Analysis of epithelial cell proliferation.

To determine the degree of epithelial cell proliferation, 5´-bromodeoxyuridine (BrdU) (Zymed Laboratories, San Francisco, CA) incorporation analysis is performed. In brief, mice are injected intraperitoneally with 0.25 ml of 5´-BrdU solution (0.75 µg BrdU) 2 hours before death. The esophagus was fixed with 10% neutral buffered formalin (Sigma) for 24 hours. After fixation, the tissue is embedded in paraffin, and 5 micron sections are processed using standard histologic approaches. Tissue is digested with trypsin (0.125%) for 3 minutes at 37°C, followed by incubation for 30 minutes at room temperature. Sections are washed with PBS 3 times for 2 minutes and further incubated with monoclonal biotinylated anti-BrdU antibody for 60 minutes at room temperature. Negative controls include replacing the primary antibody with PBS, and positive controls are provided by the manufacturer. Cells with nuclear staining for BrdU are detected with streptavidin-peroxidase and DAB substrate (Zymed Laboratories, San Francisco, CA), followed by counter staining with hematoxylin. The $BrdU^{+}$ cell quantitation is carried out with the assistance of digital morphometry.