SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Measurements of the PNS and LP. (Top) Graphical representation of average width of the primordial nasal septum (PNS) and lamina propria (LP) in wild-type and *TEC1KO* embryos. Note that these tissues were significantly thicker in the mutants compared to normal ($p \leq 0.0001$). (Bottom) Despite the increased thickness of the PNS, overall cell number in the tissue was unchanged between wild-type and *TEC1KO*.

Supplemental Figure 2. LacZ staining of transverse sections through the nasal septum. LacZ staining of E14.5 (left panels) and E17.5 (right panels) in the primordial nasal septum (PNS) and the lamina propria (LP), indicating cre expression in those tissues. The olfactory epithelium (OE) was devoid of staining, confirming morphologic observations in mutants exhibiting nasal septal defects, whereas the olfactory epithelium is essentially normal. Scale bars in the wild-type E14.5 panels apply to the remaining images in the corresponding rows. The top left panel scale bar represents 500 um, middle left represents 250 um, and bottom left represents 50 um.

Supplemental Figure 3. Proliferation and apoptosis analysis in the PNS. (A) Graphical representation of BrdU incorporation assays showing the percent of cells that were actively dividing in the primordial nasal septum (PNS). No statistically relevent change was observed between wild-type and *TEC1KO*. (B-C) Cleaved Caspase-3 staining revealed no difference between wild-type (B) and *TEC1KO* (C) septa. Magnification 40x.

Supplemental Figure 4. Phospho-Smad 1/5/8 immunohistochemistry. (Top left) 40x view of the wild-type nasal septum (PNS). Phospho-Smad 1/5/8 staining was observed throughout the PNS and lamina propria (LP). (Top right) 10x magnification of *TEC1KO* PNS. No changes in

expression were noted, however. Additionally, no difference in staining was noted in the *TEC1KO* mandible (bottom right) compared to wild-type (bottom left).

Supplemental Figure 5. PTHrP immunohistochemistry. No staining for PTHrP was observed in either the wild-type (left) or *TEC1KO* (middle) primordial nasal septa (PNS) or lamina propria (LP). Neonate liver was used as a positive control for the staining procedure (right). Magnification 40x.

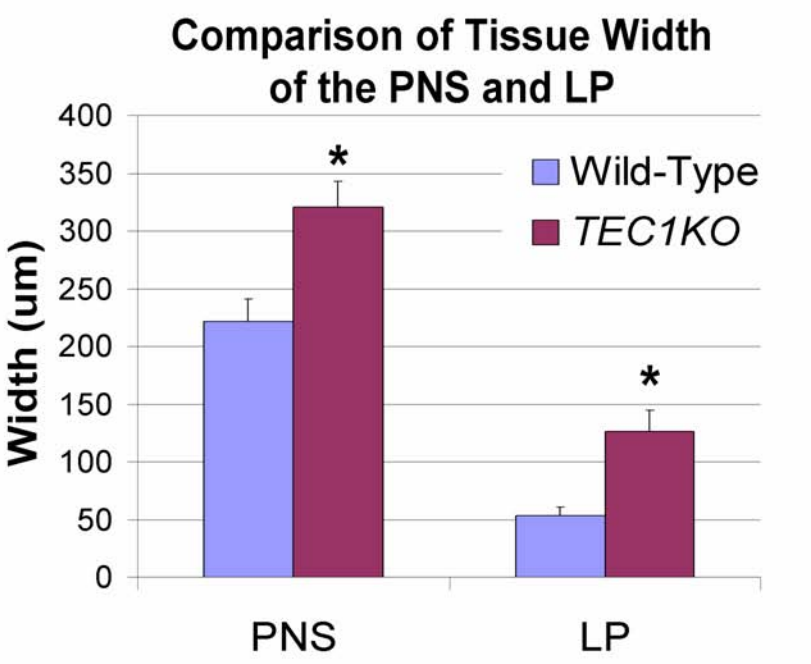
Supplemental Figure 6. Sox9 immunohistochemistry. At E14.5 the nasal septa (PNS) of wild-type (top left) and *TEC1KO* (top right) show no difference in staining pattern for Sox9. At E17.5, the staining is still unchanged between wild-type (bottom left) and *TEC1KO* (bottom right). Magnification 40x.

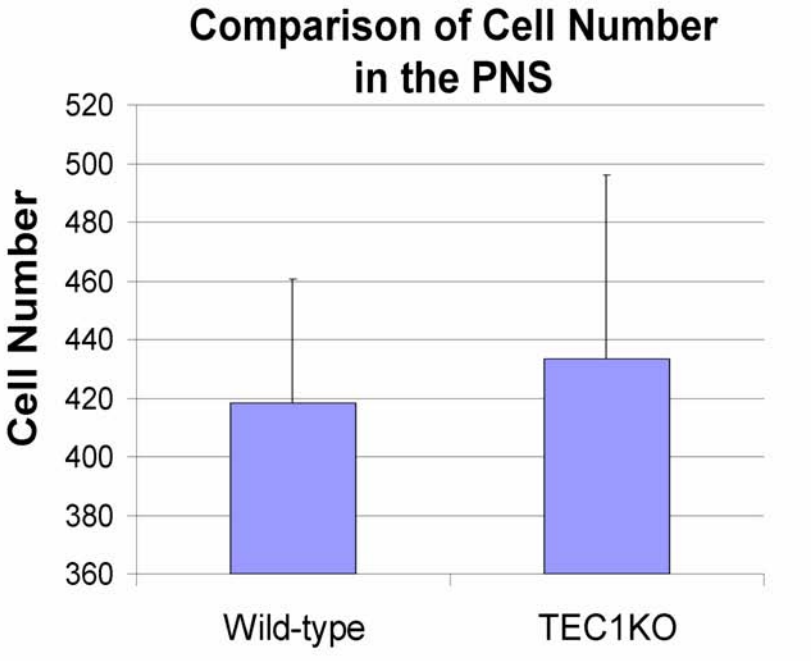
SUPPLEMENTAL MATERIALS AND METHODS

Immunohistochemistry. Primary embryonic tissues were fixed in 10% formalin, dehyrated in ethanol and xylenes and embedded in paraffin wax. Histological sections were deparaffinzed and boiled in antigen retrieval solution (Vector Labs, Burlingame, CA) for 20 min. The slides were blocked in 5% goat serum and incubated in primary antibody diluted in block solution. Secondary biotinylated antibodies were used (Vector Labs), and color development was ahieved using the ABC Elite kit (Vector Labs) and DAB chromogen (Dako, Carpinteria, CA). The following primary antibodies were used in this study: Sox9 and PTHrP (Santa Cruz Biotechnology, Santa Cruz, CA); Phospho-CREB, cleaved Caspase-3, and Phospho-Smad 1/5/8 (Cell Signaling Technology, Danvers, MA).

LacZ Enzymatic Stain. Primary embryonic tissues were harvested, embedded in Tissue-Tek O.C.T Compound (Sakura, Torrance, CA), and frozen to -80^{o}C. Histological sections were obtained using a cryostat and fixed with 0.2% glutaraldehyde. Staining was achieved using 1 mg/ml X-gal (Sigma Aldrich, St. Louis, MO) at 37^{o}C overnight. Slides were subsequently dehydrated and mounted with coverslips.

Imaging and Analysis. Histological staining was examined using an Olympus BX50 microscope and images were taken using Spot Basic software, version 4.1 (Diagnostic Instruments, Sterling Heights, MI). Cell number counts and width measurements of specific tissues (such as the PNS or LP) were taken using Meta Vue software, version 6.1 (Universal Imaging Corp, Dowingtown, PA). Numerical results were analyzed using a standard two-sample T-test to check for statistical relevance.


Comparison of Tissue Width of the PNS and LP
Width (um)
400
350
300
250
200
150
100
50
0
*
*
Wild-Type
TEC1KO
PNS
LP


Comparison of Cell Number in the PNS
Cell Number
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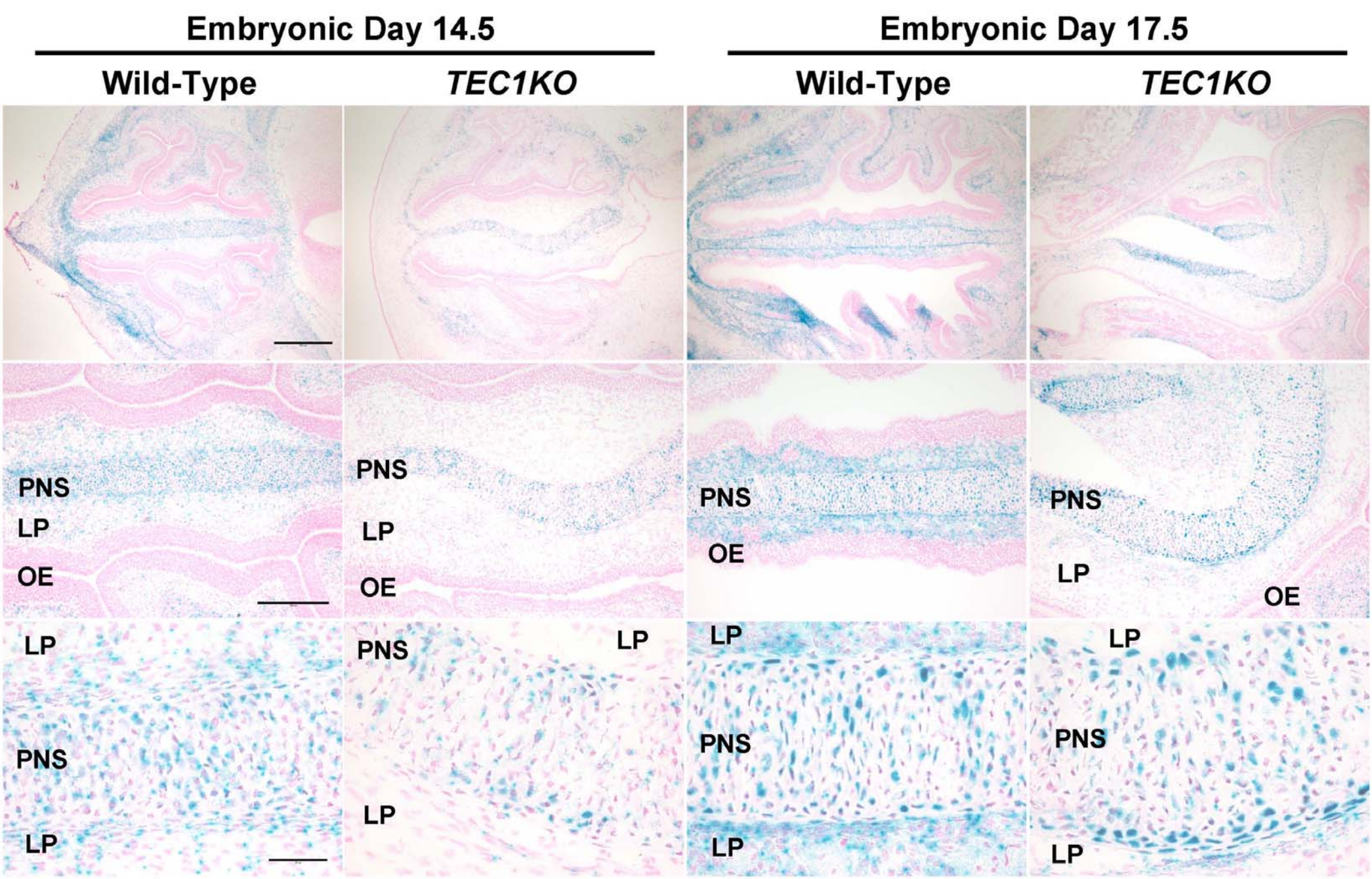
480
460
440
420
400
380
360
Wild-type
TEC1KO


Embryonic Day 14.5
Embryonic Day 17.5
Wild-Type
TEC1KO
Wild-Type
TEC1KO
PNS
LP
OE
PNS
LP
OE
PNS
OE
PNS
LP
OE
LP
PNS
LP
PNS
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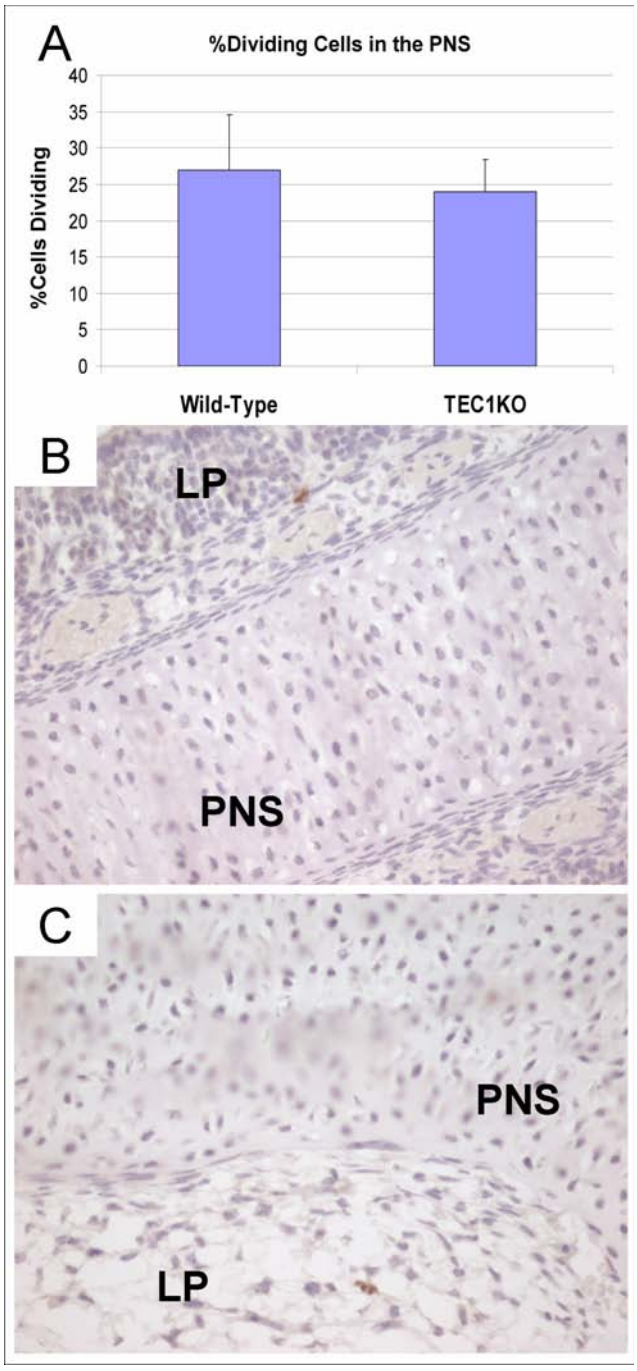
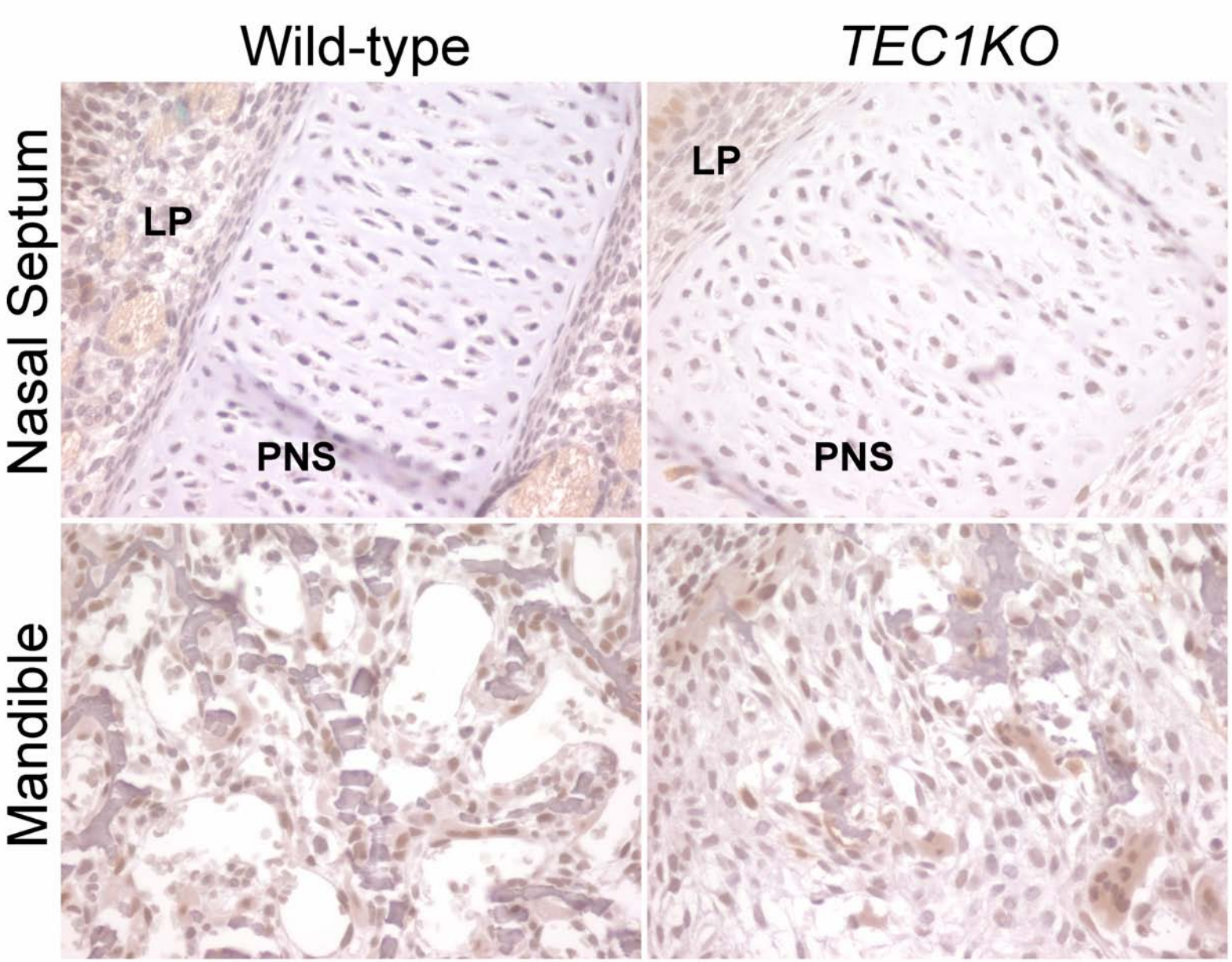
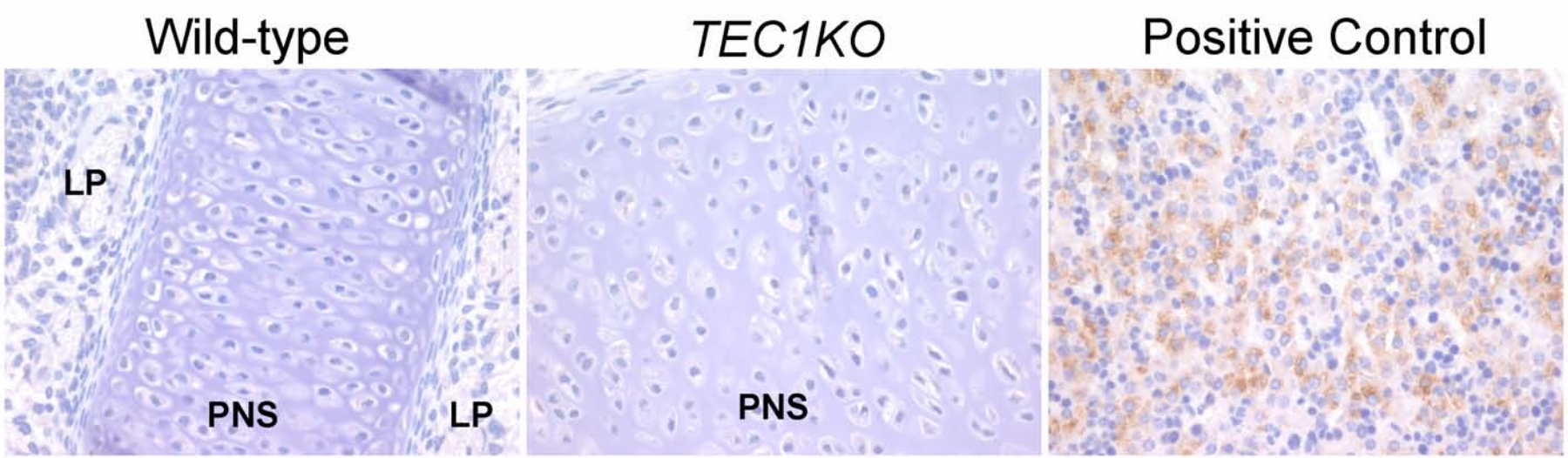
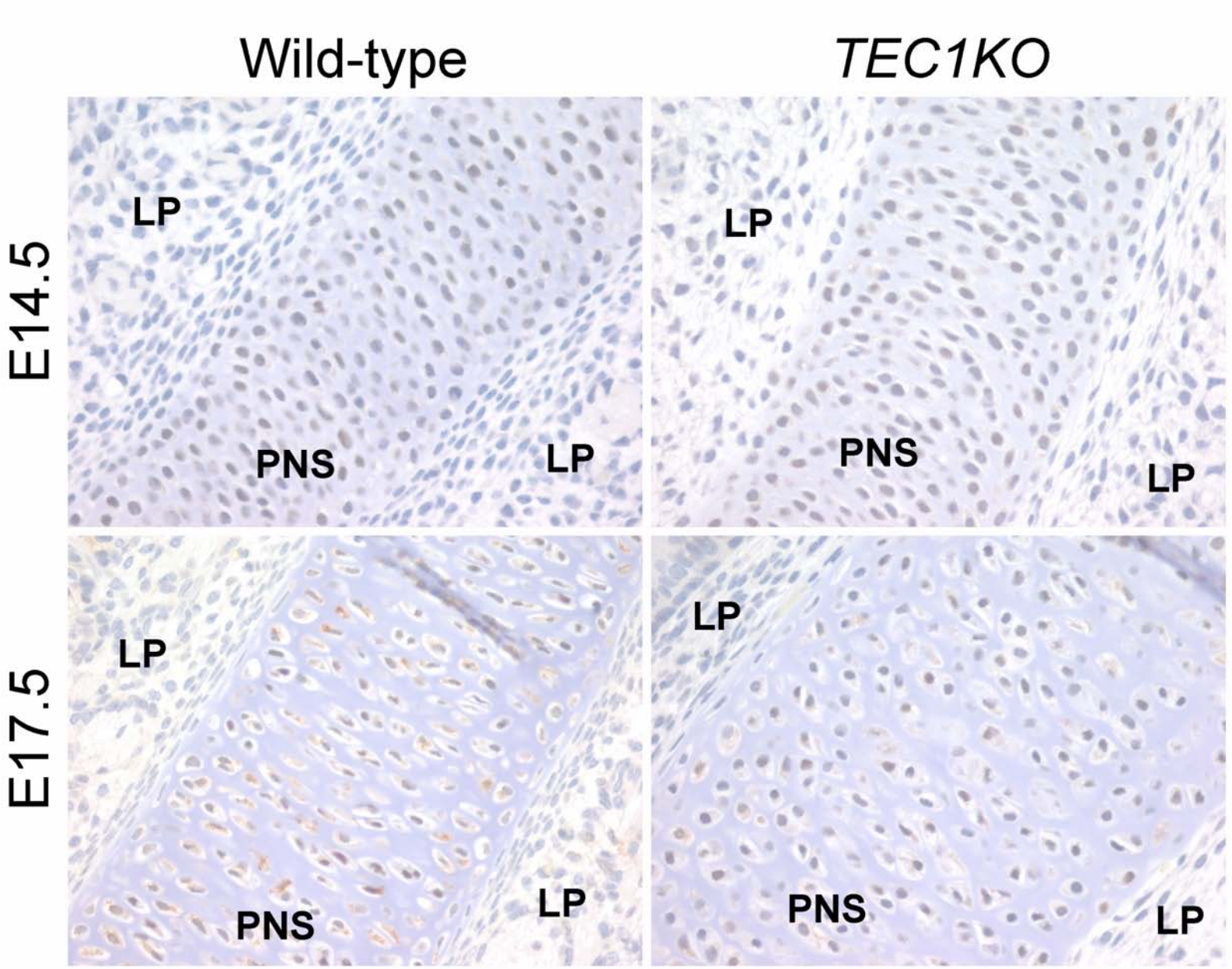
LP
PNS
LP
LP
PNS
LP


A
%Dividing Cells in the PNS
%Cells Dividing
40
35
30
25
20
15
10
5
0
Wild-Type
TEC1KO
B
LP
PNS
C
PNS
LP


Wild-type
TEC1KO
Nasal Septum
Mandible
LP
PNS
LP
PNS


Wild-type
TEC1KO
Positive Control
LP
PNS
LP
PNS


Wild-type
TEC1KO
E14.5
E17.5
LP
PNS
LP
LP
PNS
LP
LP
PNS
LP
LP
PNS
LP