Supplementary Table 1
Table shows different endogenous flouorescent properties of tissue structures compared with the literature.

Supplemental Fig. 1: Preparation and setup used in ex vivo and in vivo studies.
a-c) In the ex vivo preparation, the trachea was placed in a heated culture dish and fixed with insect needles. a) trachea before, and b) after cutting the trachealis muscle. c) Culture dish mounted on culture dish holder. d) The mouse was placed on a heated stage and the trachea was exposed. To increase the stability of the trachea during imaging, a small metal frame that contained a cover slip was placed over the trachea and held in place by metal rods attached to the stage.

Supplemental Fig. 2: Lymph vessel detection with CD90.2 antibody
a, b) Lymph vessels were not detectable by autofluorescence (a) but could be visualized by addition of labeled anti-CD90.2 antibody (yellow structures marked by red arrows). Anti-CD90.2 antibody also labeled fibroblasts (white arrows).

Supplemental Fig. 3: Comparison of epithelium visualized by bright field microscopy and multiphoton microscopy.
a) Identification of ciliated and non ciliated cells by brightfield microscopy on the level of the cilia. b, c) On the epithelial cell level these cells can be followed by brightfield microscopy (b) and multiphoton microscopy (c). For visualization of cilia-driven transport see Movie 3.

Supplemental Fig. 4: Visualization of laser-induced photodamage.
a) Epithelium before the induction of photodamage. Laser irradiation of the area marked with the red arrow in a induced hyperfluorescence immediately adjacent and a loss of fluorescence in the epithelial cells surrounding the hyperfluorescent area. c) Addition of propidium iodide only labeled the cells

surrounding the damaged site. Cross section of the damaged area shows that propidium iodide-labeled cells are expulsed from the epithelium.

Supplemental Fig. 5: Induction of hyperfluoescence during scanning.
a-d) Time series of a single focal plane. Continuous scanning induced hyperfluorescence (arrowheads) that increased in size over time. e-h) This hyperfluorescence appears mostly in the channels 495-580 nm (e-f) and 580-680 nm (g-h).

Supplemental Fig. 6: Uptake of DQ OVA in cells with dendritic cell morphology.
a, b) Time series of a single focal plane in an animal with induced airway inflammation. Addition of DQ OVA lead to increase of fluorescence (yellow) in cells with dendritic cell morphology (yellow circles, compare Movie 12). c-f) Fixation of the trachea after DQ OVA-uptake and subsequent labeling with an antibody against MHC II. c) DQ OVA (green), d) MHC II labeling (red). e) Merged image. f) Magnification of the boxed area in e) Green arrowheads = DQ OVA positive cells, red arrowheads = MHC II positive cells, yellow arrows = cells being positive for both labels.

Movie 1: Multiphoton microscopy. Z-stack of the murine tracheal wall *ex vivo* and *in vivo* from the outer part of the trachea to the airway lumen.122 optical slices over 122 µm depth, c.f. Fig. 1b-g.

Movie 2: Multiphoton microscopy. *In vivo* time series of blood perfusion in the trachea of the mouse visualized by fluorescent dextran. Single focal plane, 60 frames over 13 sec.

Movie 3: Transmitted light microscopy. Z-stack of the murine tracheal wall *ex vivo* through the airway epithelium from the luminal side.71 optical slices over 70 µm depth and cilia-driven particle transport of the airway epithelium (100 frames over 20 sec).

Movie 4: Multiphoton microscopy. A mobile intraepithelial cell can be followed over time. 3D-rendering of 4 focal planes, z = 9 µm, 30 frames, 16 min 9 sec, c.f. Fig 3b-i.

Movie 5: Multiphoton microscopy. *Ex vivo* time series showing cell dynamics in the connective tissue. Single focal plane, 100 frames over 14 min 52 sec, c.f. Fig. 4.

Movie 6: Multiphoton microscopy. Short lasting contacts between cells in the connective tissue; *ex vivo*, single focal plane, 31 frames over 10 min.

Movie 7: Multiphoton microscopy. Long lasting contacts between two motile cells and a stationary cell in the connective tissue in the trachea. Single focal plane, 29 frames over 4 min 13 sec.

Movie 8: Multiphoton microscopy. Phagocytosis of cells in the connective tissue of the trachea. Single focal plane, 100 frames over 8 min 37 sec.

Movie 9: Multiphoton microscopy. *In vivo* time series of moving cells in the connective tissue of a mouse trachea. Single focal plane, 46 frames over 19 min 20 sec.

Movie 10: Multiphoton microscopy. Cell movement towards laser-induced damage in the connective tissue of the trachea. Single focal plane, 40 frames over 6 min 31 sec.

Movie 11: Multiphoton microscopy. Comparison between non-inflamed and inflamed trachea in a 3D-rendering of z-stacks of the connective tissue over time. 10 focal planes, z = 18 µm, 18 min (non-inflamed) and 9 focal planes, z = 16 µm, 36 min (inflamed), c.f. Fig 5 a,b.

Movie 12: Multiphoton microscopy. DQ OVA-uptake of cells in the connective tissue of the inflamed trachea. 3D-rendering of z-stack over time. 10 focal planes, z = 18 µm, 38 min 1 sec.

Movie 13: Multiphoton microscopy. Contact between granulocytes and antigen-uptaking cells in a 3D-rendering of z-stack of the connective tissue of the trachea over time. 10 focal planes, z = 18 µm, 26 min, c.f. Fig. 6 a-c.

Movie 14: Multiphoton microscopy. Contact between granulocytes in a 3D-rendering of z-stack of the connective tissue of the trachea over time. 8 focal planes, z = 14 µm, 21 min 4 sec, c.f. Fig. 6 d-f.

Movie 15: Multiphoton microscopy. Z-stack of human concha nasalis. 37 frames over 72 µm depth, c.f. Fig. 7 b-f.

Movie 16: Multiphoton microscopy. 3D-stack over time of human concha nasalis after laser-induced damage of the epithelium. 25 optical slices over 48 µm depth, 11 frames over 31 min 58 sec.