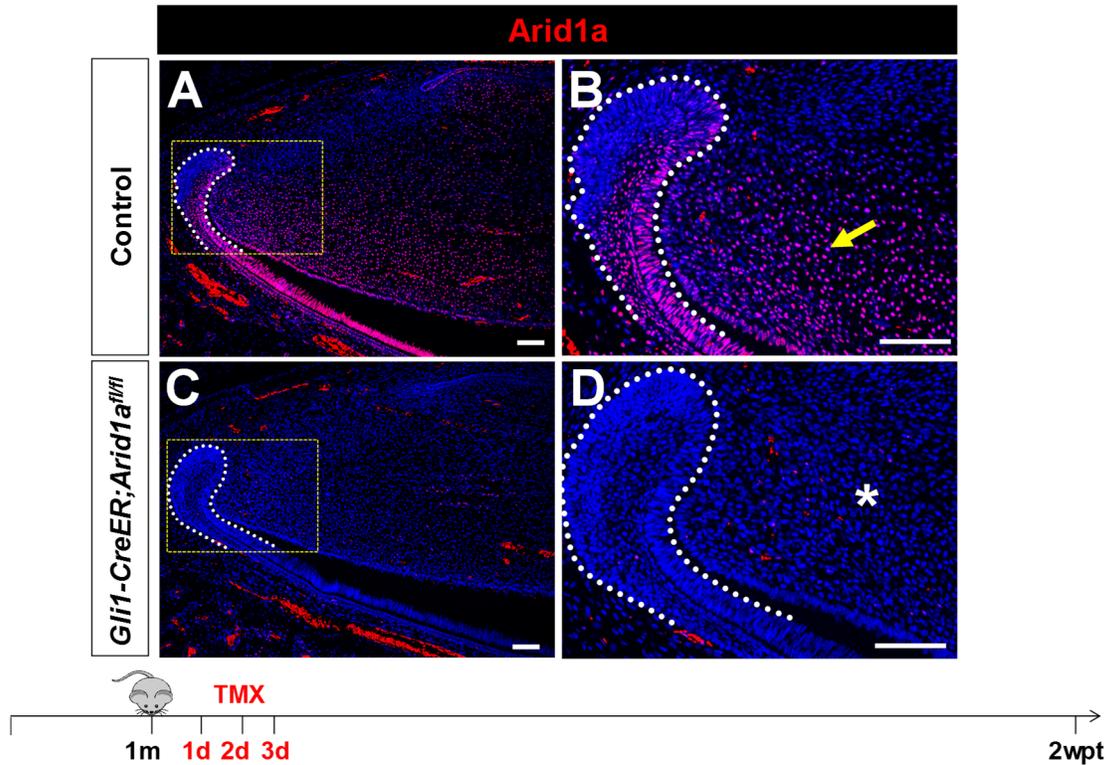


## SUPPLEMENTARY INFORMATION

### Supplementary Figures

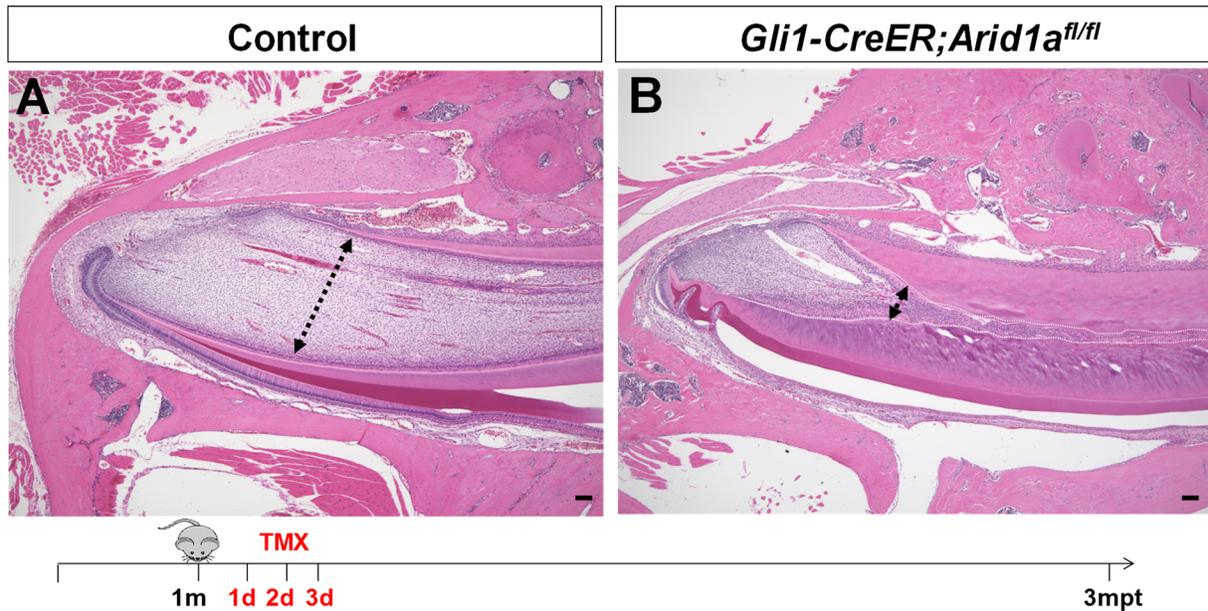
Figure S1



**Figure S1. Arid1a is knocked out efficiently in *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mouse incisor 2 weeks after induction.**

(A-D) Arid1a immunofluorescence of incisors from control (A-B) and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* (C-D) mice 2 weeks after induction. Boxes in A and C are shown enlarged in B and D. White dotted line outlines the cervical loop. Yellow arrow in B indicates positive signal in control mice; asterisk in D indicates absence of signal in *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice. Schematic at the bottom indicates induction protocol. N=3. Scale bars: 100  $\mu$ m.

Figure S2

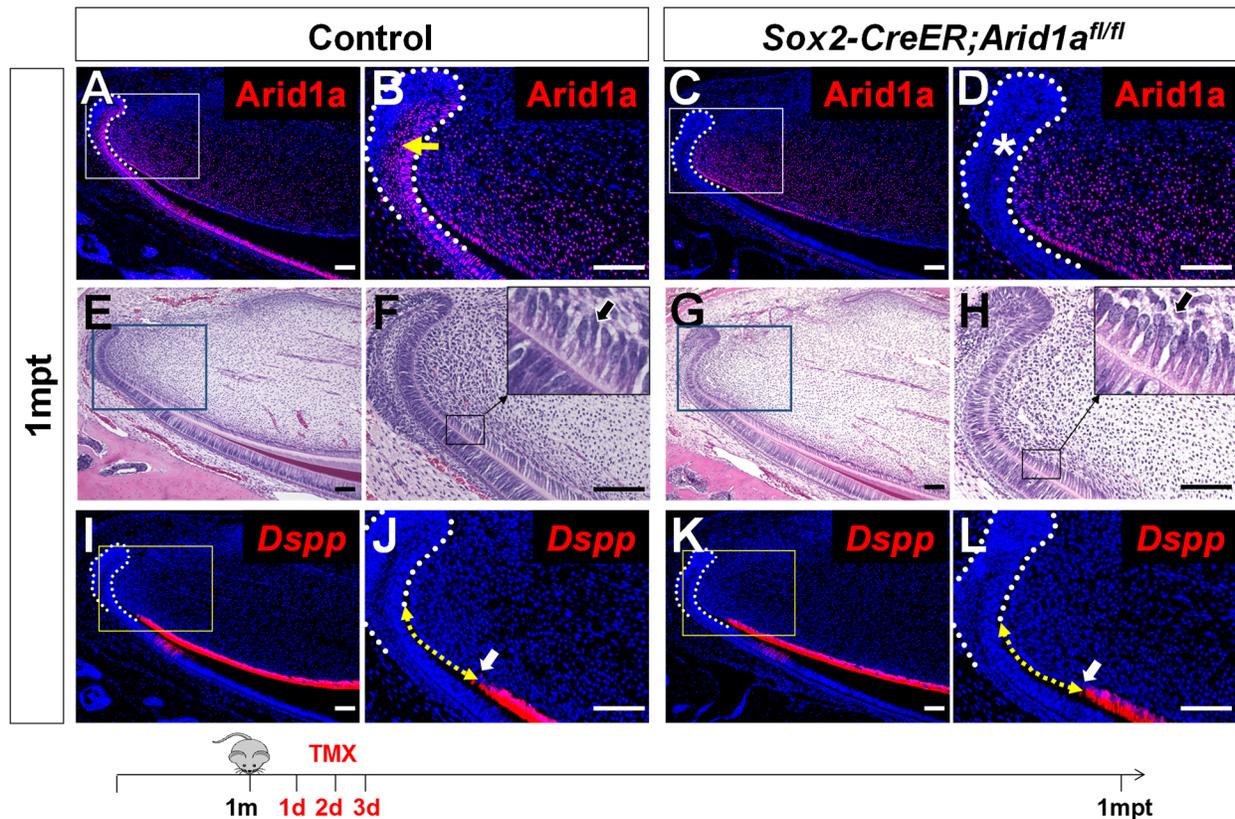


**Figure S2. Loss of *Arid1a* results in severe stacked dentin in dental pulp cavity of *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mouse incisor 3 months post-induction.**

H&E staining of incisors from control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice 3 months after induction. White dashed lines in (B) outline the stacked dentin in the dental pulp cavity. Black dotted two-way arrow shows the width of the dental pulp cavity in control (A) and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice (B). Schematic at the bottom indicates induction protocol.

N=3. Scale bars: 100  $\mu$ m.

Figure S3

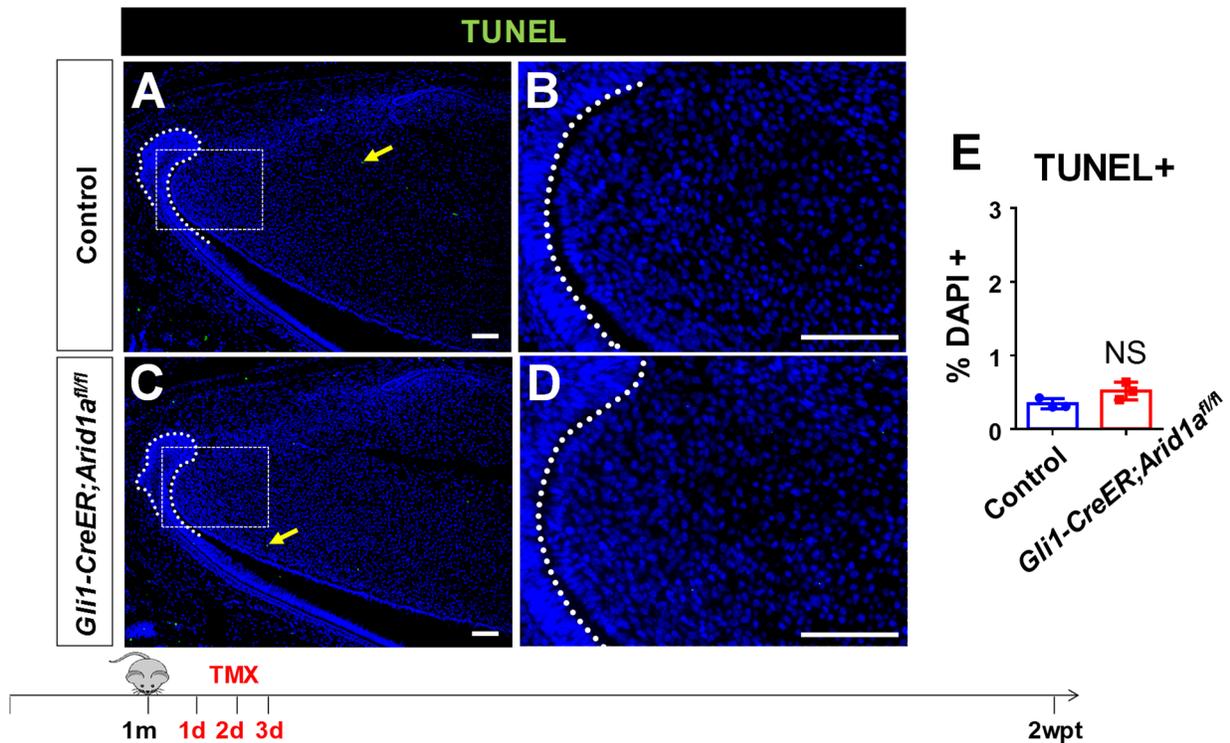


**Figure S3. Loss of *Arid1a* in the dental epithelium leads to no apparent odontoblast defects 1 month after induction.**

(A-D) *Arid1a* immunofluorescence of incisors from control (A-B) and *Sox2-CreER;Arid1a<sup>fl/fl</sup>* (C-D) mice 1 month after induction. Boxes in A and C are shown enlarged in B and D. White dotted line outlines the cervical loop. Yellow arrow in B indicates positive signal in control mice; asterisk in D indicates absence of signal in *Sox2-CreER;Arid1a<sup>fl/fl</sup>* mice. N=3. (E-H) H&E staining of incisor from control (E, F) and *Sox2-CreER;Arid1a<sup>fl/fl</sup>* (G, H) mice 1 month after induction. Boxes in E and G are shown enlarged in F and H. Black arrows indicate the initiation of odontoblast polarization. N=3. *In situ* hybridization of *Dspp* (red) in incisors of control (I, J) and *Sox2-*

*CreER;Arid1a<sup>fl/fl</sup>* (K, L) mice 1 month after induction. Boxes in I and K are shown enlarged in J and L. White dotted line outlines the cervical loop. White arrows indicate the initiation of odontoblast differentiation. Yellow dotted two-way arrow shows the distance between the bending point of the cervical loop and the initiation of odontoblast differentiation. N=3. Schematic at the bottom indicates induction protocol. Scale bars: 100  $\mu$ m.

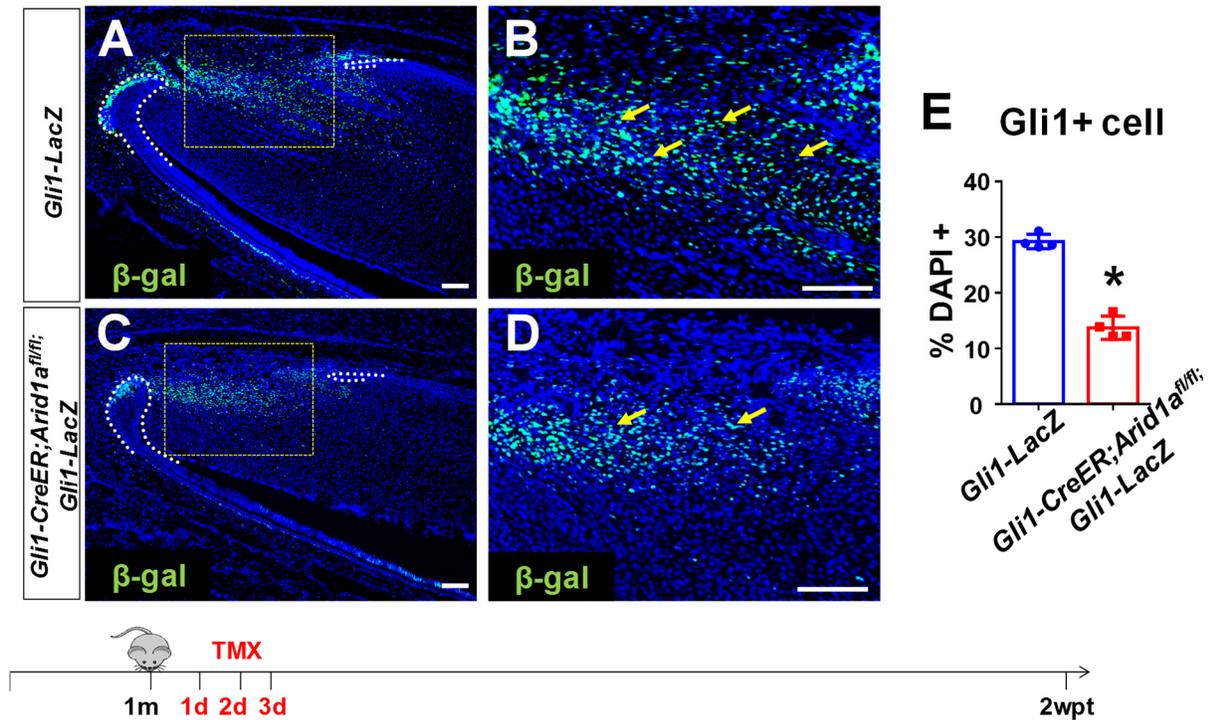
Figure S4



**Figure S4. Loss of *Arid1a* leads to no apparent change of cell apoptosis 2 weeks after induction.**

(A-D) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of incisors from control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice 2 weeks after induction. White dotted line outlines the cervical loop. Boxes in A and C are enlarged on the right. Arrows in A and C indicate positive signals. (E) Quantification of TUNEL+ cells in dental mesenchyme in control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice 2 weeks after induction. N=3, unpaired, two-tailed Student's t-test,  $p > 0.05$ , NS, no significant difference. Schematic at the bottom indicates induction protocol. Scale bars: 100  $\mu$ m.

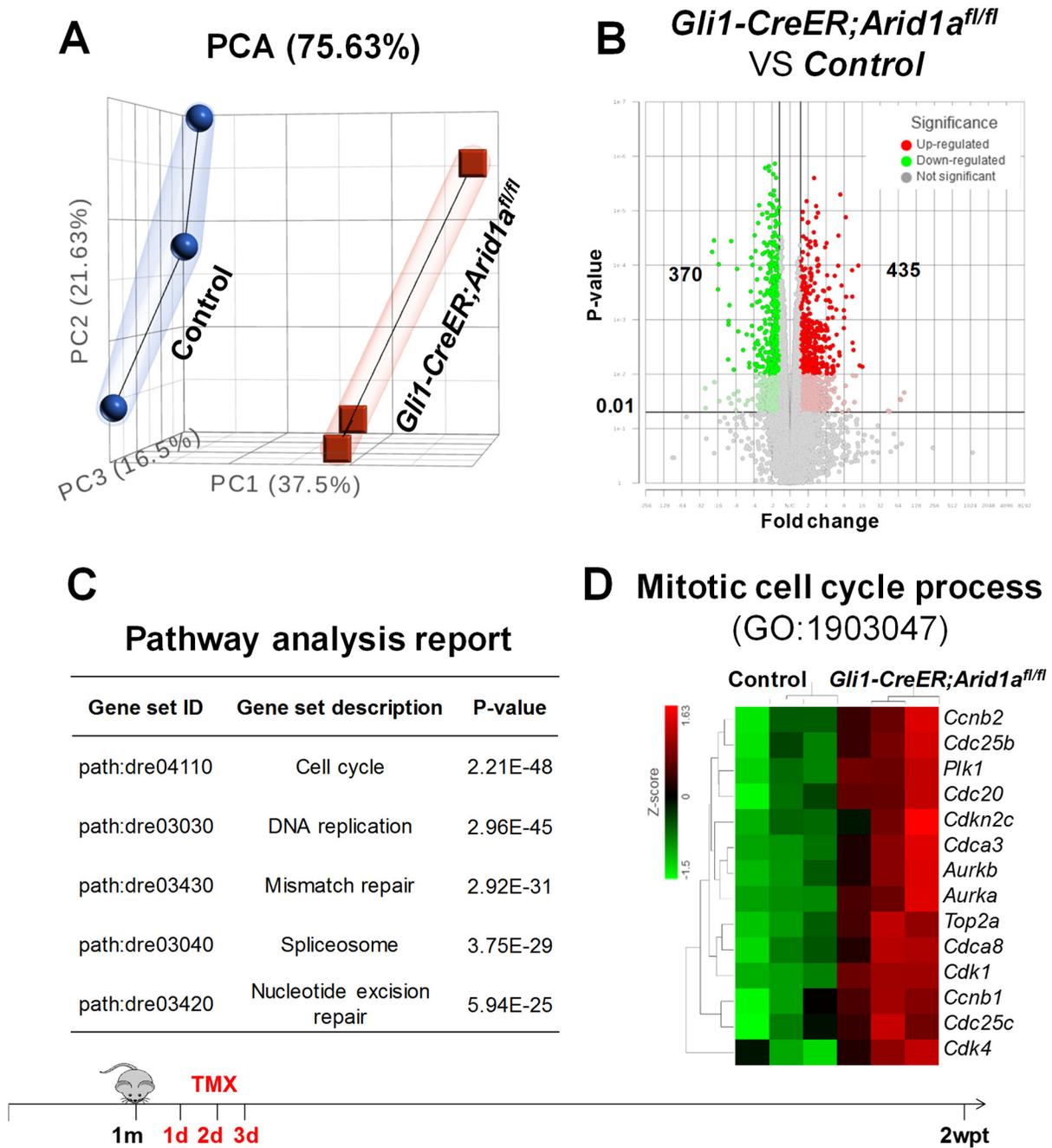
## Figure S5



**Figure S5. Loss of *Arid1a* leads to reduction of Gli1+ MSCs 2 weeks after induction.**

(A-D) Immunofluorescence of  $\beta$ -gal (green) of incisor from *Gli1-LacZ* (A-B) and *Gli1-CreER;Arid1a<sup>fl/fl</sup>;Gli1-LacZ* (C-D) mice 2 weeks after induction. White dotted line outlines the cervical loop. Boxes in A and C are shown enlarged in B and D. Arrows in B and D indicate positive signal. Quantification of Gli1+ cells in the dental mesenchyme of *Gli1-LacZ* and *Gli1-CreER;Arid1a<sup>fl/fl</sup>;Gli1-LacZ* mice 2 weeks after induction. N=4, unpaired, two-tailed Student's t-test, \* $p < 0.05$ . Schematic at the bottom indicates induction protocol. Scale bars: 100  $\mu$ m.

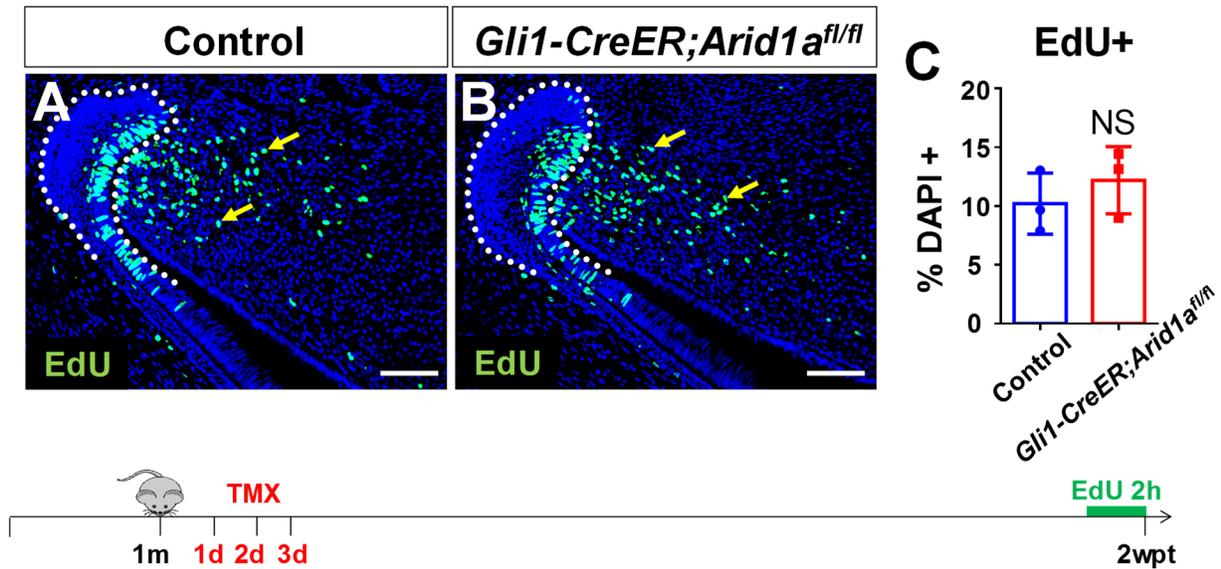
Figure S6



**Figure S6. RNA sequencing of control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mouse incisor mesenchyme.** (A) Principal component analysis showing two distinct clusters belonging to control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mouse incisor mesenchyme 2 weeks after induction.

(B) Volcano plot showing that 370 genes were downregulated and 435 genes were upregulated ( $>1.5$ -fold,  $p < 0.01$ ) in *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice compared to control samples. (C) Pathway analysis report showing the top 5 differentially expressed gene sets between control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice. (D) Heat map showing differential expression of a set of mitosis-associated genes between control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice. N=3. Schematic at the bottom indicates induction protocol.

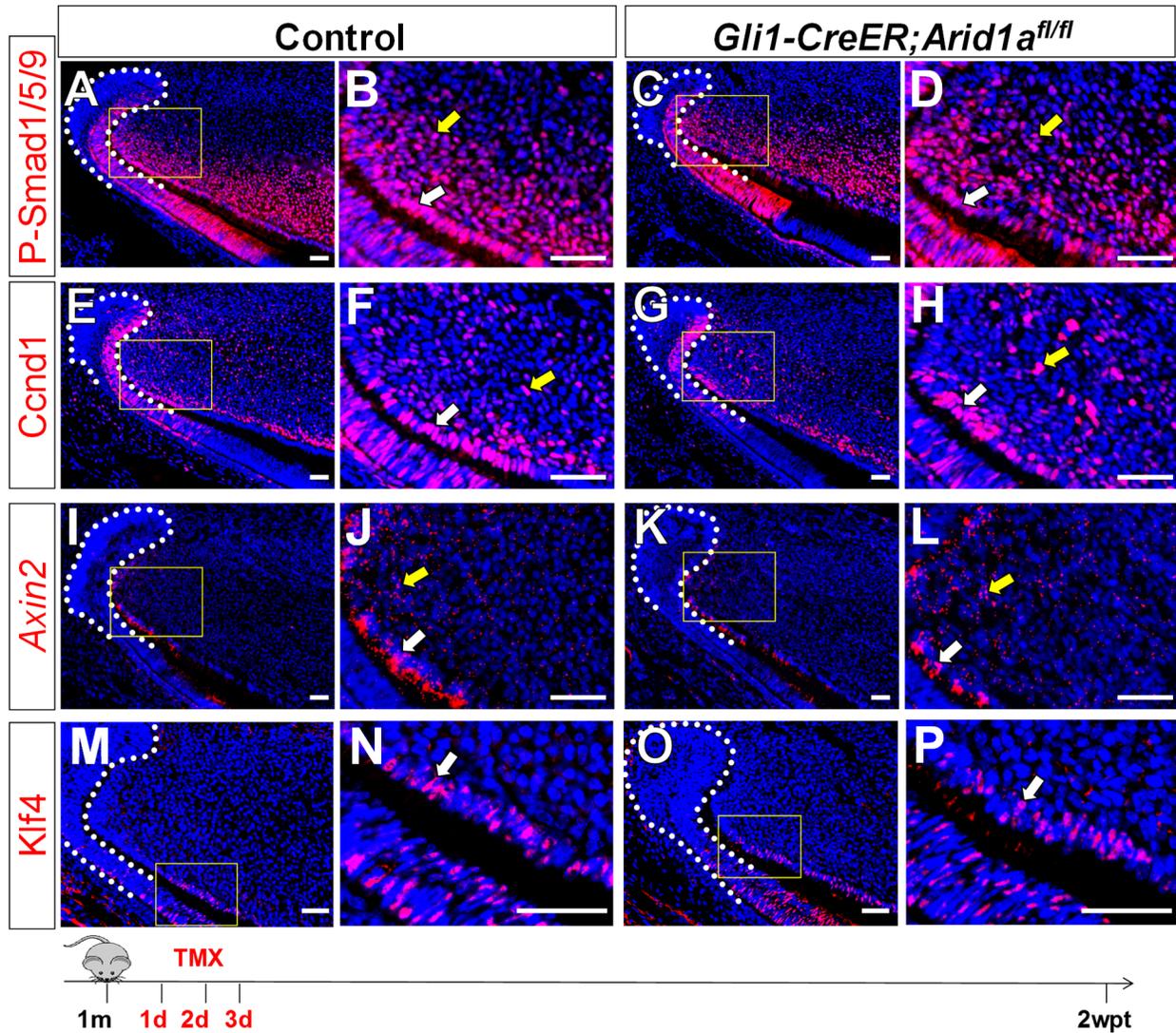
Figure S7



**Figure S7. Loss of *Arid1a* leads to no apparent change in the number of cells in the DNA synthesis phase labeled by EdU 2 weeks after induction.**

(A-B) EdU staining of incisors from control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice 2 weeks after induction. White dotted line outlines the cervical loop. Arrows in A and B indicate positive signals. N=3. (C) Quantification of EdU+ cells in dental mesenchyme in control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice 2 weeks after induction. N=3. unpaired, two-tailed Student's t-test,  $p > 0.05$ , NS, no significant difference. Schematic at the bottom indicates induction and EdU incorporation protocol. Scale bars: 100  $\mu$ m.

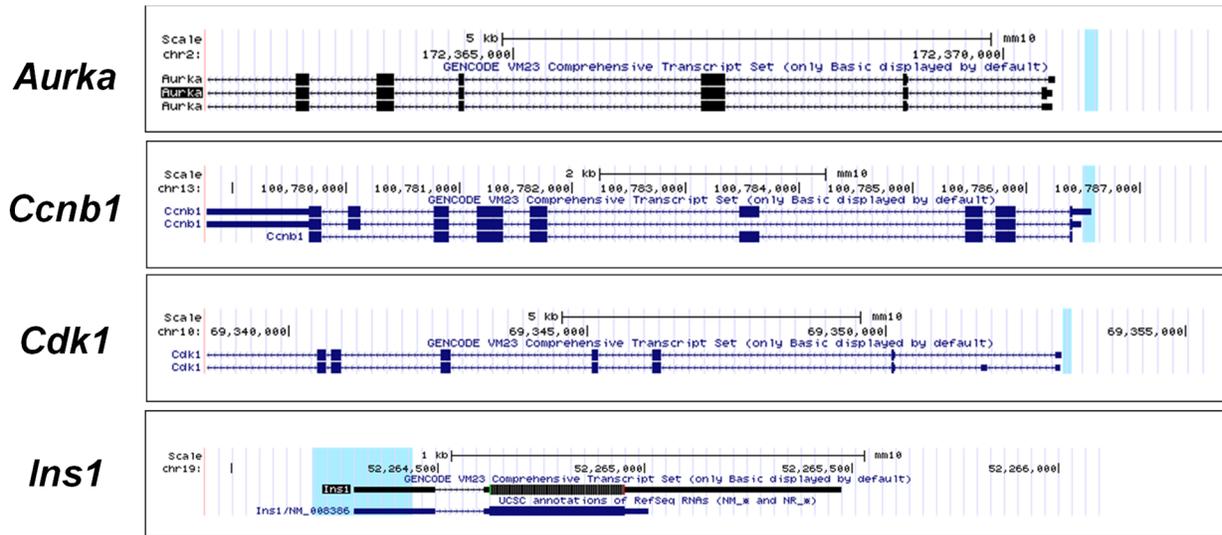
Figure S8



**Figure S8. Loss of *Arid1a* leads to no apparent change in the expression levels of p-Smad1/5/9, *Ccnd1*, *Axin2* and *Klf4* 2 weeks after induction.**

Immunofluorescence (red) of p-Smad1/5/9 (A-D), *Ccnd1* (E-H), and *Klf4* (M-P) and RNAscope *in situ* hybridization (red) of *Axin2* (I-L) on incisors from control and *Gli1-CreER;Arid1a<sup>fl/fl</sup>* mice 2 weeks after induction. White dotted line outlines the cervical loop. Boxes in A, C, E, G, I, K, M, and O are shown enlarged in B, D, F, H, J, L, N, and P, respectively. Yellow arrows indicate positive signal in TAC region; white arrows indicate positive signal in pre-odontoblast and odontoblast regions. N=3. Schematic at the bottom indicates induction protocol. Scale bars: 100  $\mu$ m.

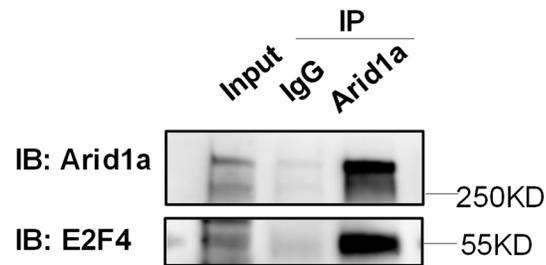
**Figure S9**



**Figure S9. Genomic location of the primer sets used in chromatin immunoprecipitation (ChIP) assay.**

Images representing the relative locations of the primer set amplicon (blue region) at the promoter regions of *Aurka*, *Ccnb1*, *Cdk1* and *Ins1* (negative genomic control) within the genome, as generated by the UCSC Genome Browser.

## Figure S10



### Figure S10. Arid1a and E2F4 interact in the proximal incisor mesenchyme.

Co-immunoprecipitation (co-IP) experiments using the proximal region of the incisor mesenchyme from control adult mice with Arid1a antibody (or IgG), followed by immunoblotting of Arid1a and E2F4. IP, immunoprecipitation. IB, immunoblotting.

### Table S1. Key resources table.

[Click here to download Table S1](#)