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Supplemental information

Arid1a-Plagl1-Hh signaling is indispensable for differentiation-associated cell cycle arrest of tooth root progenitors

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1A. Arid1a is knocked out efficiently in *Gli1-CreER;Arid1a$^{fl/fl}$* mouse molar roots at PN14.5. Related to Fig.2.

Arid1a immunofluorescence of first mandibular molars of control (Aa-Ac) and *Gli1-CreER;Arid1a$^{fl/fl}$* (Ad-Af) mice at PN14.5. Colored boxes in Aa and Ad are shown enlarged. Arrows in Ab and Ac indicate positive signals in control mice; arrowheads in Ae and Af indicate absence of signal in *Gli1-CreER;Arid1a$^{fl/fl}$* mice.

Schematic at the bottom indicates induction protocol. TAM, tamoxifen. Scale bars: 100 µm.

Figure S1B. Loss of Arid1a in Gli1+ root progenitor cells does not lead to morphological changes at PN7.5. Related to Fig.2.

Arid1a immunofluorescence of first mandibular molars of control (Ba-Bb) and *Gli1-CreER;Arid1a$^{fl/fl}$* (Be-Bf) mice at PN7.5. Boxes in Ba and Be are enlarged on the right. Arrow in Bb indicates positive signals in control; arrowhead in Bf indicates absence of signal in *Gli1-CreER;Arid1a$^{fl/fl}$*. H&E staining (Bc, Bg) and K14 immunofluorescence (Bd, Bh) of first mandibular molars of control and *Gli1-CreER;Arid1a$^{fl/fl}$* mice at PN7.5.

Schematic at the bottom indicates induction protocol. TAM, tamoxifen. Scale bars: 100 µm.

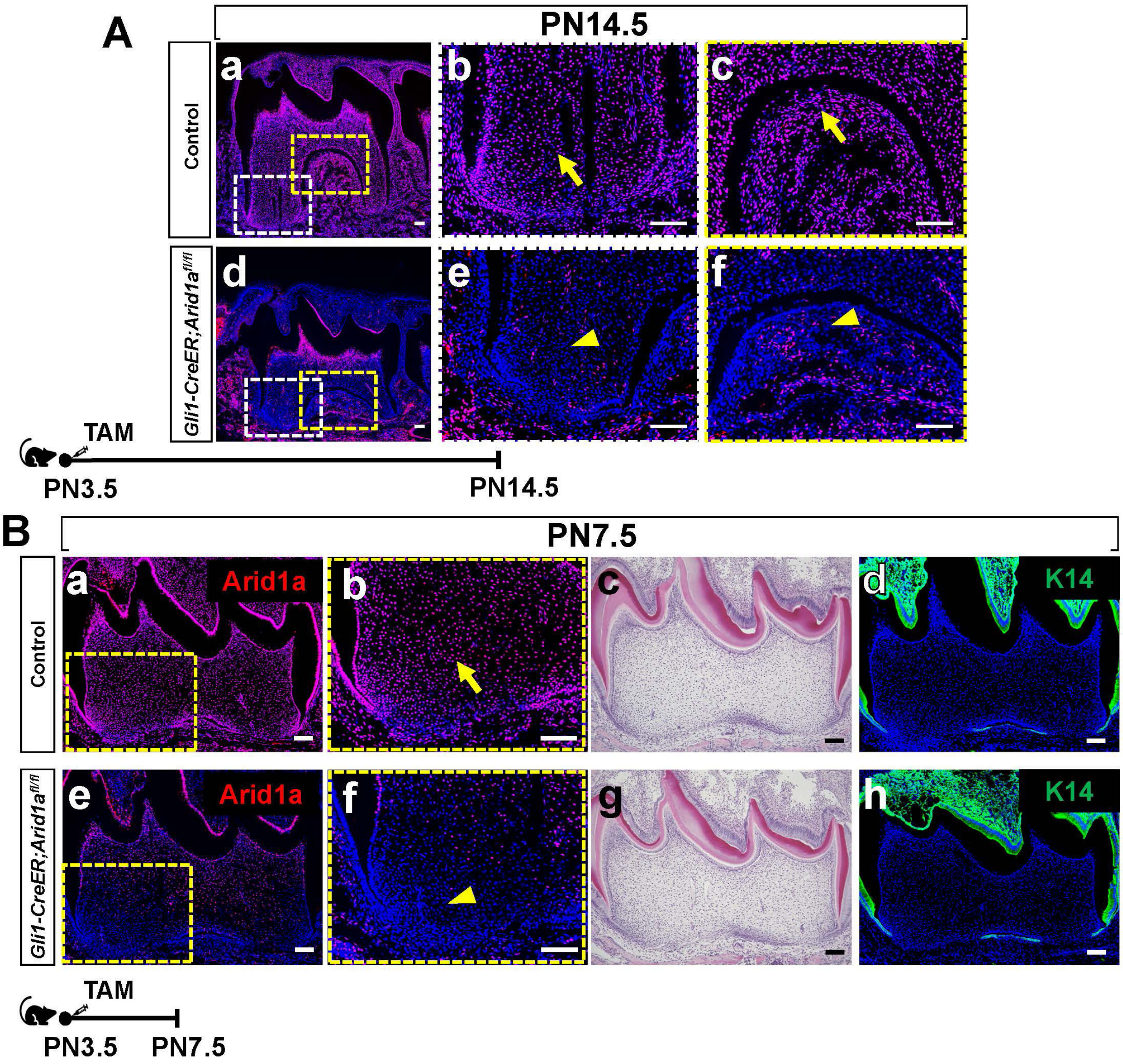

A
PN14.5
Control
a
b
c
Gli1-CreER;Arid1a$^{fl/fl}$
d
e
f
TAM
PN3.5
PN14.5
B
PN7.5
Control
a
Arid1a
b
c
d
K14
Gli1-CreER;Arid1a$^{fl/fl}$
e
Arid1a
f
g
h
K14
TAM
PN3.5
PN7.5

Figure S2A. Loss of Arid1a in the dental epithelium has no apparent effect on tooth root development. Related to Fig.2.

(Aa-Ad) Arid1a immunofluorescence of first mandibular molars of control (Aa, Ac) and *K14-rtTA;tetO-Cre;Arid1a*$^{fl/fl}$ (Ab, Ad) mice at PN7.5. Boxes in Aa and Ab are shown enlarged. Arrows in Ac indicate positive signals in control mice; arrowheads in Ad indicate absence of signal in *K14-rtTA;tetO-Cre;Arid1a*$^{fl/fl}$ mice.

(Ae-Ak) 2D (Ae, Af) and 3D (Ag, Ah) microCT images, H&E staining (Ai, Aj) and quantitative analysis of tooth root length (Ak) of first mandibular molars in control and *K14-rtTA;tetO-Cre;Arid1a*$^{fl/fl}$ mice at PN21.5. N=3. NS, no significant difference.

Schematic at the bottom indicates induction protocol. Dox, doxycycline. Data are represented as mean ± SD. Scale bars in Aa-Ad: 100 μm. Scale bars in Ae-Aj: 200 μm.

Figure S2B. Loss of Arid1a in odontoblasts leads to no apparent tooth root defects at PN21.5. Related to Fig.3.

MicroCT images (Ba, Bb) and quantitative analysis of tooth root length (Bc) of first mandibular molars in control and *Dmp1-CreER;Arid1a*$^{fl/fl}$ mice at PN21.5. N=3. NS, no significant difference.

Data are represented as mean ± SD. Scale bars: 300 μm.

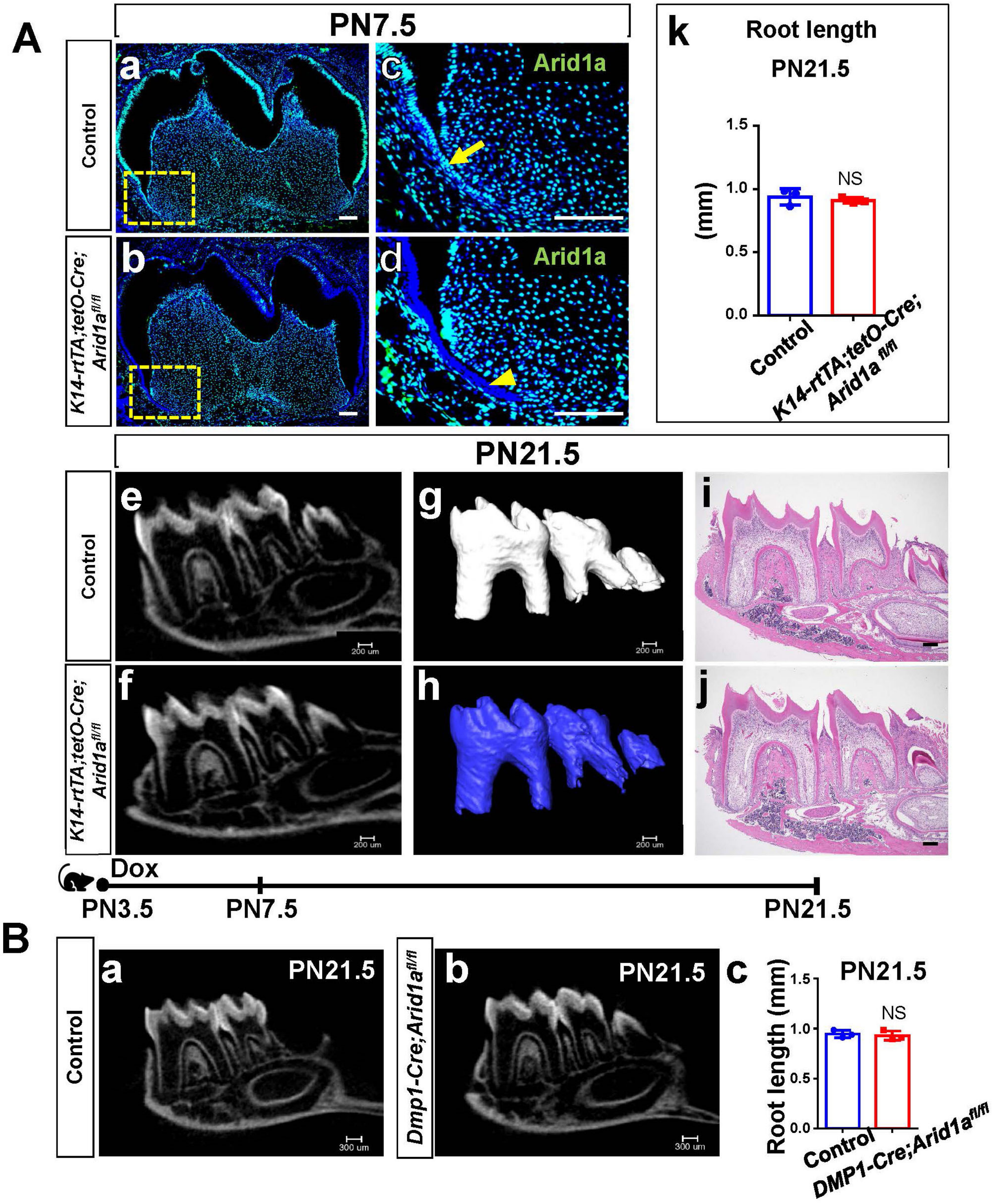

A
PN7.5
Control
K14-rtTA;tetO-Cre; Arid1a^fl/fl
a
b
c
d
Arid1a
Arid1a
k
Root length
PN21.5
(mm)
1.5
1.0
0.5
0.0
NS
Control
K14-rtTA;tetO-Cre; Arid1a fl/fl
PN21.5
Control
K14-rtTA;tetO-Cre; Arid1a^fl/fl
e
f
g
h
i
j
200 um
200 um
200 um
200 um
Dox
PN3.5
PN7.5
PN21.5
B
Control
a
PN21.5
300 um
Dmp1-Cre;Arid1a^fl/fl
b
PN21.5
300 um
c
PN21.5
Root length (mm)
1.5
1.0
0.5
0.0
NS
Control
DMP1-Cre;Arid1a^fl/fl

Figure S3. Loss of Arid1a in Gli1+ root progenitor cells leads to increased apoptosis at a later stage of tooth root development. Related to Fig.3.

(A-F) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining (green) and visualization of tdTomato (red) on sagittal sections of first mandibular molars from *Gli1-CreER;tdT* (A-C) and *Gli1-CreER;Arid1a*$^{fl/fl}$*;tdT* (D-F) mice at PN7.5, PN9.5 and PN11.5 after induction at PN3.5. The boxed areas in A-F are shown enlarged in the middle.

(G) Quantitative analysis of the ratio of TdT+TUNEL+ cells in the root apical region in *Gli1-CreER;tdT* (Con) and *Gli1-CreER;Arid1a*$^{fl/fl}$*;tdT* (Mut) mouse molars. N=3, $^*p < 0.05$, NS, no significant difference.

Schematic at the bottom indicates induction protocol. TAM, tamoxifen. Data are represented as mean ± SD. Scale bars: A-F, 100 μm.

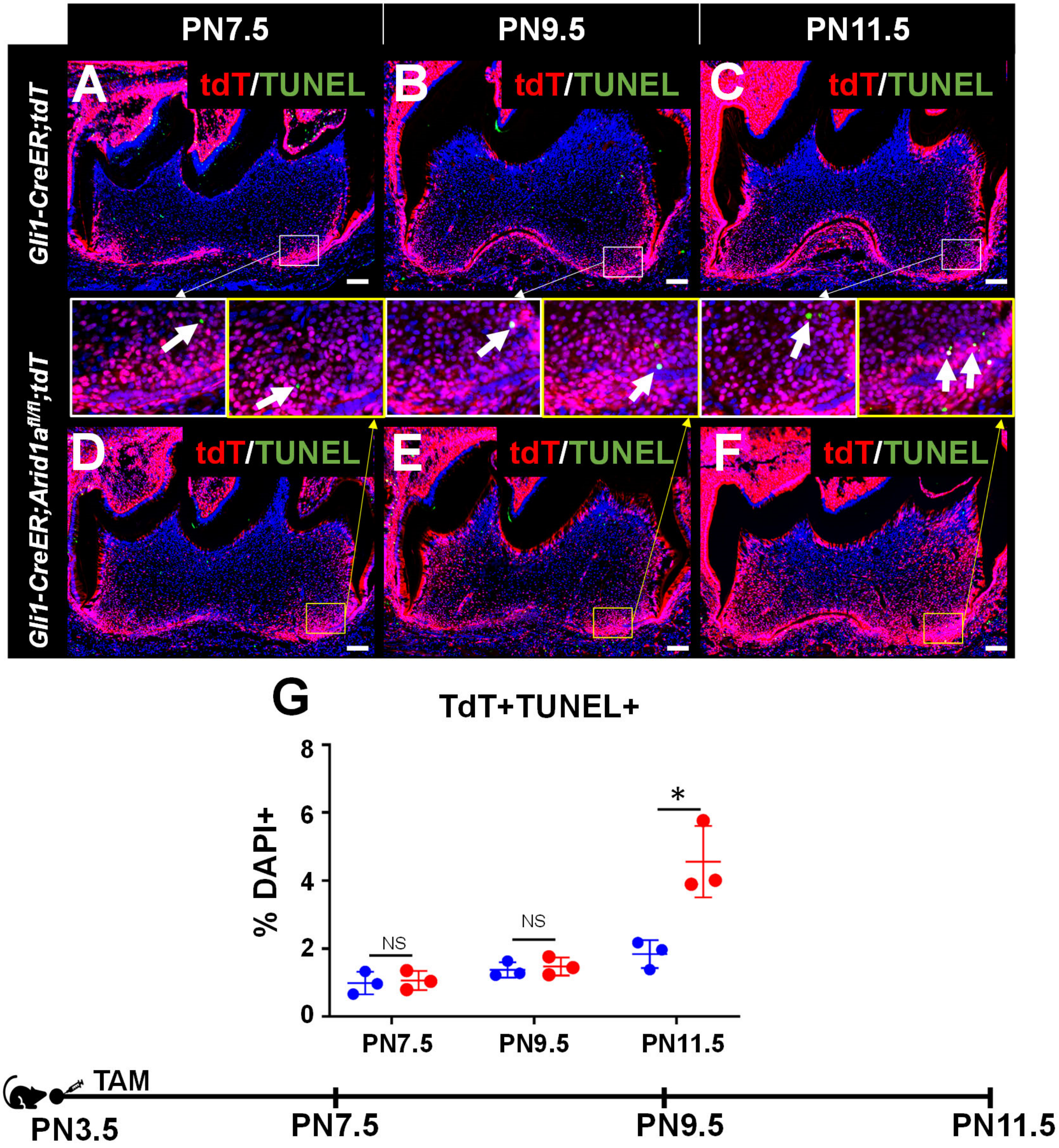

PN7.5
PN9.5
PN11.5
Gli1-CreER;tdT
Gli1-CreER;Arid1a$^{fl/fl}$;tdT
A
B
C
D
E
F
tdT/TUNEL
tdT/TUNEL
tdT/TUNEL
tdT/TUNEL
tdT/TUNEL
tdT/TUNEL
G
TdT+TUNEL+
% DAPI+
8
6
4
2
0
NS
NS
*
PN7.5
PN9.5
PN11.5
TAM
PN3.5
PN7.5
PN9.5
PN11.5

Figure S4A. PCA analysis and top 20 differentially expressed genes between control and *Gli1-CreER;Arid1a*$^{fl/fl}$ mouse molars at PN7.5. Related to Fig.4.

(Aa) PCA analysis of RNA sequencing data identified two distinct clusters belonging to control and *Gli1-CreER;Arid1a*$^{fl/fl}$ mouse molars at PN7.5.

(Ab) Top 20 differentially expressed genes between control and *Gli1-CreER;Arid1a*$^{fl/fl}$ mouse molars at PN7.5 sorted from smallest FDR value and fold change < -2.0 or > 2.0.

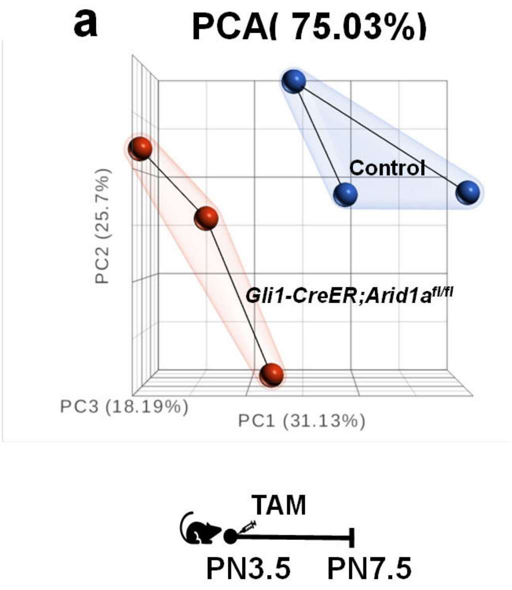
Schematic indicates induction protocol. TAM, tamoxifen.

Figure S4B. Loss of Arid1a in Gli1+ root progenitor cells leads to downregulated Hh signaling activity. Related to Fig.4.

qPCR of *Gli1* (Ba) and *Ptch1* (Bb) and western blot of Ccnd1 (Bc) in the apical third of the first mandibular molar in control and *Gli1-CreER;Arid1a*$^{fl/fl}$ mice at PN7.5. N≥3, *$p<0.05$.

Schematic indicates induction protocol. TAM, tamoxifen. Data are represented as mean ± SD.

A



b

Gene ID	FDR step up (MUT vs. WT)	Fold change (MUT vs. WT)
Ctnna2	0.010	-5.69
Ppp2r2c	0.010	-7.07
Coch	0.010	2.79
Nts	0.010	7.09
Lipm	0.010	4.77
Pstpip1	0.010	-2.97
Spock1	0.010	2.76
Ubash3b	0.010	-2.13
Ier5l	0.010	2.15
Gm13010	0.010	-6.16
Jag2	0.010	-2.26
Gm13889	0.010	-2.15
Samd11	0.010	-8.44
Gli1	0.010	-2.34
Dkk2	0.010	2.13
F2r	0.010	-2.13
Casz1	0.010	-2.17
Lrrtm3	0.020	3.83
Gcat	0.020	-2.17
Acpp	0.020	-3.01

Fold change <-2.0 or > 2.0

B

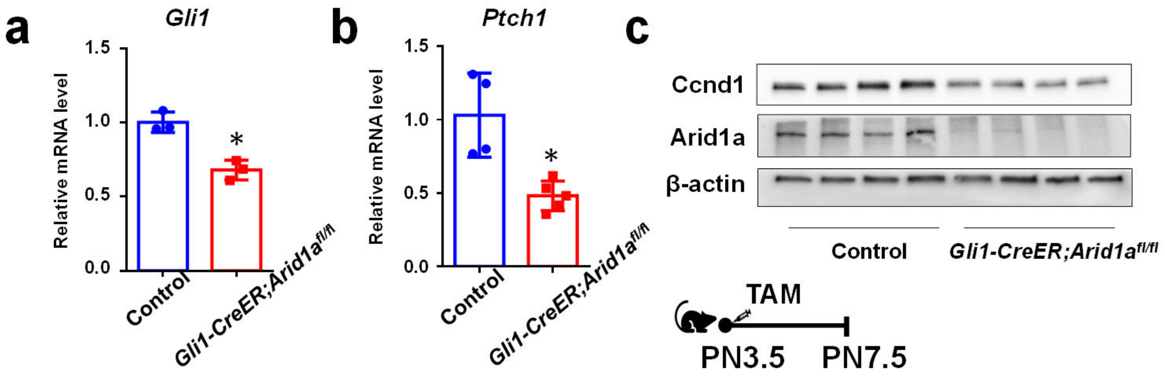


Figure S5A. Loss of Arid1a in Gli1+ root progenitor cells leads to no apparent change in Wnt signaling activity. Related to Fig.4.

RNAscope *in situ* hybridization of *Axin2* (Aa-Ad), and *Lef1* (Ae-Ah) on the first mandibular molars from control and *Gli1-CreER;Arid1a$^{fl/fl}$* mice at PN7.5. The boxed areas are shown enlarged on the right. Arrows in Ab, Ad, Af and Ah indicate positive signals.

Schematic at the bottom indicates induction protocol. TAM, tamoxifen. Scale bars: 100 µm.

Figure S5B. Loss of Arid1a leads to downregulated Hh signaling activity, overactivated mitosis and compromised osteogenesis in the mandibular condyle. Related to Fig.4.

(Ba-Bf) Arid1a immunofluorescence (Ba-Bb), RNAscope *in situ* hybridization of *Gli1* (Bc-Bd), and Ccnd1 immunofluorescence (Be-Bf) of mandibular condyle from control and *Gli1-CreER;Arid1a$^{fl/fl}$* mice at PN9.5. White dashed lines outline subchondral bone. Arrows in Ba, Bc and Be indicate positive signal. Dotted arrows in Bb, Bd and Bf indicate reduced signal.

(Bg-Bj) Immunofluorescence of pHH3 (green) and visualization of tdTomato (red) on mandibular condyle of control and *Gli1-CreER;Arid1a$^{fl/fl}$;tdT* mice at PN21.5. Boxes in Bg and Bh are enlarged in Bi and Bj. White dashed lines in Bg and Bh outline subchondral bone. Arrows Bi and Bj indicate overlapping signals.

(Bk-Bl) MicroCT images of condyles from control and *Gli1-CreER;Arid1a$^{fl/fl}$* mice at PN21.5. White and red dashed lines outline condyle in control and *Gli1-CreER;Arid1a$^{fl/fl}$* mice. White and red solid lines represented the condyle width in control and *Gli1-CreER;Arid1a$^{fl/fl}$* mice.

Schematic at the right indicates induction protocol. TAM, tamoxifen. Scale bars in Ba-Bj: 100 µm. Scale bars in Bk-Bl: 300 µm.

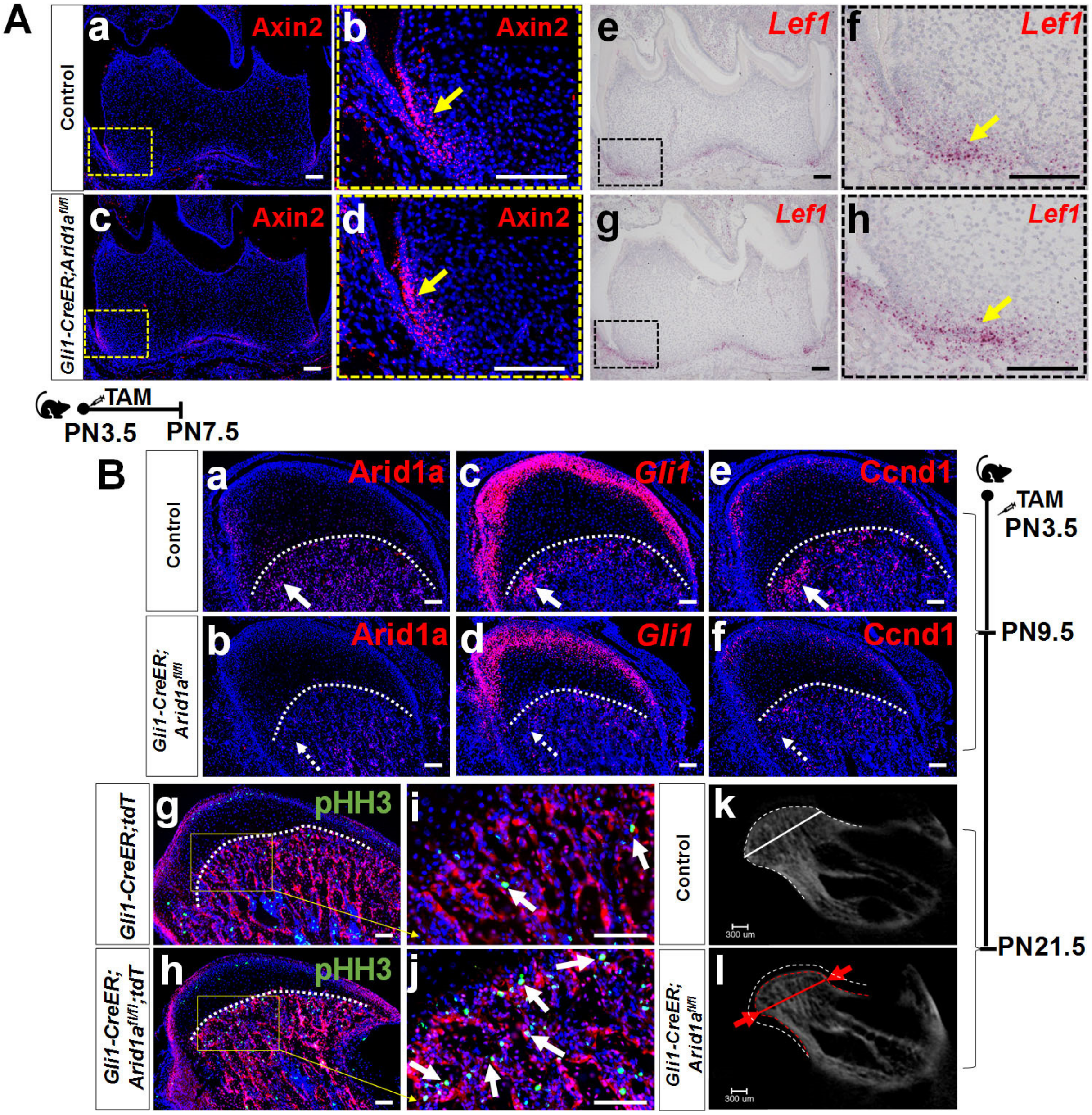

A
Control
a
Axin2
b
Axin2
e
Lef1
f
Lef1
Gli1-CreER;Arid1afl/fl
c
Axin2
d
Axin2
g
Lef1
h
Lef1
TAM
PN3.5
PN7.5
B
Control
a
Arid1a
c
Gli1
e
Ccnd1
Gli1-CreER; Arid1afl/fl
b
Arid1a
d
Gli1
f
Ccnd1
TAM
PN3.5
PN9.5
PN21.5
Gli1-CreER;tdT
g
pHH3
i
Control
k
300 um
Gli1-CreER; Arid1afl/fl;tdT
h
pHH3
j
Gli1-CreER; Arid1afl/fl
l
300 um

Figure S6. Upregulation of Hh signaling partially rescues the cell mitosis defects in *Gli1-CreER;Arid1a$^{fl/fl}$* mouse molars. Related to Fig.5.

RNAscope *in situ* hybridization of *Gli1* (red) and *Ptch1* (red) and immunofluorescence of Ccnd1 (red) and pHH3 (green) of sagittal sections of first mandibular molars of control (A-D), *Gli1-CreER;Arid1a$^{fl/fl}$* (E-H), and *Gli1-CreER;Arid1a$^{fl/fl}$;SmoM2$^{fl/+}$* (I-L) mice at PN7.5 after induction at PN3.5. Arrows indicate positive signals; arrowheads in E, F and G indicate reduced signal in targeted region.

(M) Quantification of pHH3+ cells in the apical third of first mandibular molars from control, *Gli1-CreER;Arid1a$^{fl/fl}$* and *Gli1-CreER;Arid1a$^{fl/fl}$;SmoM2$^{fl/+}$* mice at PN7.5 after induction at PN3.5 (the boxed areas in D, H and L). N=3, $^*p < 0.05$.

Schematic indicates induction protocol. TAM, tamoxifen. Data are represented as mean ± SD. Scale bars: 100 μm.

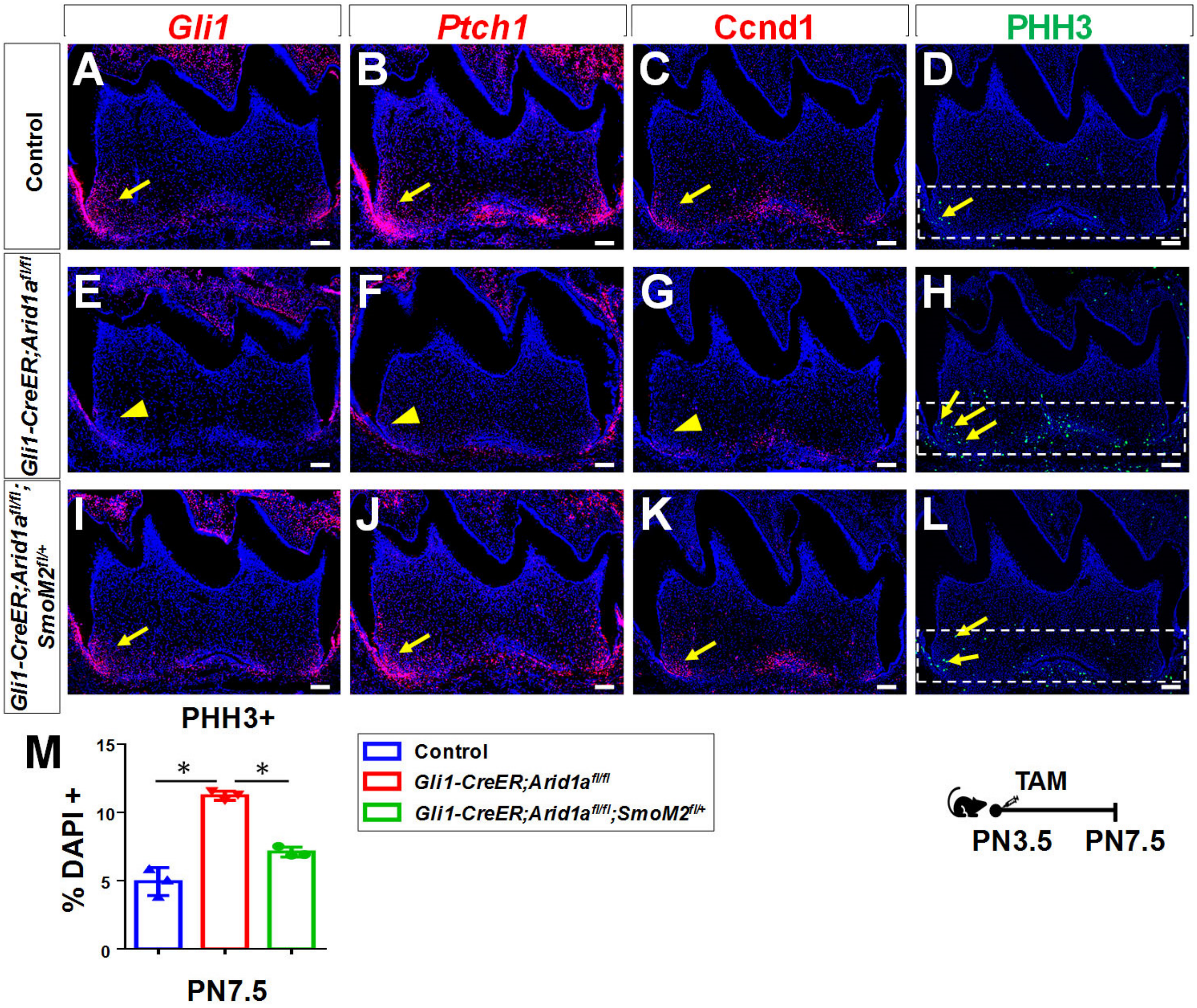

Gli1
Ptch1
Ccnd1
PHH3
Control
Gli1-CreER;Arid1a$^{fl/fl}$
Gli1-CreER;Arid1a$^{fl/fl}$;
SmoM2$^{fl/+}$
A
B
C
D
E
F
G
H
I
J
K
L
M
PHH3+
15
10
5
0
% DAPI +
*
*
PN7.5
Control
Gli1-CreER;Arid1a$^{fl/fl}$
Gli1-CreER;Arid1a$^{fl/fl}$;SmoM2$^{fl/+}$
TAM
PN3.5
PN7.5

Figure S7A. Motif analysis result of ATAC-sequencing. Related to Fig.6.

Top thirteen motifs enriched in regions with increased accessibility in control group compared to *Gli1-CreER;Arid1a*$^{fl/fl}$ mice at PN7.5 after induction at PN3.5.

Schematic at the right indicates induction protocol. TAM, tamoxifen.

Figure S7B. The mRNA and protein expression levels of Plagl1 after loss of Arid1a in Gli1+ root progenitor cells. Related to Fig.6.

(Ba-Bd) RNAscope *in situ* hybridization (red) of *Plagl1* on sagittal sections of first mandibular molars of control (Ba-Bb) and *Gli1-CreER;Arid1a*$^{fl/fl}$ mice (Bc-Bd) at PN7.5. Boxed areas in Ba and Bc are enlarged on the right. Arrows in Bb and Bd indicate positive signals.

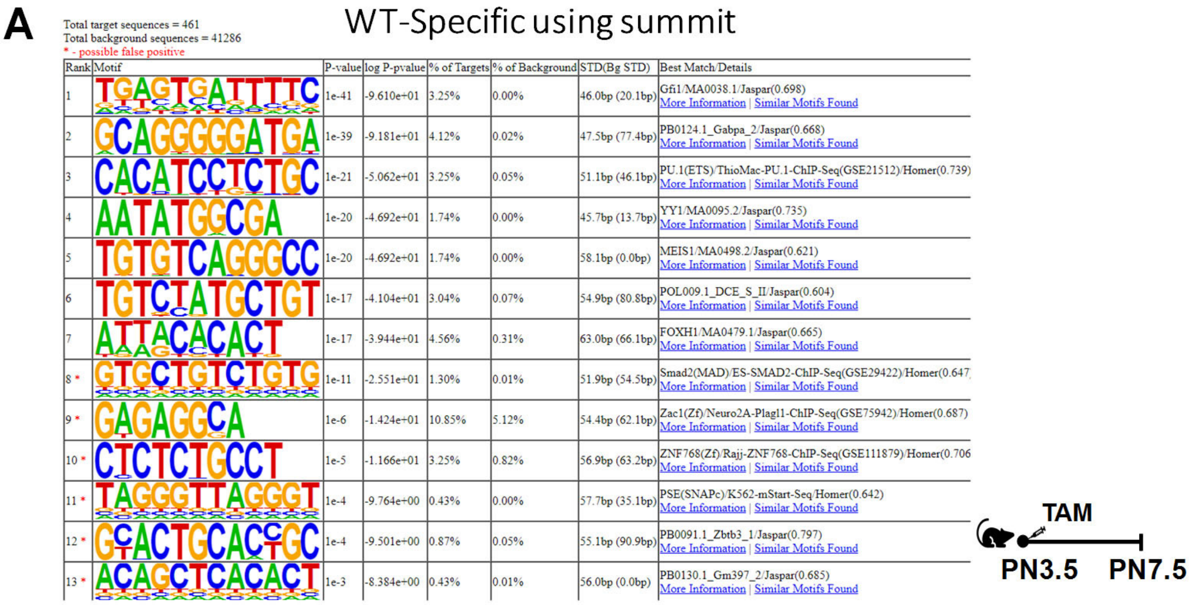
(Be) Western blot of Plagl1 in the apical third of the first mandibular molars of control and *Gli1-CreER;Arid1a*$^{fl/fl}$ mice at PN7.5.

Schematic at the bottom indicates induction protocol. TAM, tamoxifen. Scale bars: 100 μm.

Figure S7C. Colocalization of plagl1 and proliferating cells at the apical part of tooth root at PN7.5. Related to Fig.6.

RNAscope *in situ* hybridization of *Plagl1* (green) and immunofluorescence of Ki67 (red) of sagittal sections of first mandibular molars of control mice at PN7.5. Boxed area in Ca is enlarged in Cb-Cd. Arrows in Cb-Cd indicate positive signals.

Scale bars: 100 μm.

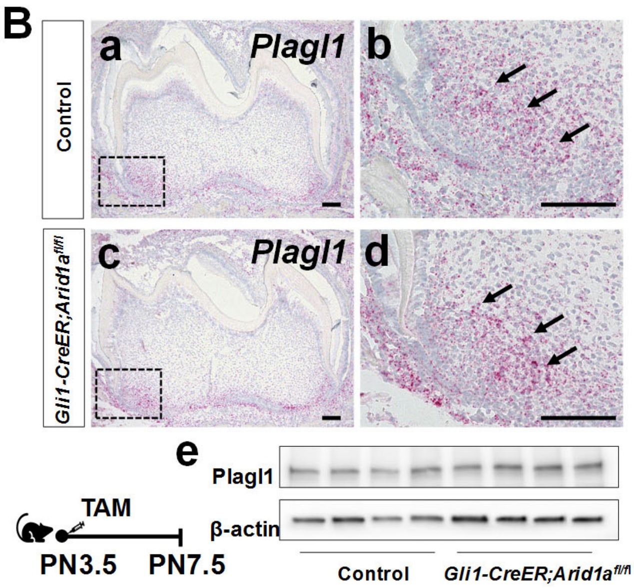


A

WT-Specific using summit

Total target sequences = 461
Total background sequences = 41286
* - possible false positive

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details
1	TGAGTGATTTTC	1e-41	-9.610e+01	3.25%	0.00%	46.0bp (20.1bp)	Gfi1/MA0038.1/Jaspar(0.698) More Information | Similar Motifs Found
2	GCAGGGGGATGA	1e-39	-9.181e+01	4.12%	0.02%	47.5bp (77.4bp)	PB0124.1_Gabpa_2/Jaspar(0.668) More Information | Similar Motifs Found
3	CACATCCTCTGC	1e-21	-5.062e+01	3.25%	0.05%	51.1bp (46.1bp)	PU.1(ETS)/ThioMac-PU.1-ChIP-Seq(GSE21512)/Homer(0.739) More Information | Similar Motifs Found
4	AATATGGCGA	1e-20	-4.692e+01	1.74%	0.00%	45.7bp (13.7bp)	YY1/MA0095.2/Jaspar(0.735) More Information | Similar Motifs Found
5	TGTGTCAGGGCC	1e-20	-4.692e+01	1.74%	0.00%	58.1bp (0.0bp)	MEIS1/MA0498.2/Jaspar(0.621) More Information | Similar Motifs Found
6	TGTCTATGCTGT	1e-17	-4.104e+01	3.04%	0.07%	54.9bp (80.8bp)	POL009.1_DCE_S_II/Jaspar(0.604) More Information | Similar Motifs Found
7	ATTACACACT	1e-17	-3.944e+01	4.56%	0.31%	63.0bp (66.1bp)	FOXH1/MA0479.1/Jaspar(0.665) More Information | Similar Motifs Found
8 *	GTGCTGTCTGTG	1e-11	-2.551e+01	1.30%	0.01%	51.9bp (54.5bp)	Smad2(MAD)/ES-SMAD2-ChIP-Seq(GSE29422)/Homer(0.647 More Information | Similar Motifs Found
9 *	GAGAGGCA	1e-6	-1.424e+01	10.85%	5.12%	54.4bp (62.1bp)	Zac1(Zf)/Neuro2A-Plagl1-ChIP-Seq(GSE75942)/Homer(0.687) More Information | Similar Motifs Found
10 *	CTCTCTGCCT	1e-5	-1.166e+01	3.25%	0.82%	56.9bp (63.2bp)	ZNF768(Zf)/Rajj-ZNF768-ChIP-Seq(GSE111879)/Homer(0.706 More Information | Similar Motifs Found
11 *	TAGGGTTAGGGT	1e-4	-9.764e+00	0.43%	0.00%	57.7bp (35.1bp)	PSE(SNAPc)/K562-mStart-Seq/Homer(0.642) More Information | Similar Motifs Found
12 *	GCACTGCACCGC	1e-4	-9.501e+00	0.87%	0.05%	55.1bp (90.9bp)	PB0091.1_Zbtb3_1/Jaspar(0.797) More Information | Similar Motifs Found
13 *	ACAGCTCACACT	1e-3	-8.384e+00	0.43%	0.01%	56.0bp (0.0bp)	PB0130.1_Gm397_2/Jaspar(0.685) More Information | Similar Motifs Found



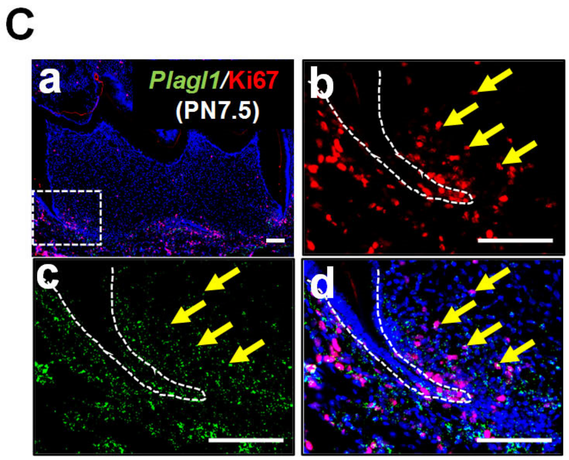


Table S1. Antibody sources and concentrations. Related to STAR Methods.

Primary antibodies	**Source**	**Dilutions**
Immunostaining		
Arid1a	Abcam, ab182561	1:100
Ki67	Abcam, ab15580	1:100
β-gal	Abcam, ab9361	1:100
K14	Abcam, ab181595	1:100
PHH3	Millipore, 06-570	1:100
Ccnd1	Abcam, ab16663	1:100
Periostin	Abcam, ab14041	1:100
Arid1b	Abcam, ab244351	1:100
Western Blot		
Arid1a	Santa Cruz, sc-32761	1:100
Arid1b	Abcam, ab244351	1:1000
Plagl1	Santa Cruz, sc-166944	1:100
Gli1	Novus, NBP1-78259	1:1000
Dspp	Santa Cruz, sc-73632 HRP	1:200
DMP-1	R&D, AF4386-SP	0.1 µg/mL
Ccnd1	Santa Cruz, sc-8396 HRP	1:200
Beta Actin	Abcam, ab20272	1:1000

Table S2. List of PCR primers. Related to STAR Methods.

Gene name	**Forward sequence**	**Reverse sequence**	**Application**
Beta-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT	qRT-PCR
Gli1	CCAAGCCAACTTTATGTCAGGG	AGCCCGCTTCTTTGTTAATTTGA	qRT-PCR
Ptch1	AAAGAACTGCGGCAAGTTTTTG	CTTCTCCTATCTTCTGACGGGT	qRT-PCR
Plagl1	ATGGCTCCATTCCGCTGTC	CTCAGCCTTCGAGCACTTGAA	qRT-PCR
Gli1 (site 1)	CGTCTCCCCGACTTTTGAGT	CCTTCCCGATTTCCCCCAAA	ChIP-qPCR
Gli1 (site 2)	ACTGAGCTTTCCCCATGTCG	ATGTTCCATAGGTCGCACCC	ChIP-qPCR