

**A**

Color Key

Normalized Read Counts

BE  
PE

BE3 BE4 BE5 PE4 PE2 PE3

**B**

external\_gene\_name

$-\log_{10}(padj)$

**C**

PI3K-Akt signaling pathway

Human papillomavirus infection

Focal adhesion

ECM-receptor interaction

Protein digestion and absorption

Axon guidance

TGF-beta signaling pathway

Estrogen signaling pathway

Small cell lung cancer

Amoebiasis

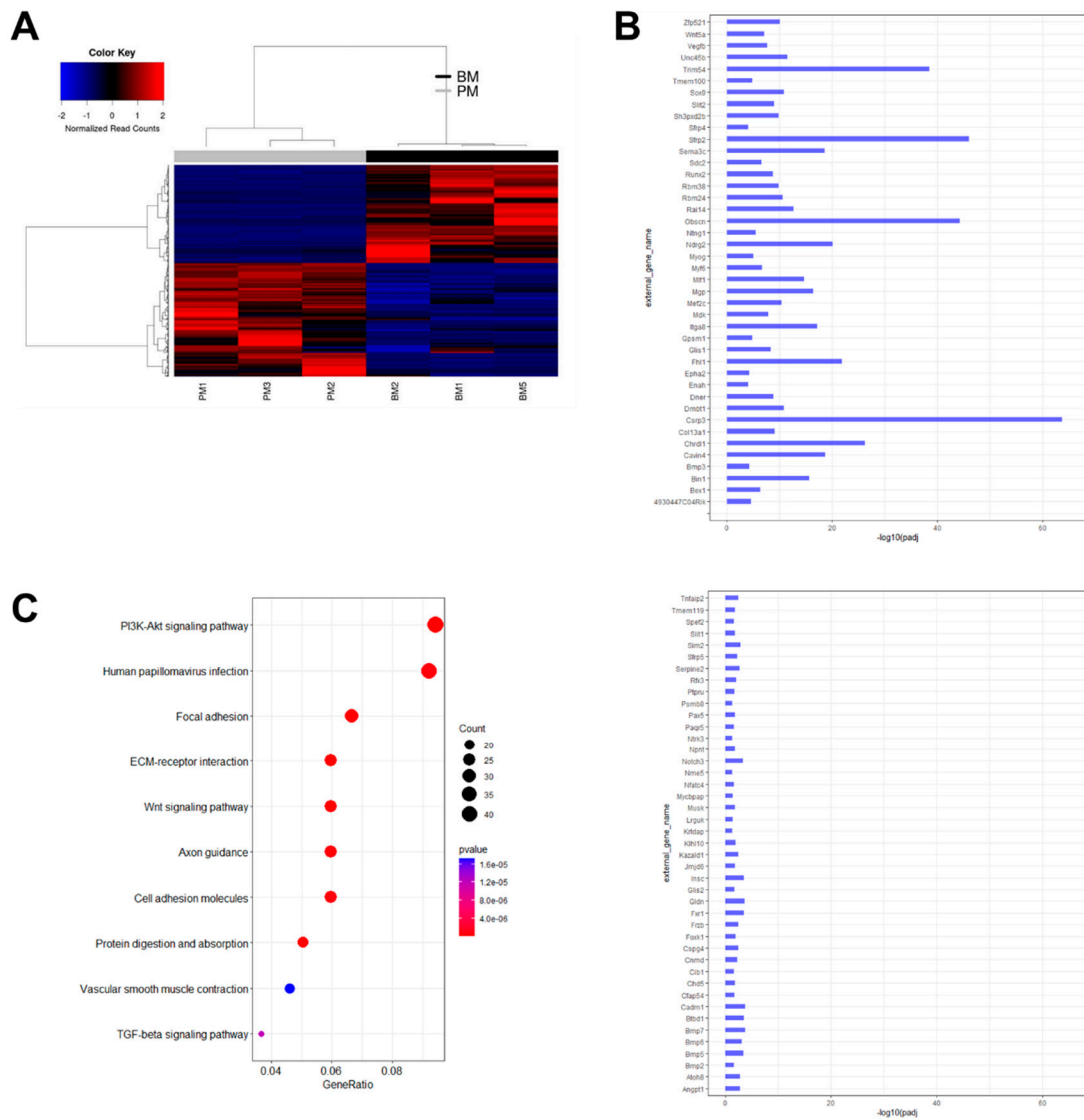
GeneRatio

Count

pvalue

**(A)** The heatmap shows the comprehensive differences in the gene expression between keratinized and non-keratinized epithelium ( $n=10$  mice/group, 3 groups).

**(C)** Over Representation Analysis revealed 10 of the most significant KEGG pathways from the upregulated DEGs.



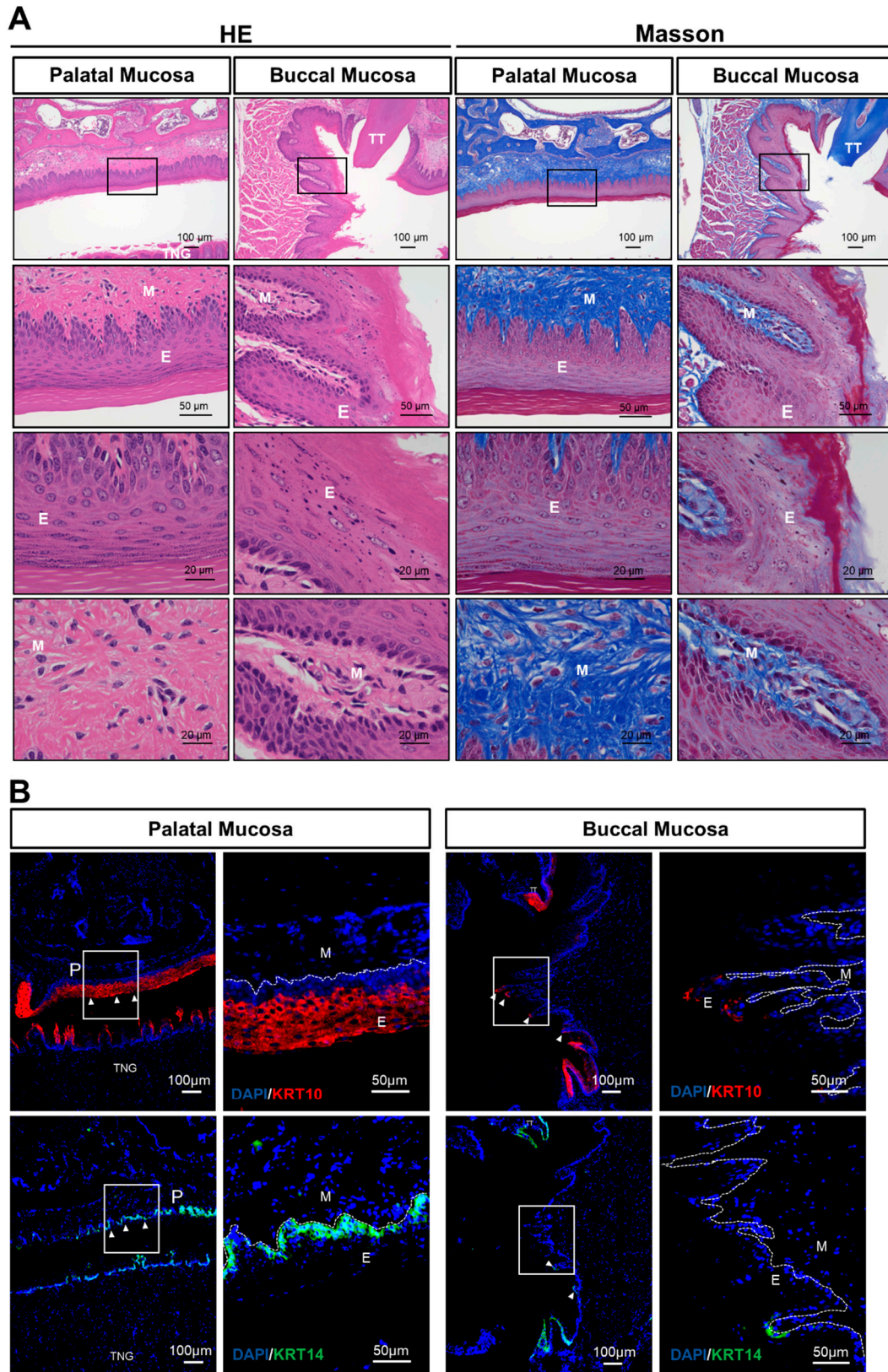
**Figure S2. Comparison of the mesenchymal tissue between keratinized and non-keratinized mucosa.**

(A) The heatmap shows the comprehensive differences in the gene expression between the mesenchyme of keratinized and non-keratinized mucosa ( $n=10$  mice/group, 3 groups).

(B) Eighty-five differentially expressed genes (DEGs) from mesenchymal comparison were associated with Uniprot keyword “Differentiation”.

(C) Over Representation Analysis revealed 10 of the most significant KEGG pathways from the upregulated DEGs.



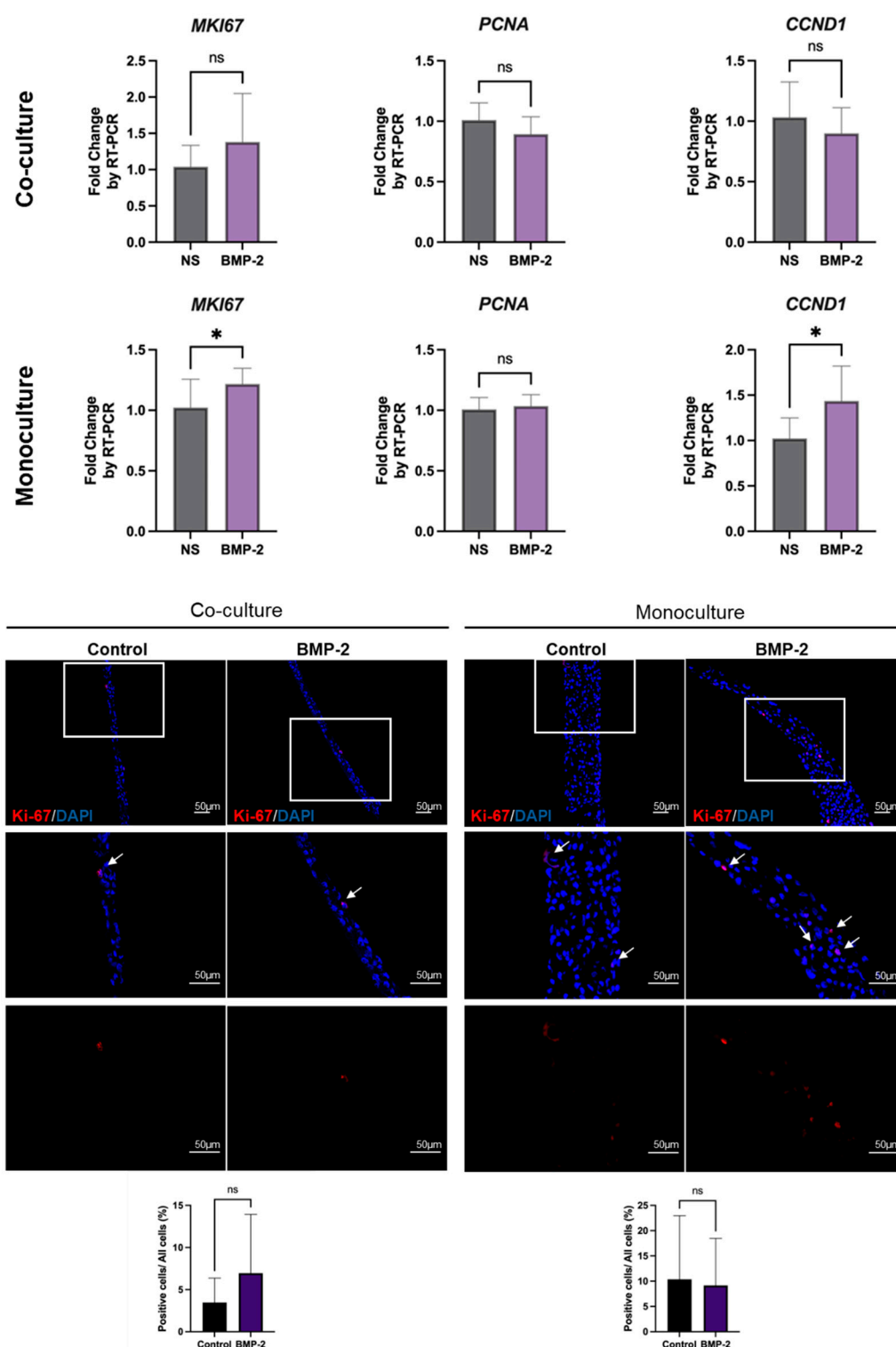


**Figure S4. Histological features of palatal and buccal mucosa.**

(A) HE and Masson's trichrome staining of palatal and buccal mucosa from 8-week-old mice. A sheet-like structure composed of accumulated dead cells was observed in the outermost

layer of both buccal and palatal mucosa. The palatal mucosa presented more flat and tightly packed cells in stratified epithelial layers, while the connective tissue of buccal mucosa was thinner and had less densely organized collagen fibers.

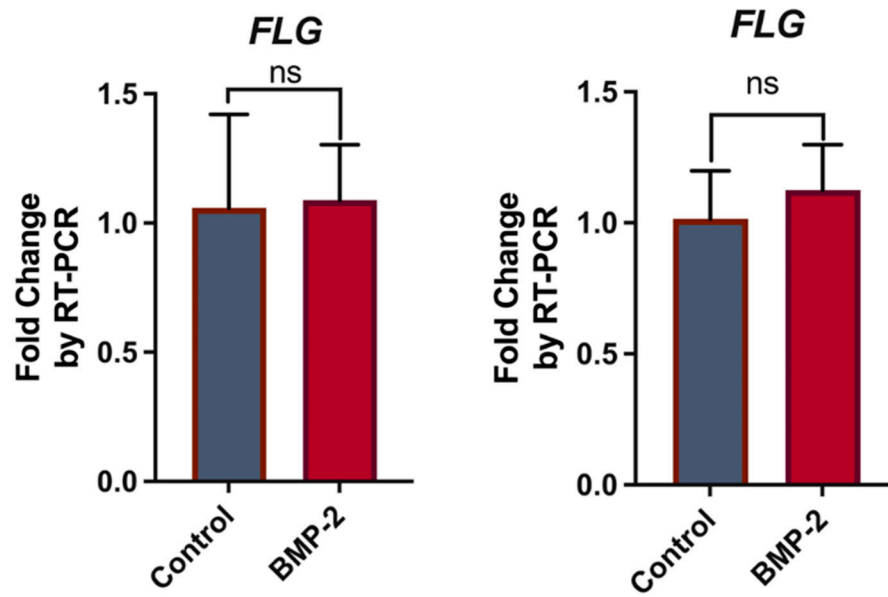
**(B)** Immunofluorescence analysis indicating the distribution of KRT10 and KRT14 in palatal and buccal mucosa. Both proteins were highly expressed in the epithelium of palatal mucosa, but only several scattered signals were detected in buccal mucosa. Arrowheads indicate positive cells for KRT10 and KRT14. E, Epithelium; M, Mesenchyme; TT, Tooth; TNG, Tongue.



**Figure S5. The expression of proliferation markers in Air-Liquid Interface (ALI) cell culture.**

Quantification of proliferation genes after treatment with BMP-2 in co-cultured and monocultured ALI by RT-qPCR and immunofluorescence staining analysis. Arrowheads indicate positive cells for Ki67. Scale bar: 50  $\mu$ m. The graphs show the number of positive cells/total cells (%). All experiments were performed in triplicate ( $n=3$ ). Data are expressed

as mean  $\pm$  SD. ns=non-significant. \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ , two-tailed unpaired t test.



**Figure S6. The gene expression of epithelial late differentiation marker in Air-Liquid Interface (ALI) cell culture.**

After stimulating by BMP-2, the gene expression of FLG in both the co-cultured and monocultured epithelial cells (TR146) was measured by RT-qPCR analysis. All experiments were performed in triplicate ( $n=3$ ). Data are expressed as mean  $\pm$  SD. Significance values were calculated with two-tailed unpaired t test. ns=non-significant.



**Table S1. The design of PCR primers.**

Target gene	Type	GeneBank registration number	Primer set
<b>S29</b>	human	BC032813	5'-TCTCGCTCTTGTCTGTCTGTTC-3'(S) 5'-ACACTGGCGGCACATATTGAGG-3'(AS)
<b>KRT10</b>	human	NM_000421	5'-ACACCGCACAGAACCACCACTC-3'(S) 5'-GGCAGGCACAGGTCTTGATGAAC-3'(AS)
<b>KRT14</b>	human	BC042437	5'-ACAGATCCCACTGGAAGAT-3'(S) 5'-AGATAATGAAGCTGTATTGATTGC-3'(AS)
<b>KRT15</b>	human	BT007261	5'-TGCTGCTTGACATAAAGACA-3'(S) 5'-CTACCACCACCTCCTGAA-3'(AS)
<b>KRT16</b>	human	AF061812	5'-TGAGATGGAGCAGCAGAG-3'(S) 5'-GCGGGAAGAATAGGATTGG-3'(AS)
<b>IVL</b>	human	BC046391	5'-CCTCAGATCGTCTCATACAAG-3'(S) 5'-ACAGAGTCAAGTTCACAGATG-3'(AS)
<b>FLG</b>	human	NM_002016	5'-AGACTCTAGTACCGCTAAGG-3'(S) 5'-CGTGACTGTATTCCTGAGTG-3'(AS)
<b>LTBP1</b>	human	NM_206943	5'-TCTTACCAGTGCCTTCCCTG-3'(S) 5'-TCTACAGTGCTTGTGGTCCG-3' (AS)
<b>SERPINE1</b>	human	NM_000602	5'-TGACTGGGTGAAGACACACAC-3'(S) 5'-GAGTCGGGGAAGGGAGTCTT-3' (AS)
<b>IGF2</b>	human	NM_000612	5'-GACACCCTCCAGTTCGTCTG-3'(S) 5'-GCGGAAACAGCACTCCTCAA-3' (AS)
<b>LOX</b>	human	NM_002317	5'-GGATTGAGTCCTGGCTGTTATGA-3'(S) 5'-GTGTAGCGAATGTCACAGCG-3' (AS)
<b>IL24</b>	human	NM_006850	5'-ATTCTACAAGCTCTGAATGTCT-3' (S) 5'-CGTGAAGTGTCCAGTGAAC-3' (AS)
<b>MMP1</b>	human	BT006874	5'-AAGCGTGTGACAGTAAGC-3' (S) 5'-CGGGTAGAAGGGATTTGTG-3' (AS)
<b>ID1</b>	human	NM_002165	5'-TGCCTAAGGAGCCTGGAAAAAG-3'(S) 5'-ATGCCGCCTGTGAAAACGAG-3' (AS)
<b>Mki67</b>	human	NM_002417	CACTCCACCTGTCTGAAGA GCAGGTTGCCACTCTTTCTC
<b>PCNA</b>	human	NM_182649	ACCAGGAGAAAGTTTCAGACTATG GTGCAAATTCACCAGAAGGCA
<b>CCND1</b>	human	NM_053056	CACTCCTACGATACGCTACTA GCATCTCATAAACAGGTCCT