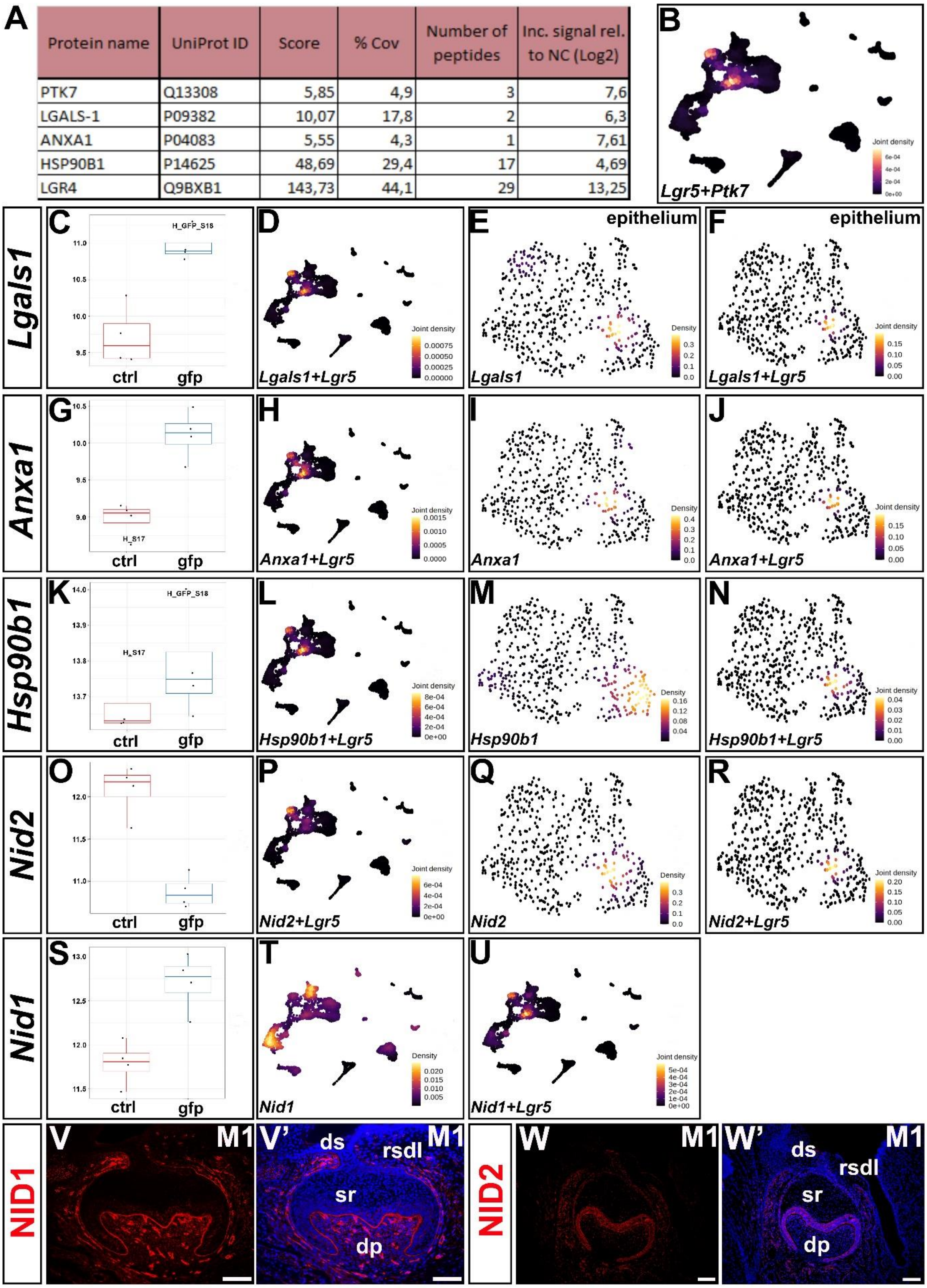
Symbols: df, dental follicle; dp, dental papilla; ds, dental stalk; iee, inner enamel epithelium; lm, labial mesenchyme; oc, oral cavity; oee, outer enamel epithelium; rsdl, rudimental successional dental lamina; sr, stellate reticulum; scale bars: 100 µm.


WT
LGR5 KO
WT
LGR5 KO
Rspo1
Rspo3
LAMININ
E-CADHERIN
β-CATENIN
A M1 ds rsdl sr
B M1 ds rsdl sr
C M2 ds sr rsdl dp
D M2 ds rsdl sr dp
E M1 ds rsdl sr
F M1 ds rsdl sr
G M2 ds sr rsdl dp
H M2 ds rsdl sr dp
I M1 ds rsdl sr
J M1 ds rsdl sr
K M2 ds rsdl sr
L M2 ds sr rsdl
M M1 ds rsdl sr
N M1 ds rsdl sr
O M2 ds rsdl sr
P M2 ds rsdl sr
Q M1 ds rsdl sr
R M1 ds rsdl sr
S M2 ds rsdl sr
T M2 ds sr rsdl

Supplementary Fig. 9 Expression patterns of *Rspo1*, *Rspo2*, LAMININ, E-CADHERIN, β-CATENIN in M1 and M2 molars of *Lgr5*-deficient (LGR5 KO) and wild-type (WT) mouse embryos at E18.5.

RNA in situ hybridization and immunofluorescence analysis was performed to evaluate the localization and expression of selected markers. ***Rspo1*** expression was detected in the DS and RSDL, with similar expression patterns observed in both WT and LGR5 KO molars **(A–D)**. ***Rspo3*** was localized to the mesenchyme on the lingual side of the DS and RSDL in WT molars **(E, G)**. In LGR5 KO embryos, *Rspo3* displayed a more dispersed pattern, with reduced spatial restriction in both the mesenchyme and epithelium of M1 and M2 **(F, H)**. **LAMININ** staining highlighted the basal lamina of the DS and RSDL in WT embryos **(I, K)**. In LGR5 KO molars, this expression was disrupted, showing discontinuities or altered intensity in the basal lamina of both M1 and M2 **(J, L)**. Arrowheads indicate the position of the basal lamina. **E-CADHERIN** was detected in the epithelial layers of the DS, RSDL, and stellate reticulum (SR) in WT molars **(M, O)**. In LGR5 KO tissues, E-CADHERIN expression was reduced or absent in the SR, while it remained detectable in the DS and RSDL **(N, P)**. **β-CATENIN** expression was present in the dental epithelium, including the DS and RSDL, in both WT and LGR5 KO molars, with no obvious differences in expression patterns **(Q–T)**.

Symbols: ds, dental stalk; rsdl, rudimental successional dental lamina; sr, stellate reticulum; scale bars: 100 μm.

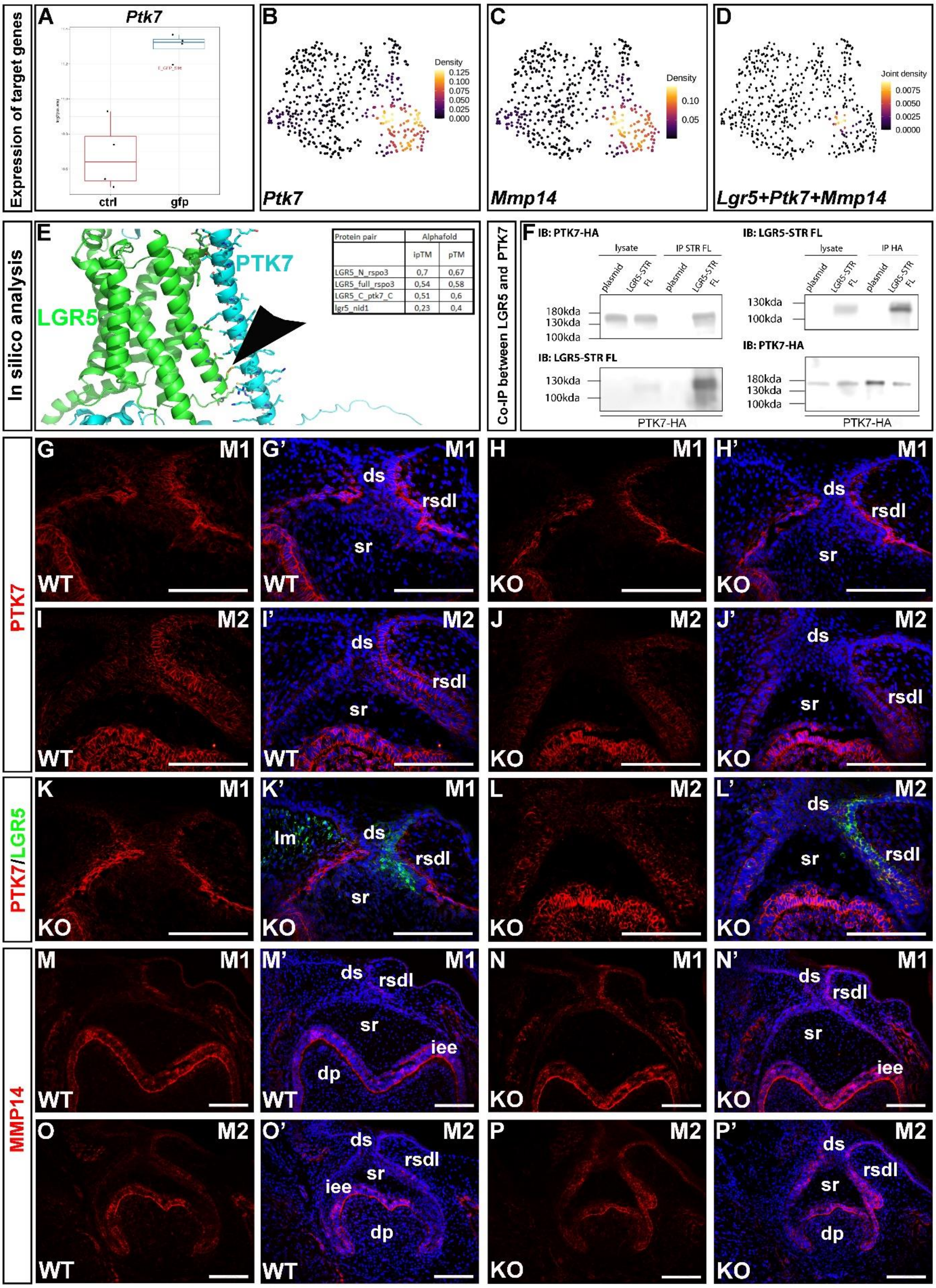


Protein name	UniProt ID	Score	% Cov	Number of peptides	Inc. signal rel. to NC (Log2)
PTK7	Q13308	5,85	4,9	3	7,6
LGALS-1	P09382	10,07	17,8	2	6,3
ANXA1	P04083	5,55	4,3	1	7,61
HSP90B1	P14625	48,69	29,4	17	4,69
LGR4	Q9BXB1	143,73	44,1	29	13,25

Supplementary Fig. 10 Identification and spatial expression of candidate LGR5-binding partners in the mouse molar epithelium.

A table summarizes selected candidate LGR5-interacting proteins identified by mass spectrometry, including UniProt IDs, peptide scores, coverage, and relative signal intensities **(A)**. A joint density UMAP shows spatial co-expression of *Lgr5* and *Ptk7* across the molar tissue **(B)**. Box plots display differential expression of selected candidate genes—*Lgals1*, *Anxa1*, *Hsp90b1*, *Nid2*, and *Nid1*—between control and *Lgr5*-EGFP-positive cells **(C, G, K, O, S)**. Joint density UMAPs illustrate spatial co-expression of *Lgr5* with *Lgals1*, *Anxa1*, *Hsp90b1*, *Nid2*, and *Nid1* in the molar region **(D, H, L, P, T)**. UMAPs show the individual expression patterns of *Lgals1*, *Anxa1*, *Hsp90b1*, *Nid2*, and *Nid1* across the molar epithelium **(E, I, M, Q, U)**. Co-expression of each candidate gene with *Lgr5* in the epithelial compartment is shown in separate joint density plots **(F, J, N, R)**. Immunofluorescence staining indicates that NID1 protein localizes to the basal lamina of M1 at E18.5 **(V, V')**. NID2 is expressed in the IEE and OEE, with weaker expression in the DS and RSDL of M1 at E18.5 **(W, W')**.

Symbols: dp, dental papilla; ds, dental stalk; rsdl, rudimental successional dental lamina; sr, stellate reticulum; scale bars: 100 μm.

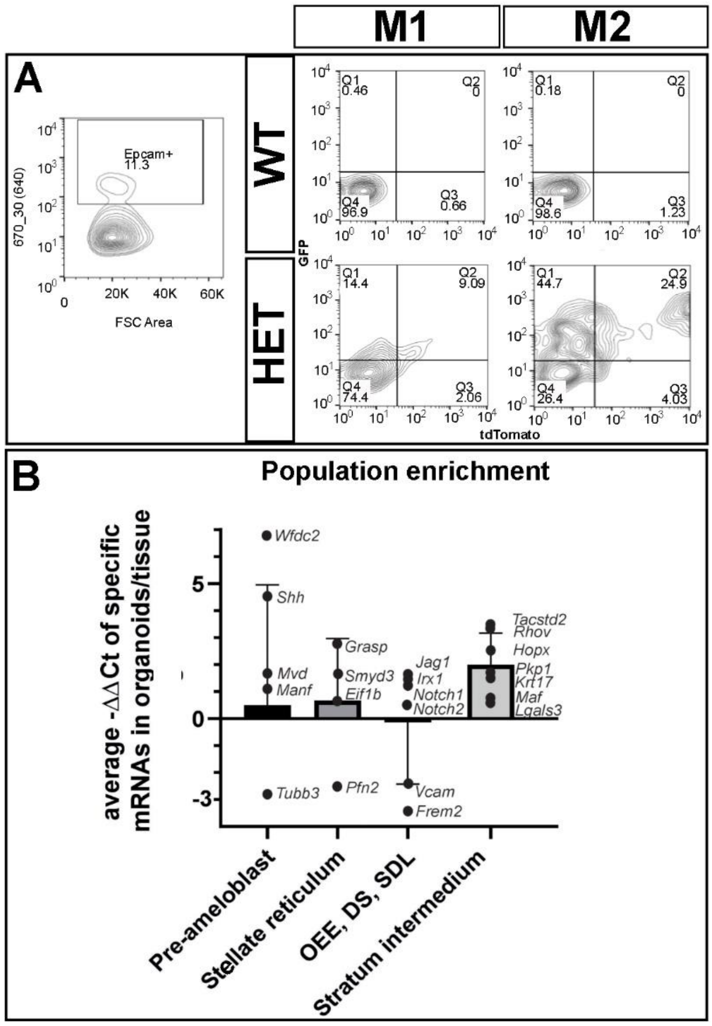


Protein pair	Alphafold	
	ipTM	pTM
LGR5_N_rspo3	0,7	0,67
LGR5_full_rspo3	0,54	0,58
LGR5_C_ptk7_C	0,51	0,6
lgr5_nid1	0,23	0,4

Supplementary Fig. 11 LGR5 interacts with PTK7 and regulates its expression in developing mouse molars

A box plot shows differential gene expression of *Ptk7* between EGFP-positive and control cells **(A)**. Density plots illustrate the spatial expression of *Ptk7* and *Mmp14,* as well as their overlap with *Lgr5* in the dental epithelial sub-cluster **(B–D)**. An *in silico* structural model predicts an interaction between LGR5 and PTK7, with the arrowhead marking the likely contact site in the extracellular/transmembrane region. The table displays the global accuracy of 3D structure prediction for the interaction of LGR5 and its selected partners by the Alphafold 3 server. The accuracy of prediction is described by PTM and ipTM values and shows that the best accuracy of prediction was achieved for N terminal (without intracellular part) of Lgr5 with Rspo3 **(E)**. Co-immunoprecipitation (co-IP) assays confirm the interaction between full-length LGR5 tagged with STREP-FLAG (STR FL) and PTK7 tagged with hemagglutinin (HA). LGR5 was used as bait, and PTK7 detected with anti-HA (upper left), with co-IP efficiency validated by FLAG detection of LGR5 (lower left). Conversely, PTK7 was used as bait, and LGR5 detected with anti-FLAG (upper right), with validation via HA detection of PTK7 (lower right) **(F)**. Immunofluorescence staining shows PTK7 expression in the mesenchyme surrounding the DS, RSDL, and IEE in M1 of WT embryos **(G, G')**. A reduced PTK7 signal is observed in M1 of *Lgr5*-deficient embryos **(H, H')**. In M2, PTK7 is detected in the epithelium and mesenchyme of the DS, RSDL, and IEE in both WT and LGR5 KO samples **(I–J')**. No clear colocalization of LGR5 and PTK7 is seen in M1 **(K, K')**; however, co-expression is evident in the lingual epithelium of the DS and RSDL in M2 **(L, L')**. MMP14 is expressed in the epithelium of the DS and RSDL in both M1 and M2 of WT and mutant embryos **(M-P**'**)**.

Symbols: dp, dental papilla; ds, dental stalk; iee, inner enamel epithelium; lm, labial mesenchyme; M1, first molar; M2, second molar; rsdl, rudimental successional dental stalk; sr, stellate reticulum; scale bar: 100µm.



Supplementary Fig. 12 Molar epithelium used for establishing organoids contains Lgr5-positive cells, but corresponding subpopulation is being lost during cultivation

Epithelial cells (EpCAM$^+$) were isolated from M1 and M2 of Lgr5-EGFP-IRES-CreERT2 x Rosa26-tdTomato embryos 6 days after tamoxifen induction and analyzed by FACS for EGFP and tdTomato expression before embedding in Matrigel **(A)**. After several passages, gene expression in M1- and M2-derived organoids was compared to freshly isolated molar epithelium by analyzing representative markers from different epithelial subpopulations identified by scRNA-seq using quantitative PCR **(B)**. To estimate the relative enrichment of individual subpopulations in the organoids, the average expression changes of specific marker genes were calculated and presented as -ΔΔCt values (first normalized to *Actb*, and then to gene expression in the starting tissue). While the genes associated with the preameloblasts, stellate reticulum, and stratum intermedium were differentially enriched, the subpopulation comprising the OEE, DS, and rudimental successional dental lamina (RSDL) — which includes the Lgr5$^+$ cells — was the only group that was not enriched in the organoids **(B)**. The individual dots show the average expression changes of each gene within the indicated subpopulation.

Supplemental Table 1 List of Abbreviations

ALDH1A2	Aldehyde Dehydrogenase 1 Family Member A2
ALPL	Alkaline Phosphatase, Biomineralization Associated
AMELX	Amelogenin X-Linked
ANXA1	Annexin A1
AP	Apical Papilla
ARID1A	AT-Rich Interaction Domain 1A
ASPRV1	Aspartic Peptidase Retroviral Like 1
ASPN	Asporin
AXIN2	Axin-related protein 2
BHLHE22	Basic Helix-Loop-Helix Family Member E22
BIRC5	Baculoviral IAP Repeat Containing 5
BMP	Bone Morphogenetic Proteins
BrdU	Bromodeoxyuridine
BSA	Bovine Serum Albumin
C1qA	Complement C1q A Chain
CACHD1	Cache Domain Containing 1
CAR2	Carbonic Anhydrase 2
CCNB2	Cyclin B2
CDC20	Cell Division Cycle 20
CDC42EP3	CDC42 Effector Protein 3
CDH1	Cadherin 1
CDK1	Cyclin Dependent Kinase 1
cDNA	complementary Deoxyribonucleic Acid
CLDN5	Claudin 5
Col1A1	Collagen Type I Alpha 1 Chain
Col4A5	Collagen Type IV Alpha 5 Chain
Col8A1	Collagen Type VIII Alpha 1 Chain
Col17	Collagen Type XVII
CPm	Coronal Papilla medium
CPu	Coronal Papilla upper
CRYM	Crystallin Mu
CUBIC	Clear, Unobstructed Brain Imaging Coctails
DAPI	4‘,6-diamidino-2‘-phenylindole, dyhydrochloride
DAPL1	Death Associated Protein Like 1
DESCs	Dental epithelial stem cells
DKK1	Dickkopf WNT Signaling Pathway Inhibitor 1
DL	Dental lamina
DLX4/5	Distal-Less Homeobox 4/5
DMEM/F12	Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-DNA
DNA	Deoxyribonucleic Acid
DP	Dental Papilla
DS	Dental Stalk
DSC	Desmocollin
DSP	Dentin sialoprotein

E	Embryonic stage
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGFP	Green Fluorescent Protein
EGFR	Epidermal Growth Factor Receptor
EIF1B	Eukaryotic Translation Initiation Factor 1B
EMB	Embigin
ENAM	Enamelin
EPAS1	Endothelial PAS Domain Protein 1
EPCAM	Epithelial Cell Adhesion Molecule
ESRP1	Epithelial Splicing Regulatory Protein 1
FABP7	Fatty Acid Binding Protein 7
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal Bovine Serum
FERMT1	FERM Domain Containing Kindlin 1
FGF3	Fibroblast Growth Factor 3
FREM2	Fras1 Related Extracellular Matrix 2
FXYD3	FXYD Domain Containing Ion Transport Regulator 3
GAS6	Growth Arrest Specific 6
GFRA1/3	GDNF Family Receptor Alpha 1/3
GLI1	GLI Family Zinc Finger 1
GPC3	Glypican 3
HA	Hemagglutinin
HBBS	Hanks‘ Balanced Salt Solution
HET	Heterozygot
HIGD1B	HIG1 Hypoxia Inducible Domain Family Member 1B
HOPX	HOP Homeobox
HSP90B1	Heat Shock Protein 90 Beta Family Member 1
IEE	Inner Enamel Epithelium
IGF1	Insulin Like Growth Factor 1
IGFBP5	Insulin Like Growth Factor Binding Protein 5
JAG2	Jagged Canonical Notch Ligand 2
JAK/STAT	The Janus Kinase/Signal Transducer and Aktivator of Transcription
KI67	Marker Of Proliferation Ki-67
KLK4	Kallikrein Related Peptidase 4
KRT5/7/14/17	Keratin 5/7/14/17
KO	Knockout
LAMA3/5	Laminin Subunit Alpha 3/5
LAMB3	Laminin Subunit Beta 3
LAMC2	Laminin Subunit Gamma 3
LARS2	Leucyl-TRNA Synthetase 2
LCL	Labial cervical loop
LEF1	Lymphoid Enhancer Binding Factor 1
LGALS1	Galectin 1
LGR4	Leucine Rich Repeat Containing G Protein-Coupled Receptor 4
LGR5	Leucine Rich Repeat Containing G Protein-Coupled Receptor 5
LGR5-D	*Lgr5*-descendant
LGR6	Leucine Rich Repeat Containing G Protein-Coupled Receptor 6

LHX6	LIM Homeobox 6
LMO1	LIM Domain Only 1
LP	Lateral Papilla
LRIG1	Leucine Rich Repeats And Immunoglobulin Like Domains 1
LRP6	LDL Receptor Related Protein 6
M1	First Molar
M2	Second Molar
M3	Third Molar
MAF	MAF BZIP Transcription Factor
MAPK	Mitogen Activated Protein Kinases
MEIS2	Meis Homeobox 2
MFAP5	Microfibril Associated Protein 5
MGP	Matrix Gla Protein
MKi67	Marker Of Proliferation Ki-67
MMP14	Matrix Metallopeptidase 14
mRNA	messenger Ribonucleic Acid
MSX1	Msh Homeobox 1
MYF5	Myogenic Factor 5
MYOD1	Myogenic Differentiation 1
NFκB	Nuclear Factor kappa B
NID1	Nidogen 1
NID2	Nidogen 2
NOTCH1	Notch Receptor 1
NOTCH2	Notch Receptor 2
NPNT	Nephronectin
oDF	outlying Dental Follicle
OEE	Outer Enamel Epithelium
P	Postnatal stage
PAMR1	Peptidase Domain Containing Associated With Muscle Regeneration 1
PBS	Phosphate-Buffered Saline
PCA	Principal Component Analysis
PCLAF	PCNA Clamp Associated Factor
PFA	Paraformaldehyde
PFN2	Profilin 2
PIEZO2	Piezo Type Mechanosensitive Ion Channel Component 2
PITX2	Paired Like Homeodomain 2
PI3K	Phosphoinositide 3-kinase
PKP3	Plakophilin 3
PLVAP	Plasmalemma Vesicle ASsociated Protein
POSTN	Periostin
pre-Am	pre-Ameloblasts
PTCH1	Patched 1
PTK7	Protein Tyrosine Kinase 7
PTPRC	Protein Tyrosine Phosphatase Receptor Type C
RAB25	RAB25, Member RAS Oncogene Family
RFP	Red Fluorescent Protein
RGS3/5	Regulator Of G Protein Signaling 3/5
RHOV	Ras Homolog Family Member V

RNA	Ribonucleic Acid
ROCK2	Rho Associated Coiled-Coil Containing Protein Kinase 2
ROR2	Receptor Tyrosine Kinase Like Orphan Receptor 2
RSDL	Rudimental successional dental lamina
RSPO1	Roof Plate-Specific Spondin-1
RSPO2	Roof Plate-Specific Spondin-2
RSPO3	Roof Plate-Specific Spondin-3
RT	Room Temperature
RT-qPCR	Reverse Transcription quantitative Polymerase Chain Reaction
RUNX2	RUNX Family Transcription Factor 2
S100A14	S100 Calcium Binding Protein A14
SCUBE1	Signal Peptide, CUB Domain And EGF Like Domain Containing 1
SCT	Secretin
sc-RNAseq	single-cell RNA sequencing
sDF	surrounding Dental Follicle
SDL	Successional Dental Lamina
SeDL	Sequential Dental Lamina
SERPINB5	Serpin Family B Member 5
SFN	Stratifin
SFRP2	Secreted Frizzled Related Protein 2
SHH	Sonic Hedgehog Signaling Molecule
SI	Stratum Intermedium
SMYD3	SET And MYND Domain Containing 3
SNAI2	Snail Family Transcriptional Repressor 2
SOSTDC1	Sclerostin Domain Containing 1
SOX2/6/10	SRY-Box Transcription Factor 2
SPINT2	Serine Peptidase Inhibitor, Kunitz Type 2
SPON1	Spondin 1
SR	Stellate Reticulum
TAC1	Tachykinin Precursor 1
TACSTD2	Tumor Associated Calcium Signal Transducer 2
TBX15	T-Box Transcription Factor 15
TCEA3	Transcription Elongation Factor A3
TCF7L2	Transcription Factor 7 Like 2
TFAP2B	Transcription Factor AP-2 Beta
TFAP2C	Transcription Factor AP-2 Gamma
TGFβ	Transforming Growth Factor beta
TNFα	Tumor Necrosis Factor alpha
TNMD	Tenomodulin
TOP2A	DNA Topoisomerase II Alpha
TRAIL	Tumour necrosis factor (TNF)-Related Apoptosis-Inducing Ligand
TTN	Titin
TUBB3	Tubulin Beta 3 Class III
TWIST1	Twist Family BHLH Transcription Factor 1
TYMS	Thymidylate Synthetase
TYROBP	Transmembrane Immune Signaling Adaptor TYROBP
UMAP	Uniform Manifold Approximation and Projection
UNG	Uracil DNA Glycosylase

VCAN	Versican
VEGF	Vascular Endothelial Growth Factor
WFDC2	WAP Four-Disulfide Core Domain 2
VIM	Vimentin
Wnt	Wingless-related integration site
WNT10A	Wnt Family Member 10A
WT	Wild Type

Supplemental Table 2 List of Antibodies used for IHC

Primary Antibody	**Dilution**	**Cat. Number**	**Company**
Beta-catenin	1:100	8480	Cell Signaling Technology (USA)
BrdU	1:30	B8434	Sigma Aldrich (USA)
c-Maf	1:100	ab243901	Abcam (UK)
E-cadherin	1:30	ab15148	Abcam (UK)
GFP	1:200	ab19970	Abcam (UK)
Ki67	1:100	RBK027	Zytomed systes (Germany)
laminin	1:500	Z0097	Dako, Agilent (Denmark)
MMP14	1:100	PA5-104459	Invitrogen (USA)
Nid1	1:100	ab254325	Abcam (UK)
Nid2	1:100	ab14513	Abcam (UK)
Notch1	1:100	4380P	Cell Signaling Technology (USA)
Notch2	1:100	4530T	Cell Signaling Technology (USA)
Pfn2	1:100	PA5-79812	Invitrogen (USA)
Ptk7	1:200	17799-1-AP	Thermo Fisher Scientific (USA)
RFP	1:200	600-401-379	Rockland (USA)
Sox2	1:100	2748S	Cell Signaling Technology (USA)
Tubb3	1:100	801201	Biolegend (USA)
vimentin	1:100	sc-73259	Santa Cruz (USA)
Secondary Antibody	Dilution	Cat. Number	Company
Alexa Fluor® 488 goat anti-chicken IgG (H+L)	1:200	A11039	Life Technologies (USA)
Alexa Fluor® 488 goat anti-mouse IgG (H+L)	1:200	A11001	Life Technologies (USA)
Alexa Fluor® 594 goat anti-rabbit IgG (H+L)	1:200	A11037	Life Technologies (USA)
Alexa Fluor® 568 goat anti-mouse IgG (H+L)	1:200	A11004	Life Technologies (USA)

Supplemental Table 3 List of probes used for RNAScope

Probe	Dilution	Cat. Number	Company
Aldh1a2		447391	Advanced Cell Diagnostics (USA)
Axin2	1:50	400331	Advanced Cell Diagnostics (USA)
Bhlhe22		467641	Advanced Cell Diagnostics (USA)
Cachd1		558751	Advanced Cell Diagnostics (USA)
Dkk1		402521	Advanced Cell Diagnostics (USA)
Dlx4		575531	Advanced Cell Diagnostics (USA)
Dlx5		478151	Advanced Cell Diagnostics (USA)
Epas1		314371	Advanced Cell Diagnostics (USA)
Lgr4	1:50	318321	Advanced Cell Diagnostics (USA)
Mm-Lgr5		312171	Advanced Cell Diagnostics (USA)
Ss-Lgr5		455391	Advanced Cell Diagnostics (USA)
Lgr6	1:50	404961	Advanced Cell Diagnostics (USA)
Lmo1	1:50	511211	Advanced Cell Diagnostics (USA)
Myod1	1:50	316081	Advanced Cell Diagnostics (USA)
Pamr1	1:50	491431	Advanced Cell Diagnostics (USA)
Ptch1		402811	Advanced Cell Diagnostics (USA)
Ptprc		318651	Advanced Cell Diagnostics (USA)
Rspo1	1:50	479591-C2	Advanced Cell Diagnostics (USA)
Rspo2	1:50	402001	Advanced Cell Diagnostics (USA)
Rspo3		483781	Advanced Cell Diagnostics (USA)
Runx2		414021	Advanced Cell Diagnostics (USA)
Scube1		488131	Advanced Cell Diagnostics (USA)
Shh		314361	Advanced Cell Diagnostics (USA)
Spon1	1:50	492671	Advanced Cell Diagnostics (USA)
Tac1		410351	Advanced Cell Diagnostics (USA)
Tbx15		558761	Advanced Cell Diagnostics (USA)
Tfap2c	1:50	488861	Advanced Cell Diagnostics (USA)
Trim9		479071	Advanced Cell Diagnostics (USA)

Supplemental Table 4 List of primers used for qPCR

Mouse target gene symbol	Forvard primer sequence (5'-3')	Reverse primer sequence (5'-3')
Maf	CAACCCTTCCTCTCCCGAAT	GCGAGTTTGTCCAAGGTACC
Hopx	TTAGTCAGACGCGCACGGACC	CACTCTGCCAGGCGCTGCTT
Rhov	CCTCATCGTCAGCTACACCT	CGGGTAGCAGAGAGAACGAA
Pkp1	GCTTTGCCCTCTGACCAAAA	TTGTCAGCCAGCCCATCATA
Lgals3	GCCCGGGGAAAAGAGTACTA	TGCACCCGGATATCCTTGAG
Krt17	GATTGGTACCAGAAGCAGGC	AGCCTGCTCTGTCTCAAACT
Notch1	TGCCCCGTGGGCTTCAATGG	CGAGTCGCACCAGCGCACAA
Notch2	AGTGTCGAGGTGGTCAAGAG	AGGGGTGAGAGGTGGAGTAT
Irx1	CCAACTACAGCGCCTTCTTG	TCCCCGTATTGAAACTGACCA
Frem2	TGGCTACCTTCTCAACCTGG	GATCCACGCCCAATTCAGTC
Vcam	AGTCCGTTCTGACCATGGAG	CTGGAGCCAAACACTTGACC
Jag1	CTTCAATCTCAAGGCCAGCC	TTTCAGTGTCTGCCATTGCC
Pfn2	CGTTGATGGTGACTGCACAA	CCTCCATGGACCCCTTCTTT
Smyd3	GCAGGGTTATCGTCAAGCTG	TGAGACGCATCCTGGATCTC
Eif1b	ACACTAGTGTCGCATGTCCA	TGCGCTGCTGGATCCTTATA
Grasp	GCAGCACTGGAGGACTATCA	TGGATCTCGAAGCCAAAGGT
Shh	TTCTGTGAAAGCAGAGAACTCC	GGGACGTAAGTCCTTCACCA
Manf	GCTGCCACCAAGATCATCAA	CTGTGCTCAGGTCAATCTGC
Wfdc2	TGGACCGAGCGAAGGAGAGC	GGGGCAGGTGCCCTGCTTTT
Tubb3	AACCTGGAACCATGGACAGT	CCCTCCGTATAGTGCCCTTT
Mvd	GGGACTCCAGCATCTCAGTT	AGGAGTTGATGGGCAGGATC
Tacstd2	GAACCCACCACATCCTCATT	ATGGTGGGCTCCTCATAGTG
Ubb	ATGTGAAGGCCAAGATCCAG	TAATAGCCACCCCTCAGACG
Ctnnb	GGCATCCTCACCCTGAAGTA	AGGTGTGGTGCCAGATTTTC

Supplemental Table 5 Genes characteristic for non-dental clusters

	Gene name	Map	Function/evidence	Reference
Surrounding non-molar clusters				
MUSCLE	***Myf5***		a transcription factor, which plays a crucial role in muscle development and differentiation	Braun et al., 1989 [1]
	Ttn		a very large protein, which plays role in the structure and function of muscle cells	Maruyama K., 1997 [2]
	Tnnt1		protein, which plays a crucial role in the regulation of muscle contraction	Filatov et al., 1999 [3]

IMMUNE CELL	***C1qa***		a component of the complement system, an important part of the immune system	Wang et al.,2012 [4]
	Tyrobp		an important transmembrane adaptor protein, it plays a crucial role in activating immune cells	Lanier et al., 1998 [5]
	Fcer1g		a high affinity IgE receptor, a key molecule involved in allergic reaction	Le Coniat et al., 1990 [6]
PERICYTES	***Rgs5***		a regulator of G protein signaling, important in vascular biology, it is highly expressed in vascular smooth muscle cells and pericytes	Bondjers et al., 2003 [7]
	Higd1b		protein, which plays a role in regulation of mitochondrial function; a potential marker of pericytes in lung and heart	Baek et al., 2022 [8]
	Cpa1		an enzym that belongs to the carboxypeptidase family with role in protein digestion in the small intestine	Clauser et al., 1988 [9]

ENDOTHELIAL CELLS	***Cldn5***		a key komponent of tight junction in epithelial and endothelial cells	Morita et al., 1999 [10]
	Plvap		an endothelial cell-specific membrane protein with role in microvascular permeability	Denzer et al., 2023 [11]
	Kdr		a receptor, also known as a VEGFR, is a major growth factor for endothelial cells	Kroll et al., 1997 [12]
GLIAL CELLS	***Sox10***		a transcription factor that is involved in the development of glial cells in the nervous system	Kuhlbrodt et al., 1998 [13]
	Gfra3		a receptor for artemin, plays an important role in glial cells, particularly astrocytes	Naveilan et al., 1998 [14]
	Fabp7		an important protein primarly expressed in various types of glial cells, with crucial role in lipid metabolism	Avraham et al., 2020 [15]

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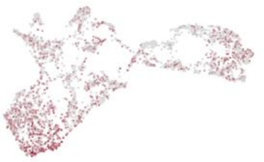
Supplemental Table 6 Genes characterisctic for molar mesenchyme

	Gene name	Map	Function/evidence	Reference
Molar mesenchyme				
MESENCHYME	***Col1a1***		a major component of conncective tissiues like bone, skin, tendons, ligaments, and the cornea	Karsenty and Park, 1995 [1]
	Twist1		a transcription factor that maintains the viability of cells and their undifferentiated mesenchymal status	Bildsoe et al., 2016 [2]
UNSPECIFIED ORAL FIBROBLASTS	***Bhlhe22***		a member of the Olig family and plays an important role in neuronal and glial differentiation and maturation	Dennis et al., 2019 [3]
	Cachd1		involved in the transmembrane transport of calcium ions and is a binding partner of the Wnt receptor FZD7 and the co-receptor LRP6. It was found to be expressed in neuron progenitors	Powell et al., 2024 [4]
	Trim9		a regulator of neuronal morphogenesis in cortical neurons, it is expressed in neural precursors	Winkle et al., 2016 [5]
UNDIFFERENTIATED OB	***Meis2***		a transcription factor, which controls skeletal formation in the hyoid region and plays a crtical role during cranial neural crest cells development	Mahon et al., 2015 [6]; Fabik et al., 2022 [7]
	Mfap5		a protein that plays a role in the regulation of bone mineralization	Li et al., 2021 [8]
	Itih5		a component of extracellular matrix, which is crucial for bone formation and remodeling; a modulator of inflammatory response	Huth et al., 2020 [9]

LGR5+ CELLS	***Tcea3***		an elongation factor that is involved in transcriptional regulation, it can control cell fate of mice embryonic stem cells	Park et al., 2013 [10]
	Emb		a transmembrane glycoprotein; it plays a role in a transition of progenitor cells ti differentiated sebocytes in the sebaceous gland	Sipilä et al., 2022 [11]
	Ptx4		a pentraxin family member, is related to PTX3, which supports osteogenic and odontogenic differentiation and stem cell migration	Kim et al., 2019 [12]
OUTLYING DENTAL FOLLICLE	***Alpl***		a member of alkaline phosphatase family, it contributes to dentin mineralization	Kramer et al., 2021 [13]
	Runx2		a transcription factor that plays role in tooth root development	Wen et al., 2020 [14]
	Pappa2		a metalloproteinase that have roles in the regulation of growth factors, particularly IGFs and it is ivolved in various biological processes	Barios et al., 2021 [15]
SURROUNDING DENTAL FOLLICLE	***Ptch1***		a receptor of the HH signalling pathway, which is important in embryonic development; it is expressed in mice dental mesenchyme at E14.5	Cobourne et al.,2009 [16]
	Tfap2c		a transcription factor, which was described highly expressed in the early mice molar development, mainly in the dental mesenchyme	Liu et al., 2024 [17]
	Ibsp		a non-collagenous protein found in cementum; it is expressed during early tooth root development by cells on the surface of the teeth root	Lao et al., 2006 [18]

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Supplemental Table 7 Genes characterisctic for dental papilla

APICAL PAPILLA	***Postn***		an extracellular matrix protein, primarily expressed in the periosteum and PDL of mice molar, but it was also found to be expressed in the pre-odontoblasts and in cells surrounding the root close to the cervical loop	Kruzynska-Frejtag et al., 2004 [6] Du and Li, 2019 [7]
	Lhx6		a transcription factor, expressed in the dental papilla during mice molar development to the postnatal stages, moreover from E16.5 its expression was enriched in the apical region	He et al., 2021 [8]
CORONAL PAPILLA MEDIUM	***Crym***		Crystallin, which is primarily known for its function in maintaining lens transparency and refraction in the eye, but it is expressed also in dental mesenchyme, where it regulates cellular metabolism and differentiation of odontoblasts	Wistow et al., 2012 [9] Hallen et al.,2011 [10]
	Piezo2		a component of a mechanosensitive channel and it mediates mechanotransduction in the peripheral pulp of molars	Han et al., 2022 [11]
CORONAL PAPILLA UPPER	***Gfra1***		acts as a co-receptor for glial cell line-derived neurotrophic factor (GDNF), during teeth development it is linked to the growth and patterning of the dental mesenchyme, particularly dental papilla and follicle	Leda et al., 2007 [12]
	Sct		a member of glucagon family of peptides which may regulate cellular signaling pathway that influence the differentiation and activity of odontoblasts and dental pulp cells	Wang et al., 2020 [13]
LATERAL PAPILLA	***Snai2***		a zinc finger transcription factor that can trigger EMT during the formation of the mesoderm and of the neural crest in vertebrate embryos; it is widely present in stem cells and essential for osteoblast differentiation	Jiang et al., 2021 [14] Martínez-Alvarez et al., 2004 [15]
	Rgs3		a transcription factor, known for its role in vascular biology; it is expressed in pericytes of the dental pulp of rat postnatal molars and incisors; it is important for vascular-derived stem cells during pulp healing	Lovschall et al., 2007 [16]

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Supplemental Table 8 Genes characterisctic for dental epithelium

	Gene name	Map	Function/evidence	Reference
Dental epithelium				
STRATUM INTERMEDIUM	***Hopx***		a transcription factor which is expressed in both embryonic and adult stem cells in various tissues to regulate biological processes	Caspa Gokulan et al., 2022 [1]
	Rhov		a GTPase of the Rho family required for the full differentiation of NCS and it is necessary for the migration process. In the dental context, it is found to be expressed in cells of the stratum intermedium	Faure et al., 2015 [2] Hermans et al., 2022 [3]
PRE-AMELOBLASTS	***Tubb3***		a member of beta tubulin protein family forming microtubules; during odontogenesis it is expressed in both ameloloblasts and odontoblasts	Oshima and Yawaka, 2019 [4]
	Wfdc2		a component of the innate immune difences of the lung, nasal and oral cavities	Bingle et al., 2006 [5]
STELLATE RETICULUM	***Smyd3***		a histon methyltransferase works as a chromatin regulator with oncogenic activity; it is implicated as a transcriptional activator in various types of cancers	Yang et al., 2023 [6]
	Eif1b		a translation factor, which is involved in the initiation phase of translation; its expression was found in the uveal melanoma	Demirci et al., 2013 [7]
TYMS+ CELLS	***Tyms***		an enzym, which catalyzes the methylation of deoxyuridylate to deoxythymidylate	Costi et al., 2005 [8]
	Birc5		a member of the inhibior of apoptosis gene family; highly expressed during fetal development	Ambrosini et al., 1997 [9]
	Pclaf		a regulator of DNA repair during DNA replication; involved in cell cycle progression	Povlsen et al., 2013 [10] Emanuele et al., 2011 [11]

PROLIFERATING CELLS	***Lars2***		a synthetase, which catalyzes the attachment of leucin to its cognate tRNA	Riley et al., 2015 [12]
	Arid1a		a member of the SWI/SNF family, which is involved in transcriptional activation and repression of select genes by chromatin remodeling	Li et al., 2010 [13]
OEE, DS, SDL	***Frem2***		an extracellular matrix protein that plays a role in epidermal-dermal interaction; mutations in this gene are associated with Fraser syndrom, cryptophthalmos, dental anomalies and oral vestibule defects; it was shown to be expressed in the developing tooth and vestibular lamina	Kunz et al., 2020 [14] Kantaputra et al., 2022 [15]
	Vcan		a large proteoglycan and a major component of the extracellular matrix; it was found to be expressed in the dental epithelium during mice molar development	Jiang et al., 2010 [16]
	Notch1		a transmembrane receptor that plays a role in the development of numerous cell and tissue types; during molar development it is expressed in epithelial structures such as outer enamel epithelium or stratum intermedium	Mitsiadis et al., 1995 [17]

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Supplemental Table 9 Markers of GFP dataset at E16.5 and E18.5

	Gene name	Map	Function/evidence	Reference
GFP dataset E16.5 and E18.5				
DENTAL EPITHELIM	***Epcam***		a protein that is primarily expressed on the surface of epithelial cells. It plays an important role in various cellular processes such as cell adhesion, proliferation, differentiation, and migration.	Balzar et. al., 1999 [1]
	Krt7		a protein that is well-known to be situated in dental epithelium in both incisors and molars	Zhao et al., 2024 [2]
	Dsp		an extracellular matrix protein primarily observed in odontoblasts and dentin, but it is found to be located also in pre-ameloblasts. It is important for regulation of mineralization or matrix formation.	Bronckers et al., 1993 [3]
SURROUNDING DENTAL FOLLICLE	***Ptch1***		a receptor of the HH signalling pathway, which is important in embryonic development; it is expressed in mice dental mesenchyme at E14.5	Cobourne et al.,2009 [4]
	Car2		an enzyme, which catalyzes reversible hydration of carbon dioxide. It is expressed in both mesenchyme and epithelium during tooth development.	Reibring et al., 2014 [5] Wang et al., 2022 [6]
	Tnmd		a cartilage-specific glycoprotein, which acts on the maturation and maintenance of the periodontal ligaments by regulating cell adhesion	Komiyama et al., 2013 [7]

OUTLYING DENTAL FOLLICLE	***Npnt***		an extracellular matrix protein localizes in a basal lamina. During tooth development it is expressed in the basal lamina on the buccal side of the tooth germ.	Arai et al., 2017 [8]
	Gas6		a member of the vitamin K-dependent protein family, which is described to be expressed in the outer layers of the oral epithelium during odontogenesis.	Nassar et al., 2017 [9]
	Mgp		an extracellular matrix protein that plays a role as a negative regulator for mineral apposition in the tooth, specifically in the dental cementum.	Hashimoto et al., 2001 [10]
PROLIFERATING CELLS	***MKi67***		a protein associated with cellular proliferation	Gerdes et al., 1983 [11]
	Top2a		an important enzyme that plays a role in the controlling of DNA structure during replication, transcription or chromosome segregation.	Downes et al., 1994 [12]
	Pclaf		a nucleoprotein involved in DNA replication and repair processes	Yu et al., 2001 [13]

LABIAL MESENCHYME	***Sostdc1***		a protein, which is studied in the context of tooth development, kidney disease, hair follicle formation and ect. It is decribed as a BMP and Wnt antagonis and it influences the behaviour of mesenchymal stem cells in response to bone injury.	Collette et al., 2016 [14]
	Tcea3		a transcription elongation factor, which controls self-renewal and/or pluripotent differentiation potential of mice embryonic stem cells	Park et al., 2013 [15]
	Tfap2b		a transcription factor known to regulate nerual crest and melanocyte development in the mouse. It is reguired for melanocyte regeneration from Mesenchymal Stem Cells, providing a functional role in stem cell potential.	Brombin et al., 2022 [16]
UNSPECIFIED ORAL FIBROBLASTS	***Sp9***		a transcription factor studied in the context of bone development. Its paralog is SP8, which influences craniofacial malformations and proliferation of neural crest cells	Kasberg et al., 2013 [17]
	Tbx18		a transcription factor, which plays a role in embryonic developmental processes such as a regulation development of the epicardium, craniofacial area or angiogenesis	Wu et al., 2013 [18] Sun et al., 2023 [19]
	St8sia2		an enzyme synthesizes polysialic acid, which is essential for brain development	Ikegami et al., 2019 [20]
CILP+ CELLS	***Cilp***		a matrix glycoprotein that is primarily found in the cartilage, where it plays an important role in regulating the metabolism of the extracellular matrix, specifically in an invertebral disc	Liu et al., 2021 [21]
	Scx		a transcrtiption activator that plays an important role in a growth of tendons after mechanical loading by production of extracellular matrix components and the commitment and the expansion of new tenocytes	Gumucio et al., 2020 [22]
	Prg4		a large proteoglycan that is synthesized by chondrocytes. It is needed to prevent protein deposition onto cartilage from synovial fluid, the protection of joints and tendons surfaces, and the control of synovial cell growth	Rhee et al., 2005 [23]

SIM2+ CELLS	***Sim2***		a transcription factor, which is an important regulator of neurogenesis. It was described as an important player in limb muscle formation.	Coumailleau and Duprez, 2009 [24]
	Foxd1		a transcription factor, which is selectively expressed in neuroepithelial cells of the prethalamus and hypothalamus	Newman et al., 2018 [25]
	Scn3a		a voltage-gated sodium channel important for cortical organization and neuronal migration, especially in speech and language areas	Smith et al., 2018 [26]
LATE PRE-OSTEOBLASTS	***Tnxb***		en extracellular matrix glycoprotein, The deficiency of this protein causes bone resorption and significant bone loss	Kajitani et al., 2019 [27]
	Ccn3		a small secreted cysteine-rich protein, which is associated with the extracellular matrix protein. It inhibits osteoblast differentiation and plays a role in bone regeneration.	Matsushita et al., 2013 [28]
	Fst		a glycoprotein, which was reported to inhibit bone formation and enhances crucial processes needed for bone repair	Fahmy-Garcia et al., 2019 [29]
EARLY PRE-OSTEOBLASTS	***Bmp7***		a member of the TGFb superfamily, which plays role in the formation and repair of endochondral bone.	Salazar et al., 2016 [30]
	Hand2		a protein that is implicated in the development of the neural crest, and involved in craniofacial development. It plays role in regulation of osteoblast differentiation in branchial arch development.	Funato et al., 2009 [31]
	Thy1		a cell surface glycoprotein, which plays role as a positive regulator of osteoblast differentiation and modulates bone homeostasis.	Paine et al., 2018 [32]

NEFL+ CELLS	***Nefl***		a neurofilament protein important for intracellular transport to axons and dendrites. It plays a role in the maturation of regenerating myelinated axons.	Zhu et al., 1997 [33]
	Gfra1		a member of the glial cell line-derived neurotrophic factor receptor, which promotes dendritic growth in hippocampal neurons and it is essential for proper hippocampal circuit development	Irala et al., 2016 [34]
	Nefm		a neurofilament protein, which is specifically expressed in large myelinated axons and is important for normal axonal function and remyelination.	Zhang et al., 2023 [35]

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Supplemental Table 10 Genes expressed in the epithelium of LGR5-positive cells

	Gene name	Map	Function/evidence	Reference
Epithelial Lgr5-positive cells from E16.5 and E18.5 scRNAseq				
TYPICAL COMPONENT OF EPITHELIAL CELLS	***Krt15***		Keratin 15, as other keratins it is responsible for the structural integrity of epithelial cells.	Leube et al., 1988 [1]
	Dapl1		Death Associated Protein Like 1, early epithelial differentiation-associated protein.	Ma et al., 2017 [2]
	Asprv1		Filaggrin is a structural protein that is crucial for the development and maintenance of the skin.	Hildenbrand et al., 2010 [3]
DESMOSOME-DEPENDENT ADHESION	***Pkp3***		Plakophilin 3, contains numerous armadillo repeats, localizes to cell desmosomes and nuclei, and participates in linking cadherins to intermediate filaments in the cytoskeleton	Schmidt et al., 1999 [4]
	Dsp		Desmoplakin, a component of functional desmosomes	Jones et al., 1986 [5]
	Rab25		a protein that maintains intergrin alpha-V/beta-1 at the tips of extending pseudopodia	Caswell et al., 2007 [6]
	Klc3		Kinesin Light Chain 3, molecular motors involved in the transport of cargo along microtubules	Junco et al., 2001 [7]
	Cdh1		Cadherin 1, calcium-dependent cell-cell adhesion protein	Vestweber et al., 1987 [8]

ACTIN-ASSOCIATED GENES	***Fermt1***		FERM Domain Containing Kindlin 1, involved in integrin signaling and linkage of the actin cytoskeleton to the ECM, required for normal keratinocyte proliferation	Siegel et al., 2003 [9]
ION CHANNELS	***Tacstd2***		*Trop2*, cell surface receptor that transduces calcium signals	Smith et al., 2024 [10]
	S100A14		S100 Calcium Binding Protein A14, which contains an EF-hand motif and binds calcium	Pietas et al., 2002 [11]
	Fxyd3		FXYD Domain Containing Ion Transport Regulator 3, a membrane protein that regulates the function of ion-pumps an ion-channels and the activity of the sodium/potassium-transporting ATPase	Crambert et al., 2003 [12]
STEM CELLS MARKERS	***Sox2***		a transcription factor which is involved in a deteramination of cell fate	Ellis et al., 2004 [13]
	Sox6		a transcription factor, which plays an important role in cell proliferation, differentiation and cell fate determination. It's not a typical stem cells marker, but it is implicated in the regulation of certain types of stem and progenitor cells such as adipose and neural tissues or skin.	Du et al., 2023 [14]
	Pitx2		Paired Like-Homeodomain 2, a transcription factor, which is not a typical stem cell marker, but it regulates progenitor oral/dental epithelial cells together with SOX2. Moreover, it plays a role in a regulation of muscle stem cells during muscle regeneration.	Yu et al., 2020 [15] L ' honoré et al., 2018 [16]
CELL CYCLE REGULATORS	***Sfn***		Stratifin, encodes a cell cycle checkpoint protein, a regulator of mitotic translation, plays a role in preventing DNA errors during mitosis, it regulates protein synthesis and epithelial cell growth by stimulating Akt/mTOR pathway	Bridges and Moorhead, 2005 [17]
	Serpinb5		Maspin, plays a role in regulation of epithelial cell proliferation and extracellular matrix organization	Zoe et al., 1994 [18]

BASAL MEMBRANE	***Lama5***		an extracellular matrix glycoproteins, major non-collagenous constituents of basement membranes	Hohenester and Yurchenco, 2013 [19]
	Lamb3			
	Lamc2			
	Col17a1		a structural component of hemidesmosomes, multiprotein complexes at the dermal-epidermal basement membrane zone, that mediate adhesion of keratinocytes to the underlying membrane were is also expressed.	Franzke et al., 2003 [20]
REGULATORS OF OTHER SIGNALING PATHWAYS	***Esrp1***		Epithelial Splicing Regulatory Protein 1, specifically regulates the expression of FGFR2-IIIb, an epithelial cell-specific isoform of FGFR2	Warzecha et al., 2009 [21]
	Spint2		a transmembrane protein that inhibits a HGF activator, which prevents the formation of active hepatocyte growth factor, it inhibits serine protease activity of ST14/matriptase	Qin et al., 1998 [22]
	Jag2		a member of the Notch gene family	Shawber et al., 1996 [23]

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List of additional supplemetary materiál:

Supplementary material 1 Top 150 markers of the main clusters
Supplementary material 2 Top 150 markers of the mesenchymal clusters
Supplementary material 3 Top 150 markers of the dental papilla clusters
Supplementary material 4 Top 150 markers of the dental epithelium clusters
Supplementary material 5 Top 30 markers of the GFP dataset