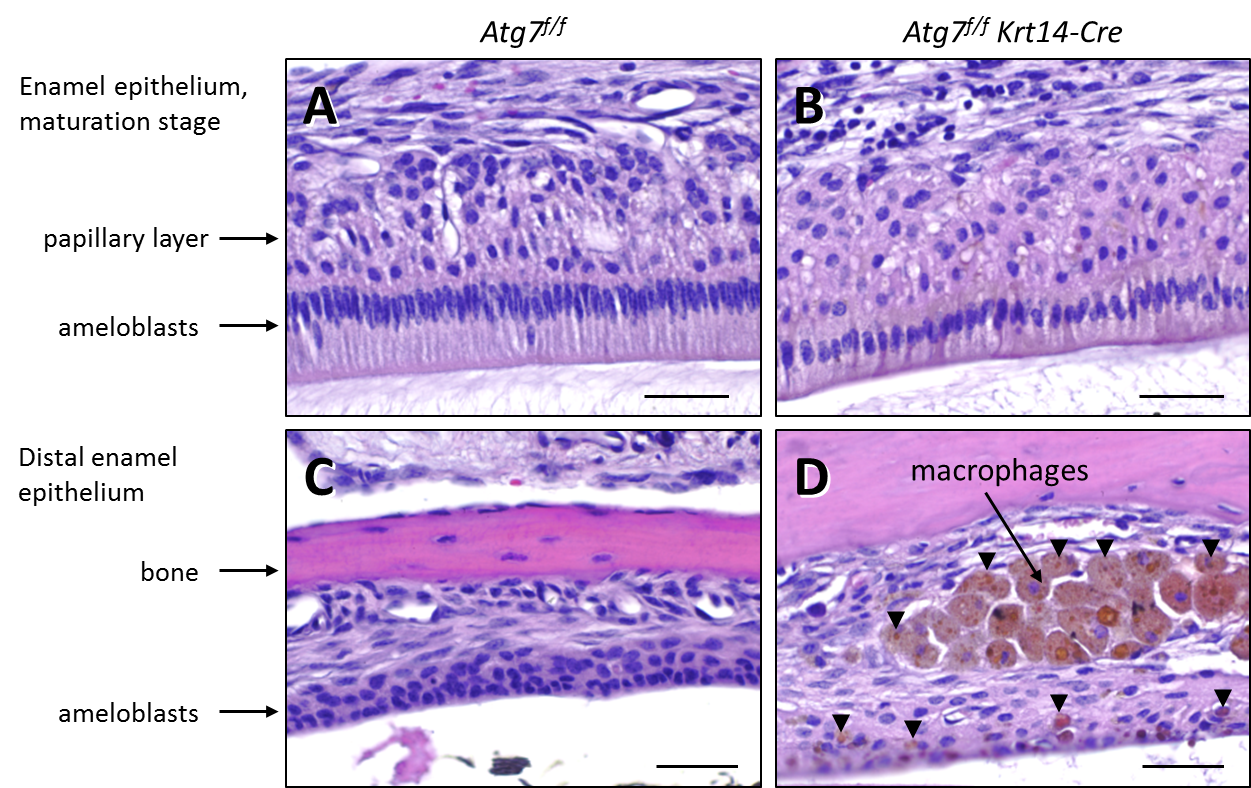
*sqstm1-/-*

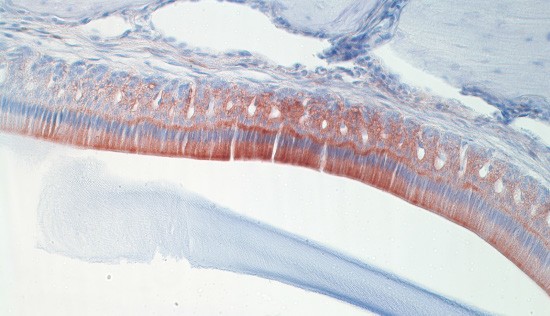


**Figure S1.** Deletion of *Sqstm1*/*p62* does not compromise pigmentation of murine incisors. The image is representative for n=5 *sqstm1*-deficient mice.



**Figure S2.** Histology of the maxillary enamel epithelium in fully autophagy-competent and epithelial autophagy-deficient mice. Tissue sections of *Atg7f/f* (**A**, **C**) and *Atg7f/f Krt14-Cre* (**B**,

**D**) mice were stained with hematoxylin and eosin. Brown color indicates accumulation of iron (arrowheads). Scale bars: 20 µm.



**A**

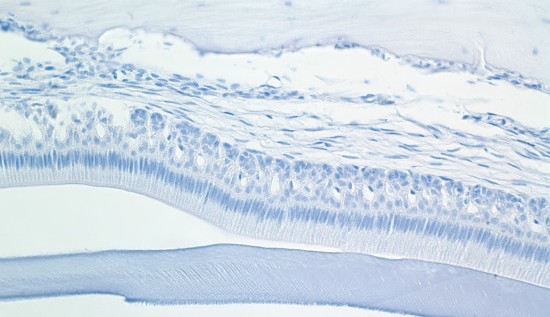
*Atg7f/f*

**ferritin**

pl

am

early pigmentation stage



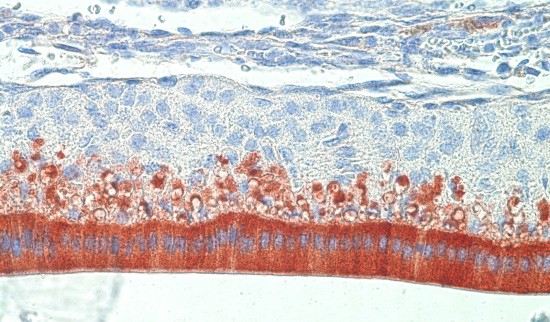
**B**

*Atg7f/f*

**neg. con.**

pl

am



**C**

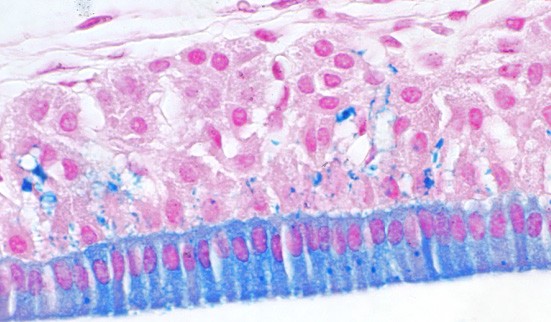
*Atg7f/f Krt14-Cre*

**ferritin**

pl

am

early pigmentation stage



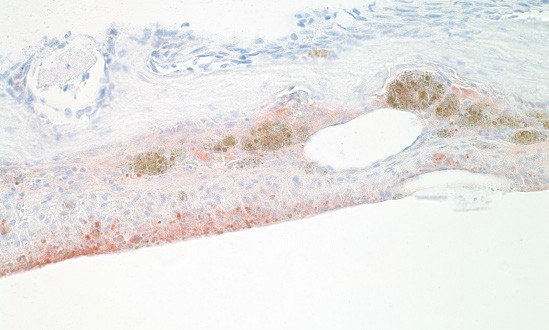
**D**

*Atg7f/f Krt14-Cre*

**iron**

pl

am

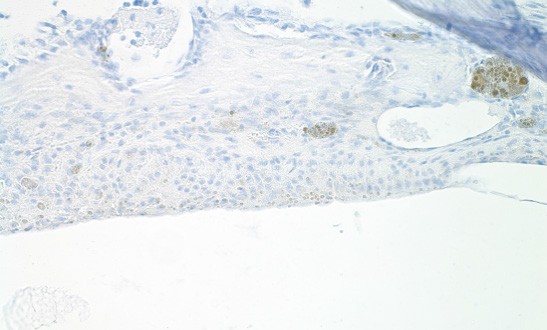


**E**

*Atg7f/f Krt14-Cre*

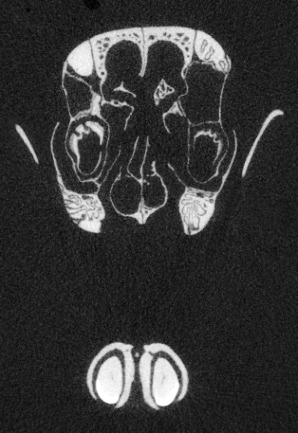
**ferritin**

distal epithelium



**F** *Atg7f/f Krt14-Cre* **neg. con.**

**Figure S3.** Control experiments and ferritin immunostaining of early pigmentation stage enamel epithelium. Tissue sections of maxillary incisors of *Atg7f/f* (**A**, **B**) and *Atg7f/f Krt14-Cre* (**C**-**D**) mice were investigated. These results show a similar distribution of ferritin in the early pigmentation stage enamel epithelium (including ameloblasts, am, and papillary layer, pl) of both genotypes (**A**, **C**), presence of both ferritin and iron in the papillary layer of the early pigmentation stage (**C**, **D**), and specificity of ferritin immunostaining (**A**, **B**, **E**, **F**). Ferritin was detected by immunohistochemistry with a primary antibody against FTH1 (**A**, **C**, **E**). In negative control staining the primary antibody was replaced by an isotype immunoglobulin (**B**, **F**). Iron was detected by staining with Perls’ Prussian blue (**D**). Nuclei were counterstained with hematoxylin (**A**-**C**, **E**, **F**) and nuclear fast red (**D**). Scale bars: 50 µm (**A**-**C**, **E**, **F**), 20 µm (**D**).



**C**

*Atg7f/f*



**A**



**B**



**F**

*Atg7f/f*

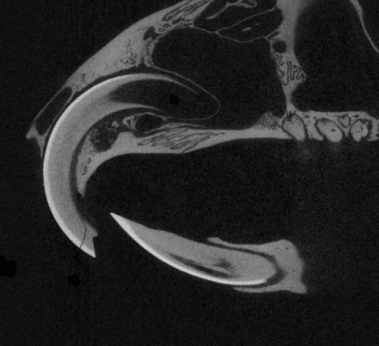


**D**



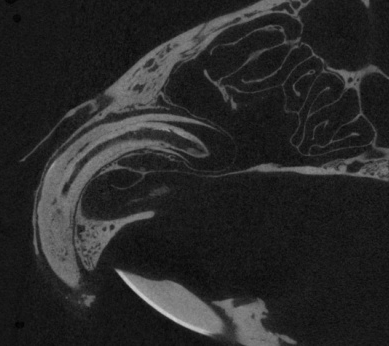
**E**

*Krt14-Cre*



**G**

*Atg7f/f*

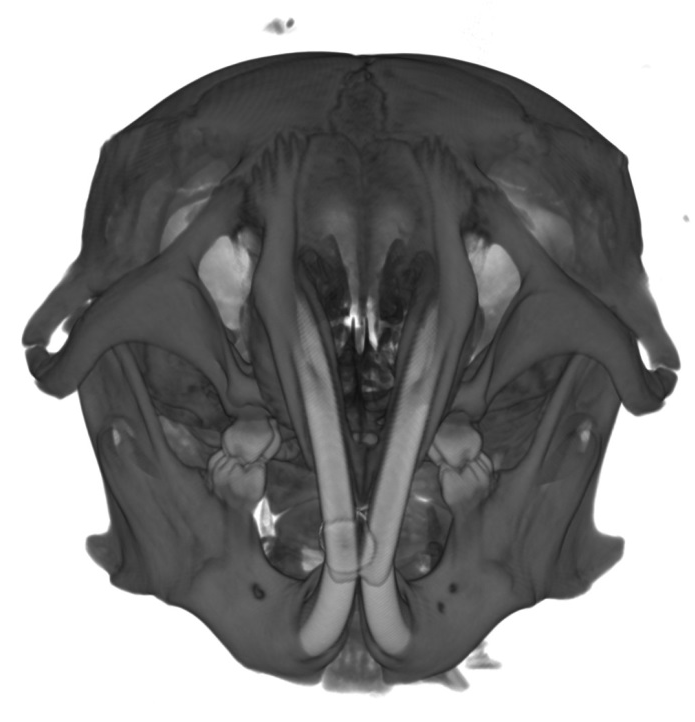


**H**

*Atg7f/f Krt14-Cre*

**Figure S4.** Micro-CT analysis of old mice. The heads of *Atg7f/f* (**A**-**C**, **G**) and *Atg7f/f Krt14-Cre* (**D**-**F**, **H**) mice aged >1.5 years were investigated by micro-CT. Frontal (**A**-**F**) and sagittal (**G**, **H**) sections are shown. Yellow arrowheads indicate the enamel surface of primordial maxillary incisors. Arrows indicate ectopic incisor tubes. The number of ectopic incisor tubes was significantly different between the 2 genotypes (0.0±0.0 *versus* 4.7±0.9, mean ± standard deviation, *P*=0.02, 2-sided t-test).

*Atg7f/f Atg7f/f Krt14-Cre*



**A**



**B**

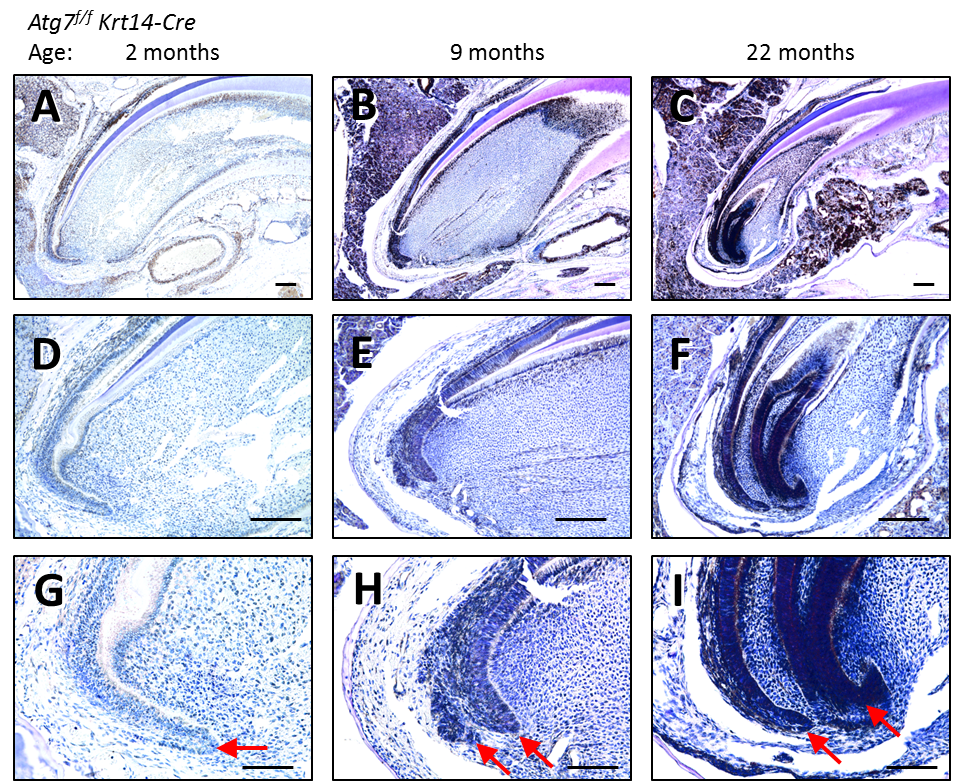


**C**



**D**

**Figure S5.** Micro-CT analysis of young mice. The heads of *Atg7f/f* (**A**, **C**) and *Atg7f/f Krt14-Cre* (**B**, **D**) mice aged <0.5 years were investigated by micro-CT. 3D reconstructions (**A**, **B**) and frontal (**C**, **D**) sections are shown. Yellow arrowheads indicate the enamel surface of maxillary incisors (**C**, **D**).



**Figure S6.** Histology of the cervical loops of maxillary incisors of *Atg7f/f Krt14-Cre* mice at different ages. Tissue sections were prepared from *atg7f/f Krt14-Cre* mice aged 2 (**A**, **D**, **G**), 9 (**B**, **E**, **H**), and 22 (**C**, **F**, **I**) months, and stained with hematoxylin and eosin. Red arrows indicate cervical loops. Scale bars: 200 µm (**A**-**F**), 100 µm (**G**-**I**).

*Ncoa4fl/fl; CMV-Cre*

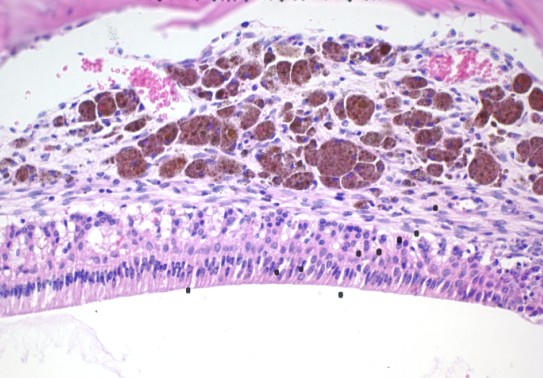
*Ncoa4fl/fl*

**Figure S7.** Deletion of the ferritinophagy receptor NCOA4 does not compromise pigmentation of murine incisors. The images show the incisors of *Ncoa4fl/fl; CMV-Cre* and *Ncoa4fl/fl* (representative for n=9) mice.



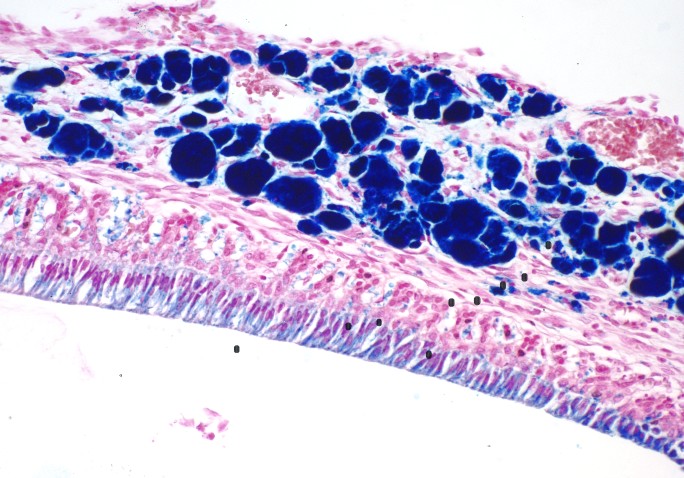
**A**

*nfe2l2-/-*



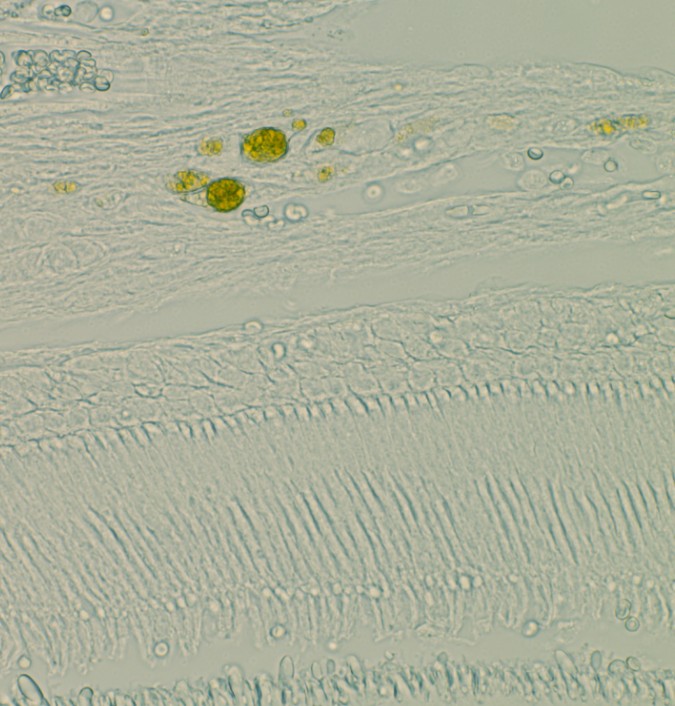
**B**

H&E



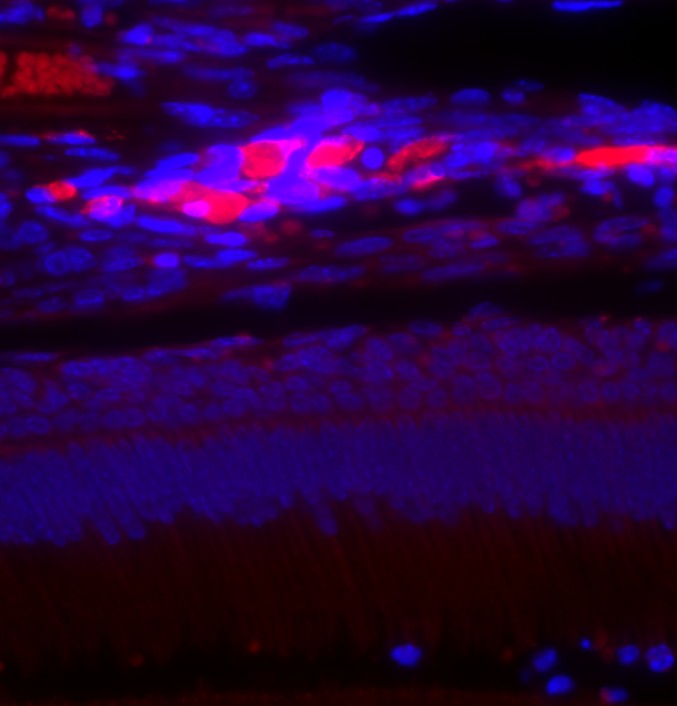
**C**

Perls' blue



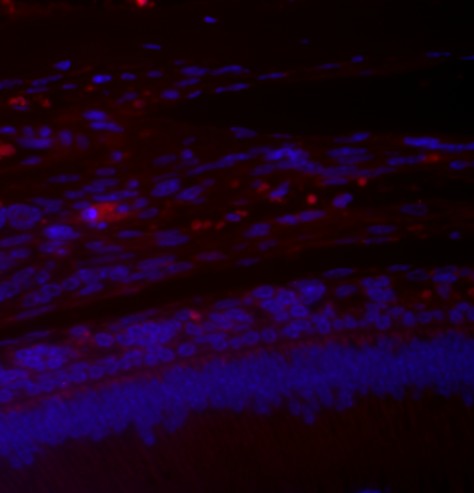
**D**

Bright field



**E**

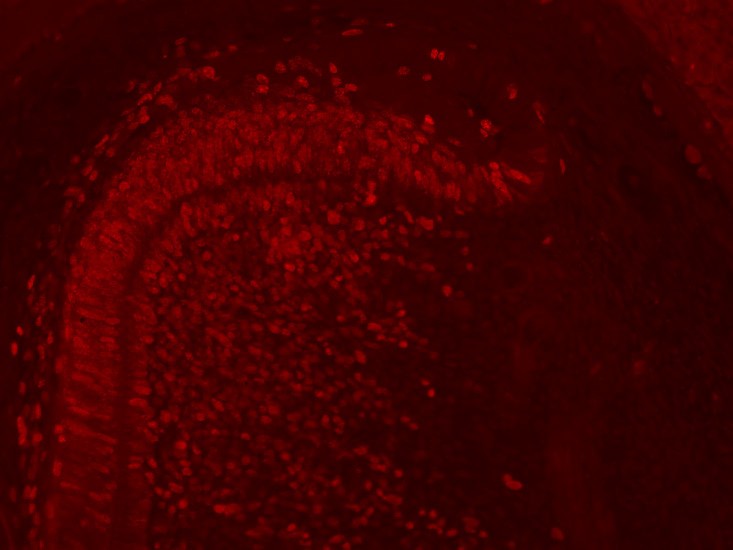
IF: LGASL3/Mac-2



**F**

IF: isotype control

**Figure S8.** *nfe2l2*/*nrf2* knockout incisors and enamel epithelium. (**A**) Macroscopic appearance of incisors of *nfe2l2* knockout mouse, representative for n=10. (**B**) Hematoxylin and eosin staining and (**C**) Perls' blue staining of the maxillary enamel epithelium. (**D**) Bright field image and (**E**) immunofluorescence (IF) labeling for the macrophage marker LGALS3/Mac-2 (red) of the same site. Arrows indicate macrophages containing iron. (**F**) Isotype control of immunofluorescence labeling. Scale bars: 50 µm (**B**, **C**), 20 µm (**D**-**F**).

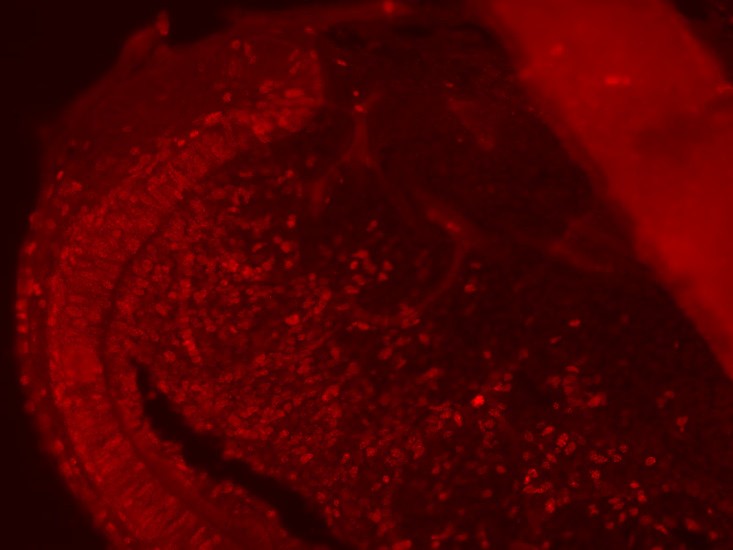


**A**

*Atg7*

*f/f*

\*

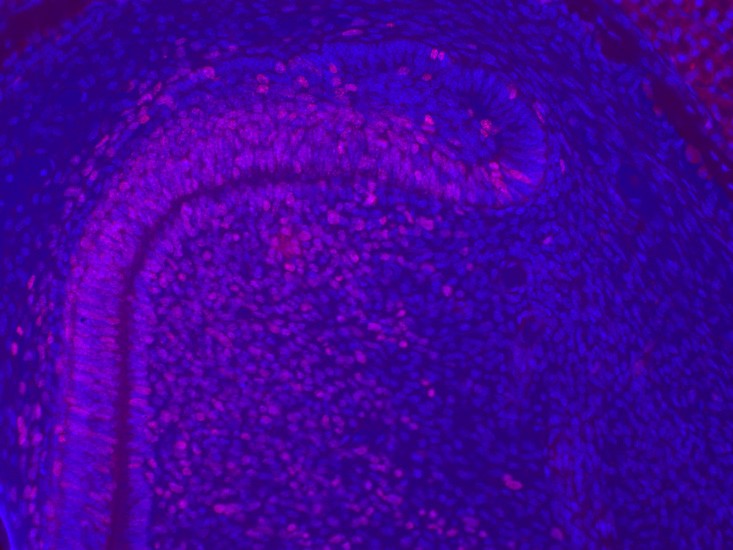


**B**

*Atg7f/f Krt14-Cre*

\*

**D** *Atg7f/f Krt14-Cre*



**C**

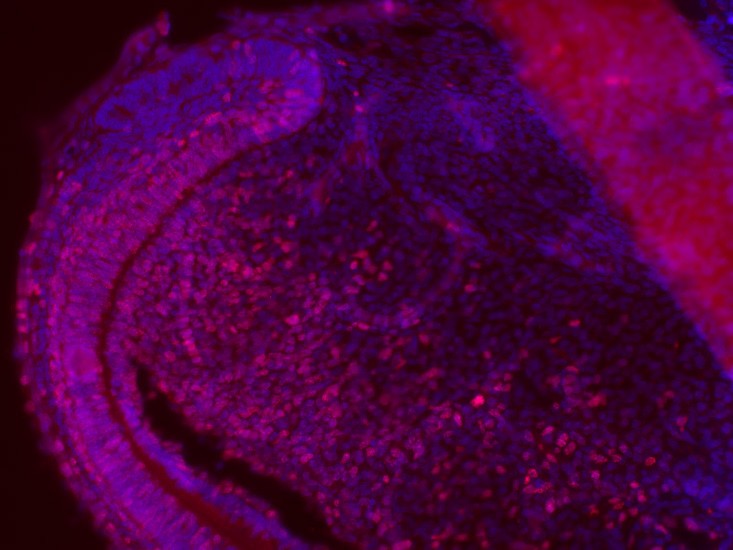
\*

*Atg7*

OEE SR

IEE

*f/f*



\* OEE

SR IEE

**Figure S9.** Proliferating cells show a normal distribution in the cervical loops of *Atg7f/f* and *Atg7f/f Krt14-Cre* mice. Tissue sections of the labial cervical loop of maxillary incisors of *Atg7f/f* (**A**, **C**) and *Atg7f/f Krt14-Cre* (**B**, **D**) mice (age 1 month) were immunolabeled for the proliferation marker MKI67 (red). Nuclear DNA was labeled with Hoechst dye (blue) (**C**, **D**). Note that cell proliferation is largely restricted to the inner enamel epithelium (IEE) and only sparse MKI67-positive cells are detected in the outer enamel epithelium (OEE) and the stellate reticulum (SR) of both genotypes (\*). A broken line marks the outer border of the cervical loop. Scale bars: 100 µm.