Appendix 1

Method

Immunofluorescence assay

The primary cultured rat tracheal epithelial cells were grown on the glass coverslips for 3 days. The cells were incubated with a mouse monoclonal antibody against pan cytokeratin (1:100, Wuhan Boster, Wuhan, China) overnight at 4 ℃ and incubated with a secondary antibody against mouse IgG conjugated to fluorescein isothiocyanate (FITC; 1:100, Wuhan Boster, Wuhan, China) for 90 min at room temperature. Cells were visualized using a fluorescence microscope (Eclipse 50i, Nikon, Tokyo, Japan). The negative control was obtained by omitting the primary antibody.

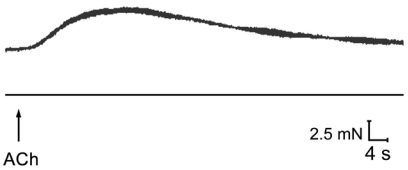

Figure S1 Effect of acetylcholine (ACh) on rat tracheal rings. Representative trace showing the transient contraction response induced by ACh (20 nM) in rat tracheal ring. The experiment was repeated at least three times.

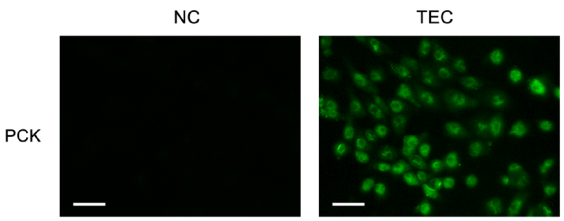

Figure S2 Characterization of the primary cultured rat tracheal epithelial cells. Fluorescence images showing the fluorescein isothiocyanate (FITC) immunoreactivity for PCK, the epithelial cell marker. Scale bars, 25 μm. NC, negative control; TEC, tracheal epithelial cell; PCK, pan-cytokeratin.

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