Supplementary

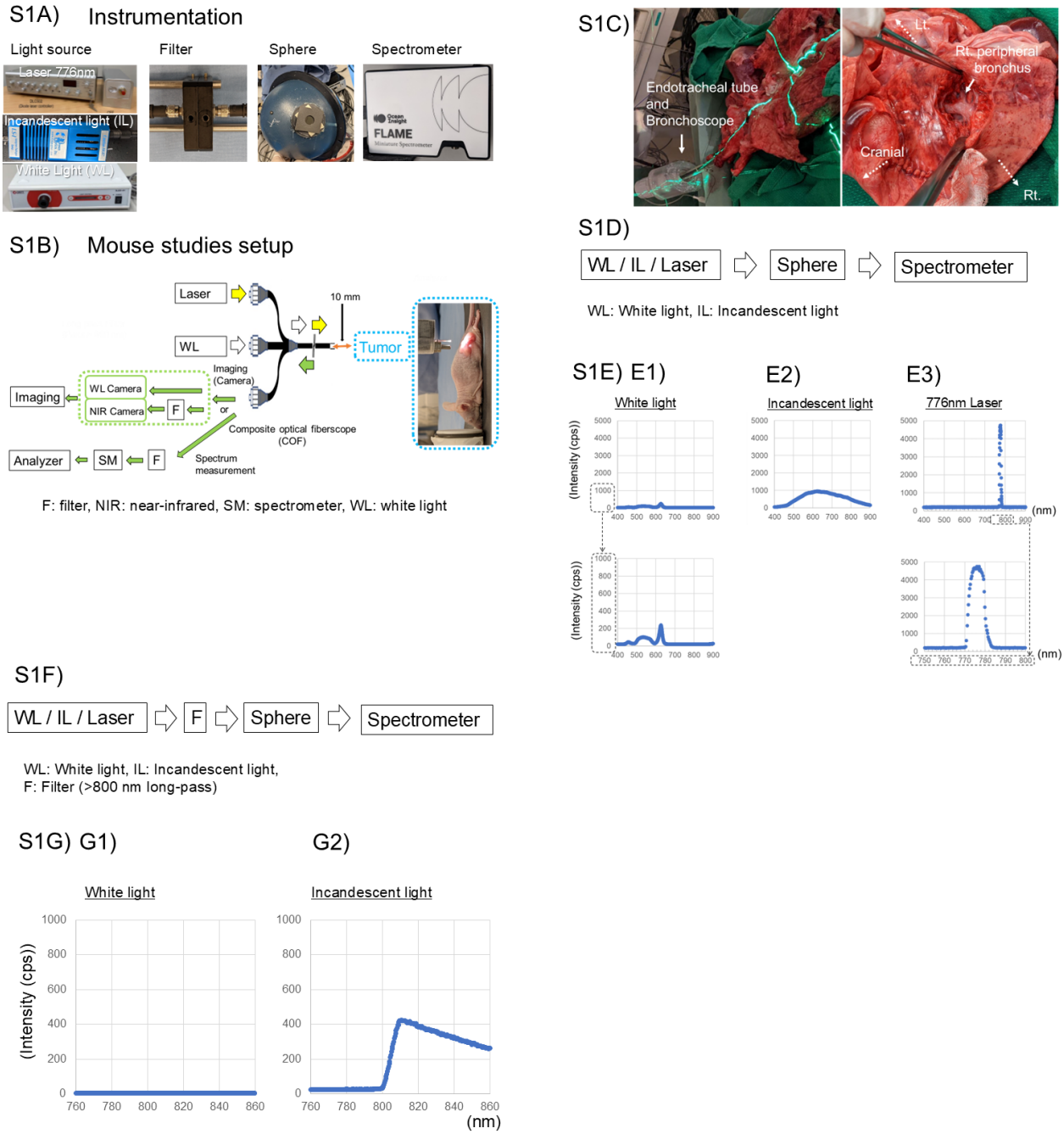

Figure S1 Experimental setup and spectroscopic results. (A) Instrumentation setup showing the light source, filter, integrating sphere and spectrometer. (B) Setup for mouse studies with the optical axes aligned. The optical fiber bundle for image collection was connected to either to the CCD camera or the spectrometer. (C) *Ex vivo* swine lung preparation: mediastinal soft tissue was removed around the carina to allow placement of the tumor and an incision was made along the peripheral bronchus to expose the bronchial wall. The bronchoscope was inserted through an endotracheal tube. (D) Instrumentation of the light sources for direct connection. (E) Corresponding spectra: (E1) white light without the long-pass filter showing the 3 LED source peaks in the visible range, (E2) incandescent source showing a continuum peaking at 600–700 nm, (E3) laser source showing the peak centered at 776 nm. (F) Instrumentation of the light sources for direct connection with long-pass filter. (G) Corresponding spectra for white light (G1) and incandescent light (G2). F, filter; IL, incandescent light; NIR, near-infrared; SM, spectrometer; WL, white light.

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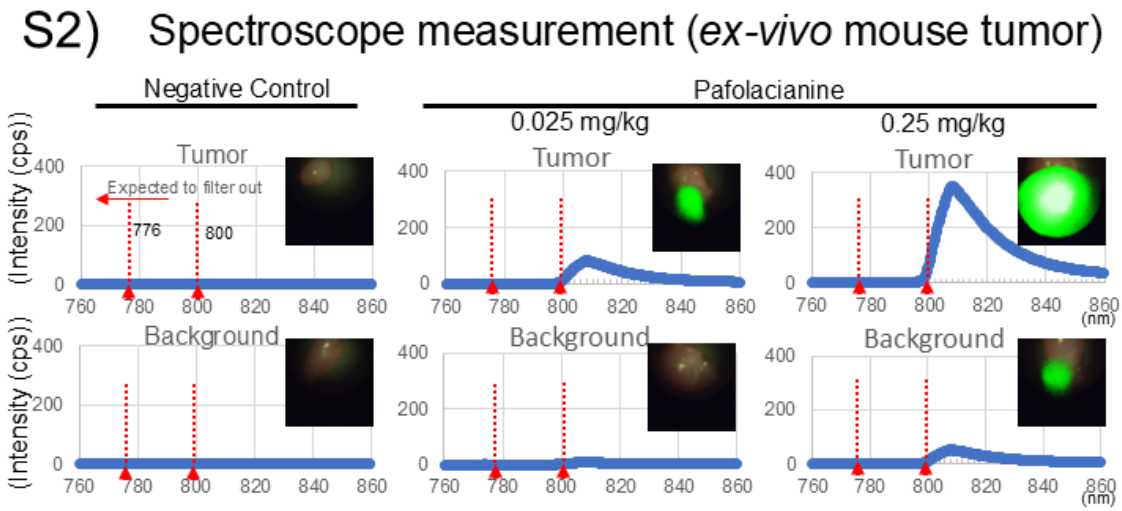

Figure S2 Spectra from *ex vivo* mouse tumor showing absence of the 776 nm signal, independent of pafolacianine administration. The 810 nm peak increased with fluorophore concentration but was absent in the negative control.

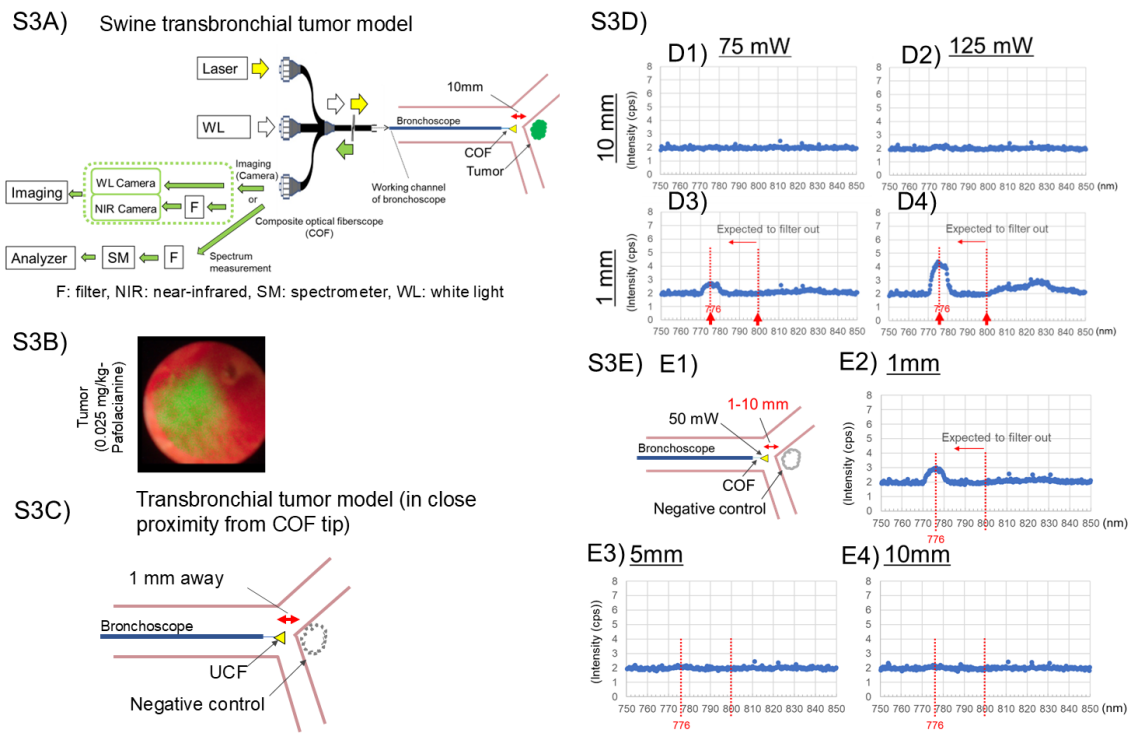

Figure S3 Setup of the swine transbronchial tumor model and spectroscopic and imaging results. (A,B) Schematic diagram of measurements in the swine transbronchial tumor model in the same geometry as the mouse model: (A) COF tip placed at 10 mm from the bronchus; (B) images from 0.025 mg/kg pafolacianine-infused tumor at 10 mm distance. (C) COF tip placed at 1 mm from the bronchus. (D) Resulting spectra for the negative control tumor, showing the 776 nm peak detected at the short distance, increasing with laser power (75 or 125 mW). (E) Corresponding negative control spectra at 1, 5 or 10 mm distance (laser at 50 mW): the 776 nm peak is evident only in the 1 mm case.

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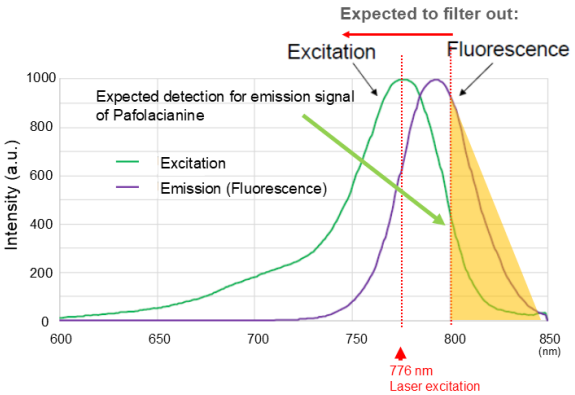
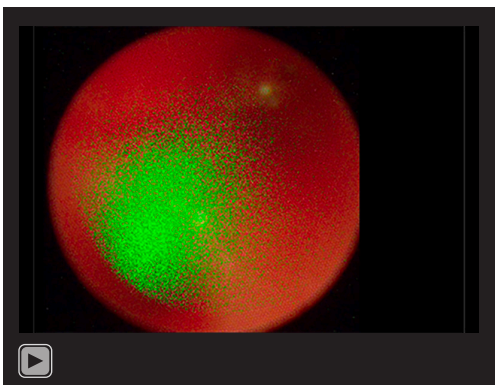
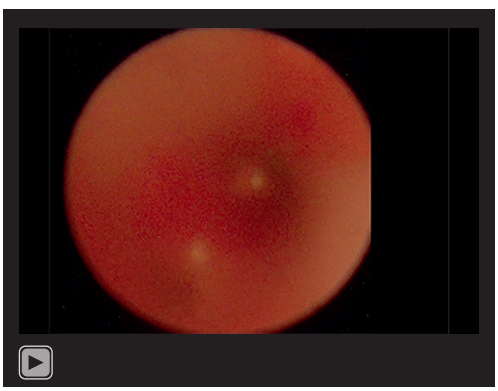


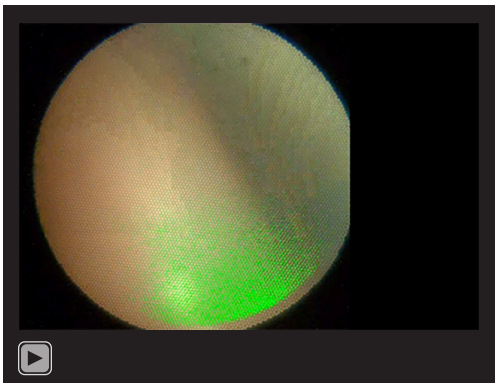
Figure S4 Pafolacianine excitation and emission spectra (19) with the 776 nm laser and long-pass filters indicated.



Video S1 Video image from pafolacianine-infused tumor at 10 mm distance.



Video S2 Video images in the lung from negative control tumor at 10 mm distance.



Video S3 Video images in the lung from negative control tumor at 1 mm distance.

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