Supplementary Material 3. Microscopic and histological evaluation of the airway and epithelium

The harvested trachea and lung were fixed in 4% paraformaldehyde for a day, and the fixed tissues were then washed with distilled water. The tissues were dehydrated by serial ethanol washing and embedded in paraffin. Sections of 4-μm thickness of the paraffin-embedded tissues were used for histological analyses. Hematoxylin and eosin, Masson's trichrome, Alcian Blue, and TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining were all performed according to standard procedures. Immunohistochemical staining was performed using anti-cytokeratin (CK5, 10 μg/mL, monoclonal immunoglobulin G [IgG]; Antibodies Online, Aachen, Germany) and anti-β-tubulin (1 μg/mL, monoclonal IgG; Thermo Fisher Scientific, Rockford, IL, USA) antibodies according to the manufacturer's instructions (Vector, Burlington, CA, USA) with minor modifications. Briefly, after deparaffinization and rehydration, tissues were put into citrate buffer (1×, pH 6.0) and then that was heated using a microwave. After washing with phosphate buffered saline (PBS; pH 7.4), the tissues were incubated with each primary antibody at 22°C for 1 hour. After extensive washing with PBS (pH 7.4), the tissues were subsequently incubated with the pre-diluted Universal Pan-specific secondary antibody (R.T.U. Vectastain, Vector) at room temperature for 10 minutes. After washing three times with PBS for 5 minutes each, the tissues were incubated with a ready-to-use streptavidin and peroxidase complex for 5 minutes. After another three washes with PBS, the tissues were incubated with peroxidase substrate solution until the desired stain intensity developed.