Supplementary information

Real-time imaging of hydrogen peroxide dynamics in vegetative and pathogenic hyphae of *Fusarium graminearum*

Michael Mentges and Jörg Bormann

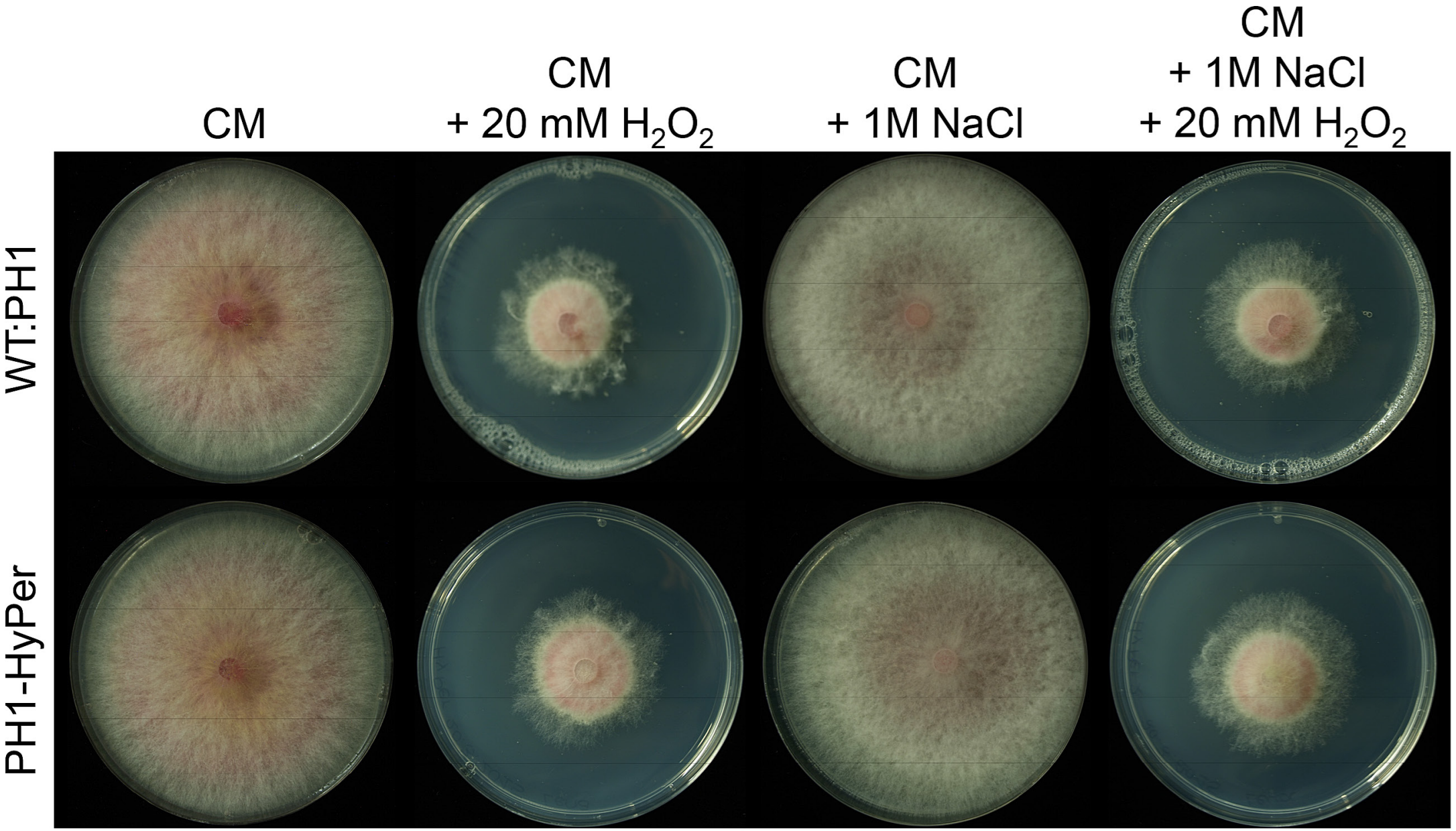

Figure S1. Colony morphology of wild type (WT:PH1) and HyPer-2-expressing strain (PH1-HyPer). Plate assay (4 dpi) of the wild type and PH1-HyPer on CM and CM supplemented with 20 mM H_2O_2 and 1 M NaCl, respectively, and both chemicals in combination.

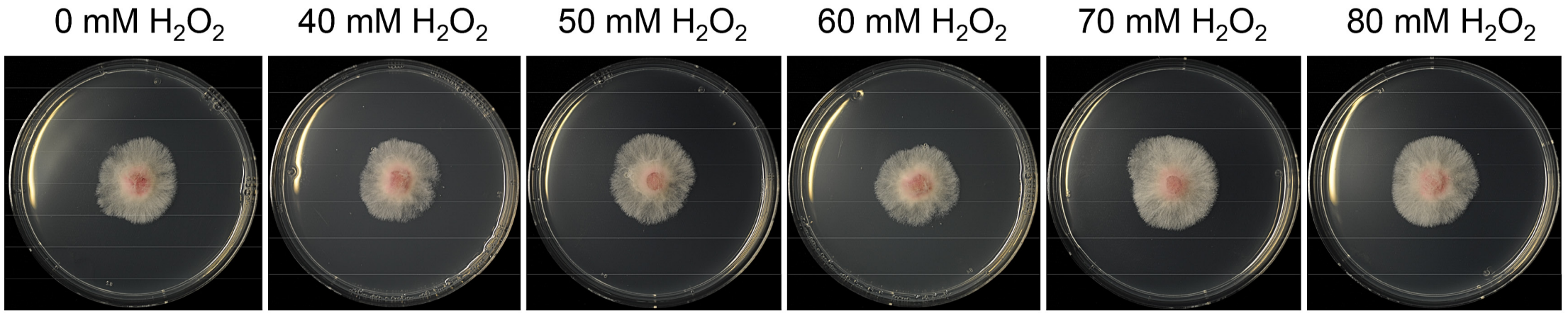

Figure S2. Fungal viability assay. Colony morphology (2 dpi) of the *F. graminearum* wild type on CM after treatment with increasing concentrations of H_2O_2. Inoculum was taken from microtiter plate assay after injection of H_2O_2 and fluorescence measurement for 45 min. Treatment with up to 80 mM H_2O_2 did not compromise fungal viability.

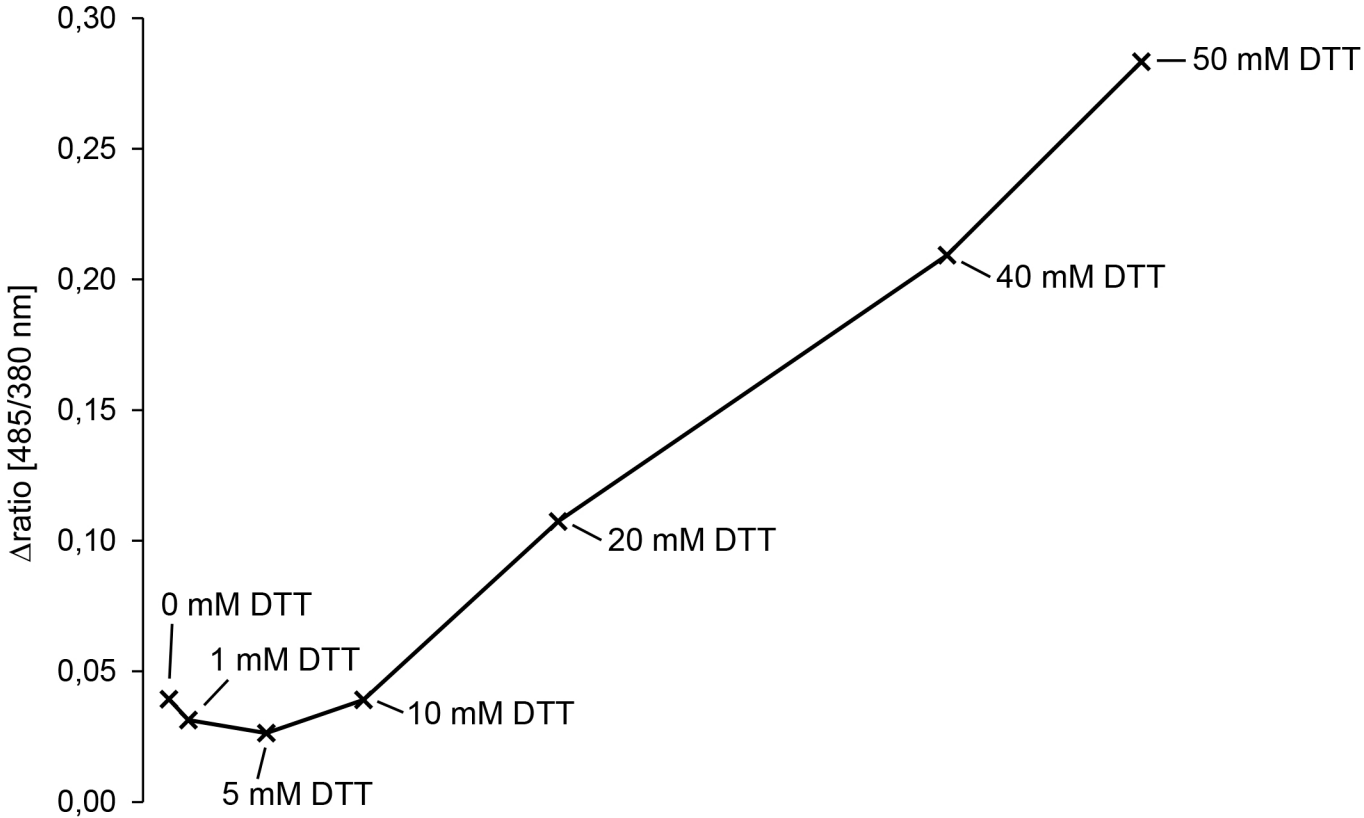

Figure S3. HyPer sensitivity assay. Assay for the relative changes in the ratio [485/380nm] in response to increasing dithiothreitol (DTT) concentrations. Mycelia were raised in a 96-well plate filled with 100 µl minimal agar medium and analyzed in a fluorometer. Prior to DTT injection 50 mM H_2O_2 were injected in each well.

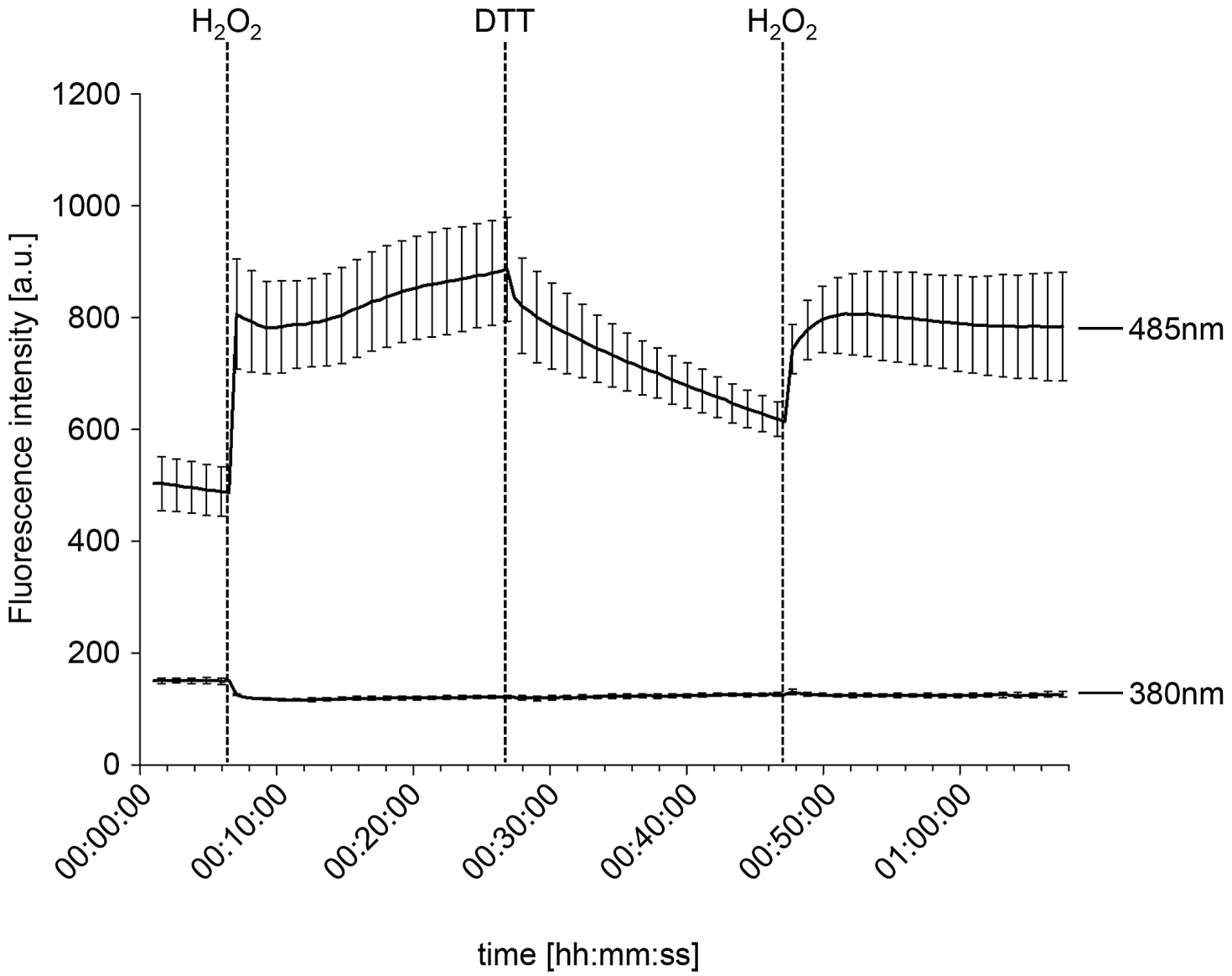

Figure S4. Fluorescence time course assay of the fungal response to external H_2O_2 and dithiothreitol (DTT). Timing of H_2O_2 (50 mM) and DTT (50 mM) induced changes in HyPer-2-fluorescence intensity after excitation at 380 nm and 485 nm, respectively. Mycelia were raised in a 96-well plate filled with 100 µl minimal agar medium and analyzed in a fluorometer. Prior to the first H_2O_2 injection 100 µl H_2O were injected in each well. Error bars represent the standard deviation (n=24).

Flow chamber preparation

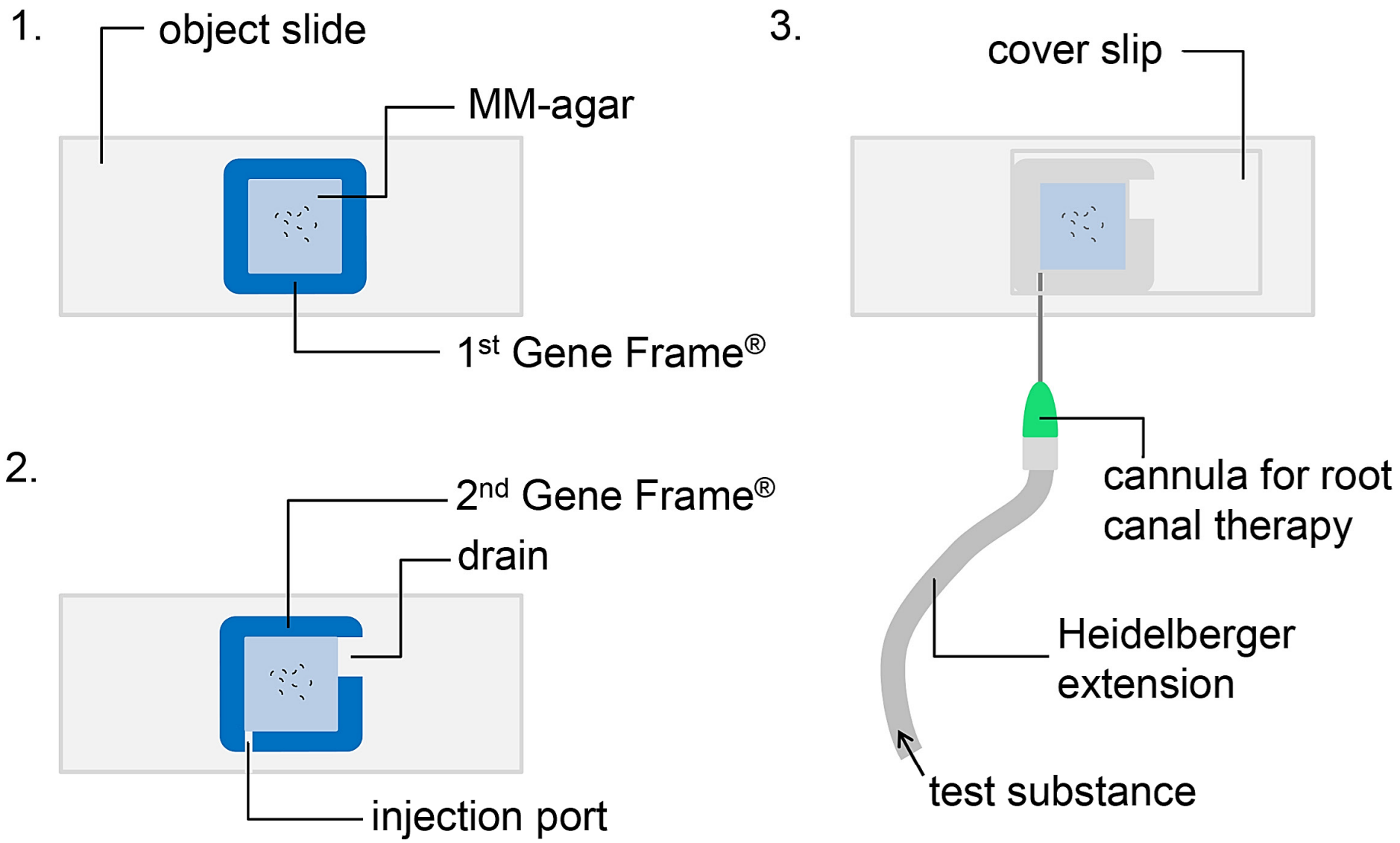

Figure S5. Flow chamber preparation. To monitor HyPer-2-responses using confocal laser scanning microscopy a flow chamber was designed that allows simultaneous image acquisition and injection of assay substances, e.g. H_2O_2.

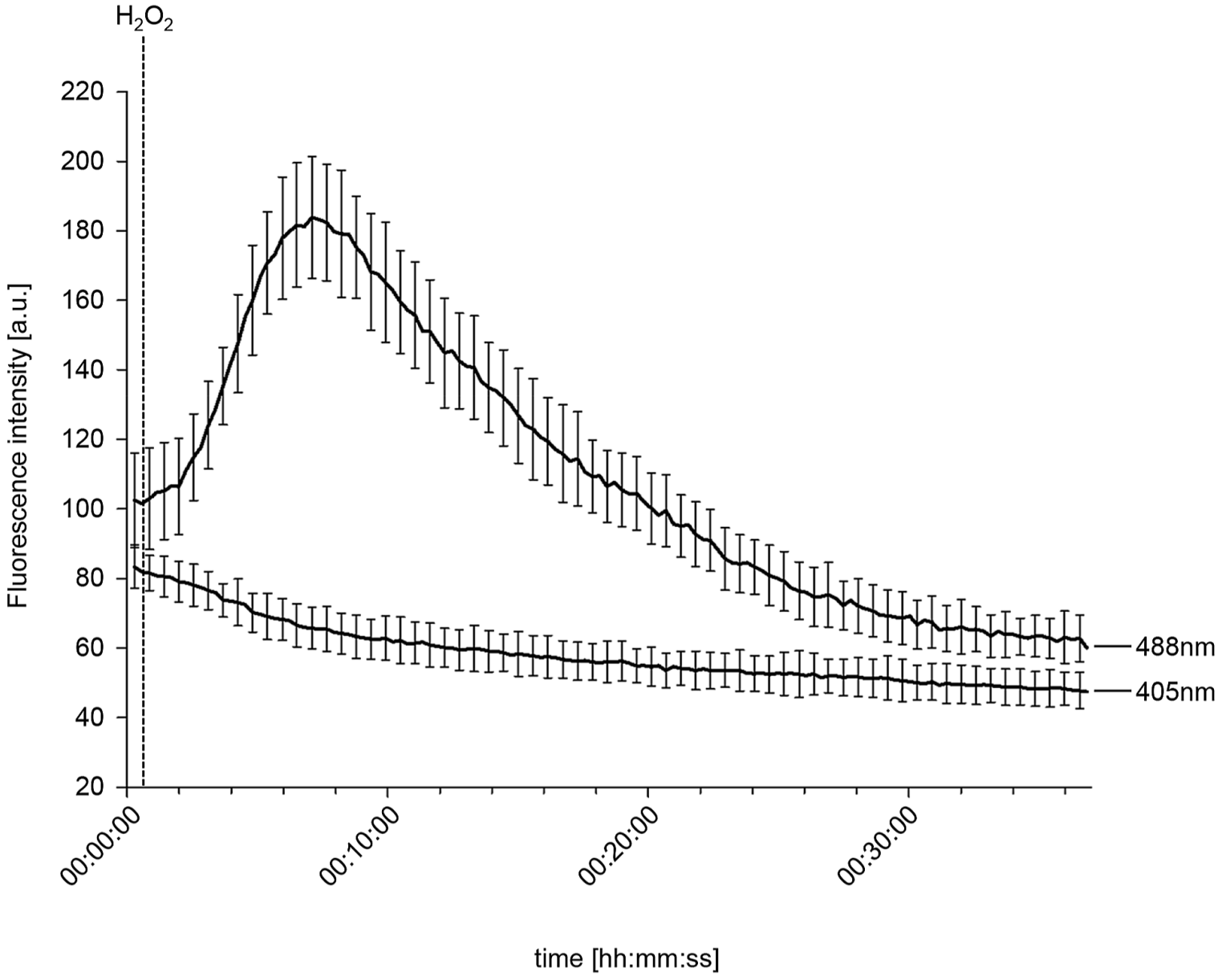

Figure S6. Fluorescence time course assay of the fungal response to external H_2O_2. Timing of H_2O_2 (50 mM) induced changes in HyPer-2-fluorescence intensity after excitation at 405 nm and 488 nm, respectively. Mycelia were raised in a flow chamber (see figure S3) and imaged using confocal laser scanning microscopy. Pixel intensities were measured in three regions of interest marked in a confocal laser scanning time series (see video S1). Error bars represent the standard deviation (n=3).

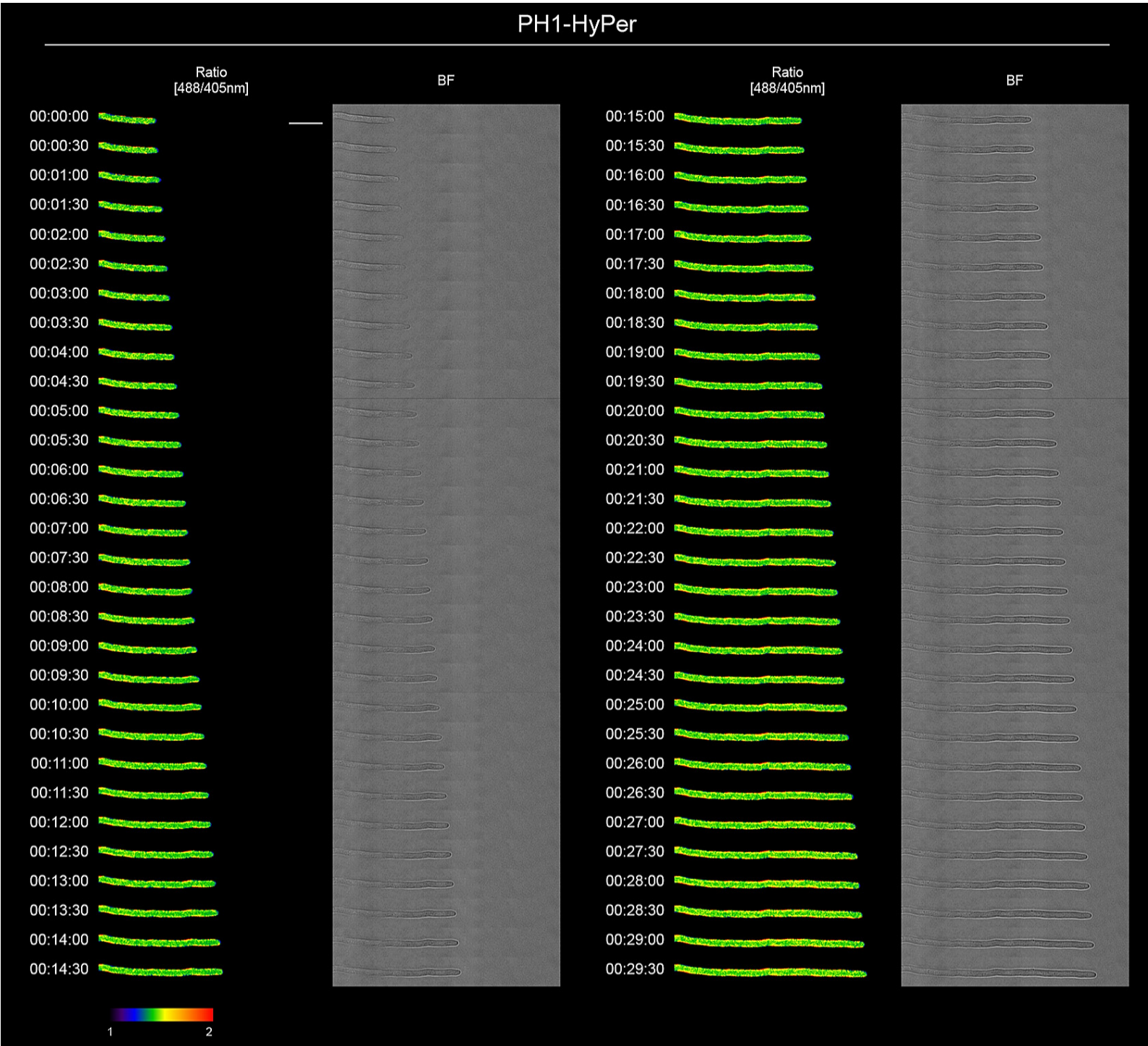

Figure S7. Ratiometric time course assay of fungal tip growth. Timing of H_2O_2 fluctuations (represented by the ratio [488/405 nm]) during tip growth of hypha expressing HyPer-2. See also video S2. Scale bar: 10 µm.

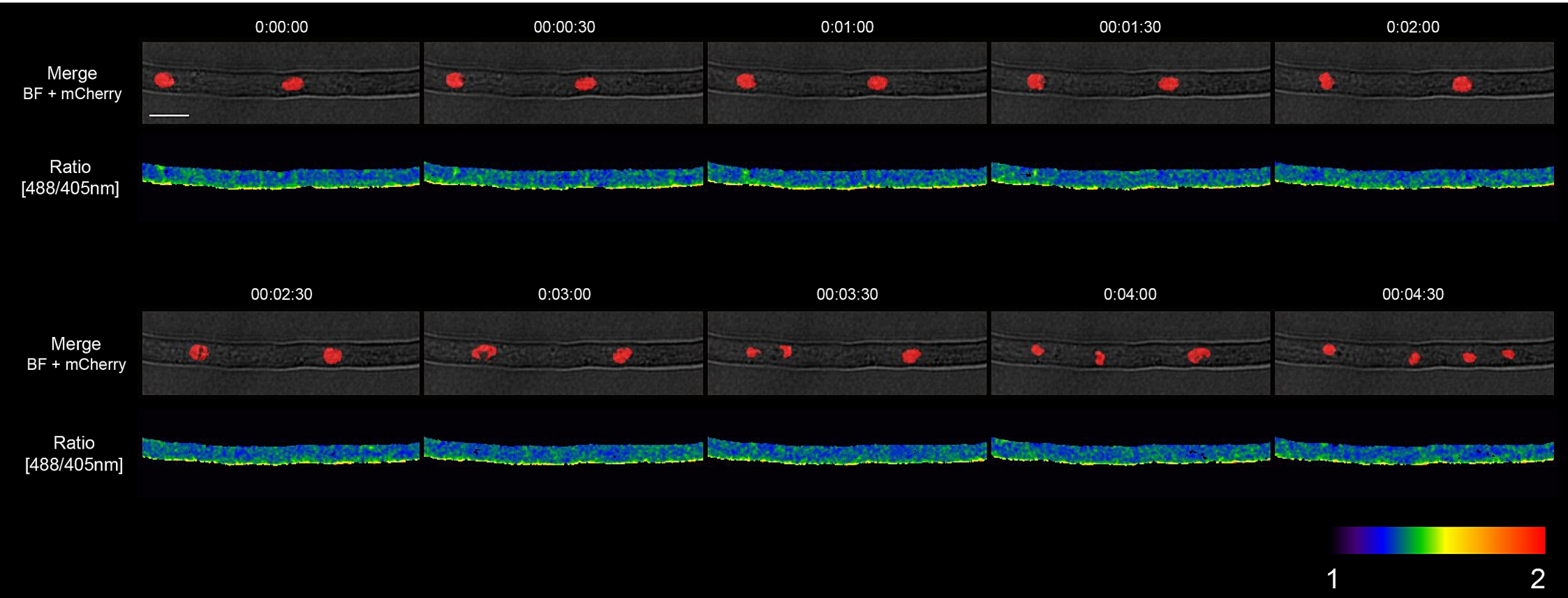

Figure S8. Ratiometric time course assay of nuclear divisions. Timing of H_2O_2 fluctuations (represented by the ratio [488/405 nm]) during nuclear divisions in hypha expressing HyPer-2. See also video S3. Scale bar: 5 µm.

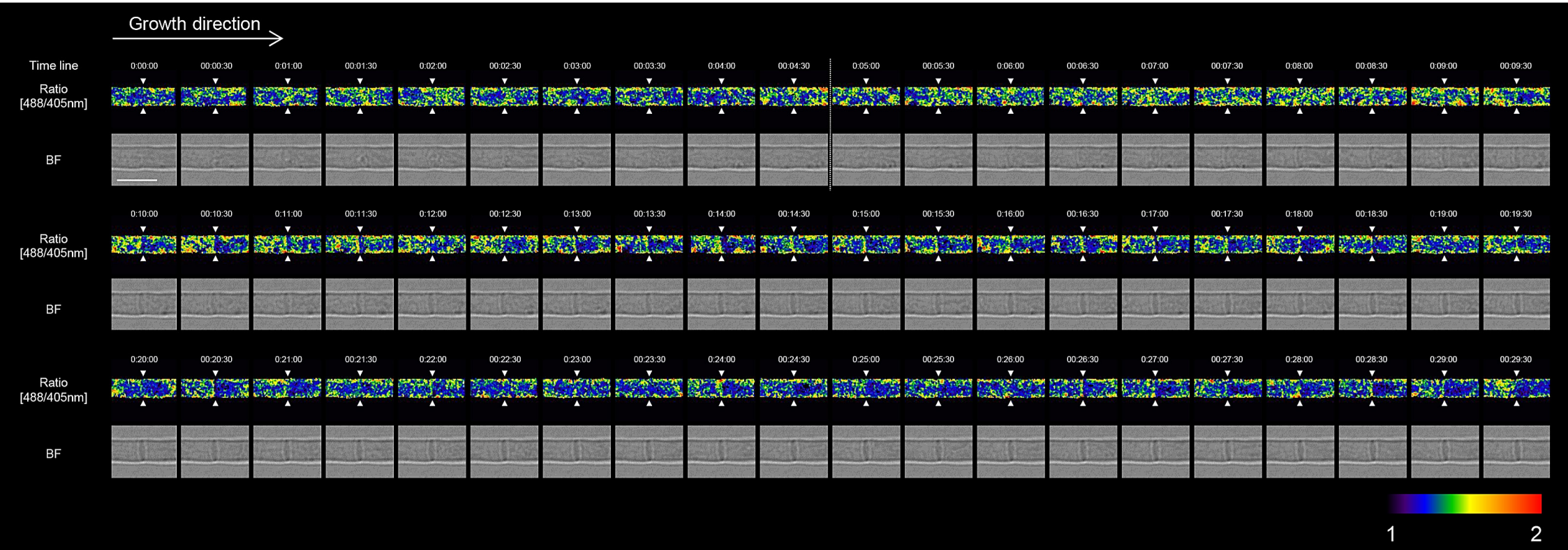

Figure S9. Ratiometric time course assay of septum formation. Timing of H_2O_2 fluctuations (represented by the ratio [488/405 nm]) during septum formation in hypha expressing HyPer-2. See also video S4. The dotted vertical line represents the initiation of septum formation. Scale bar: 5 µm.

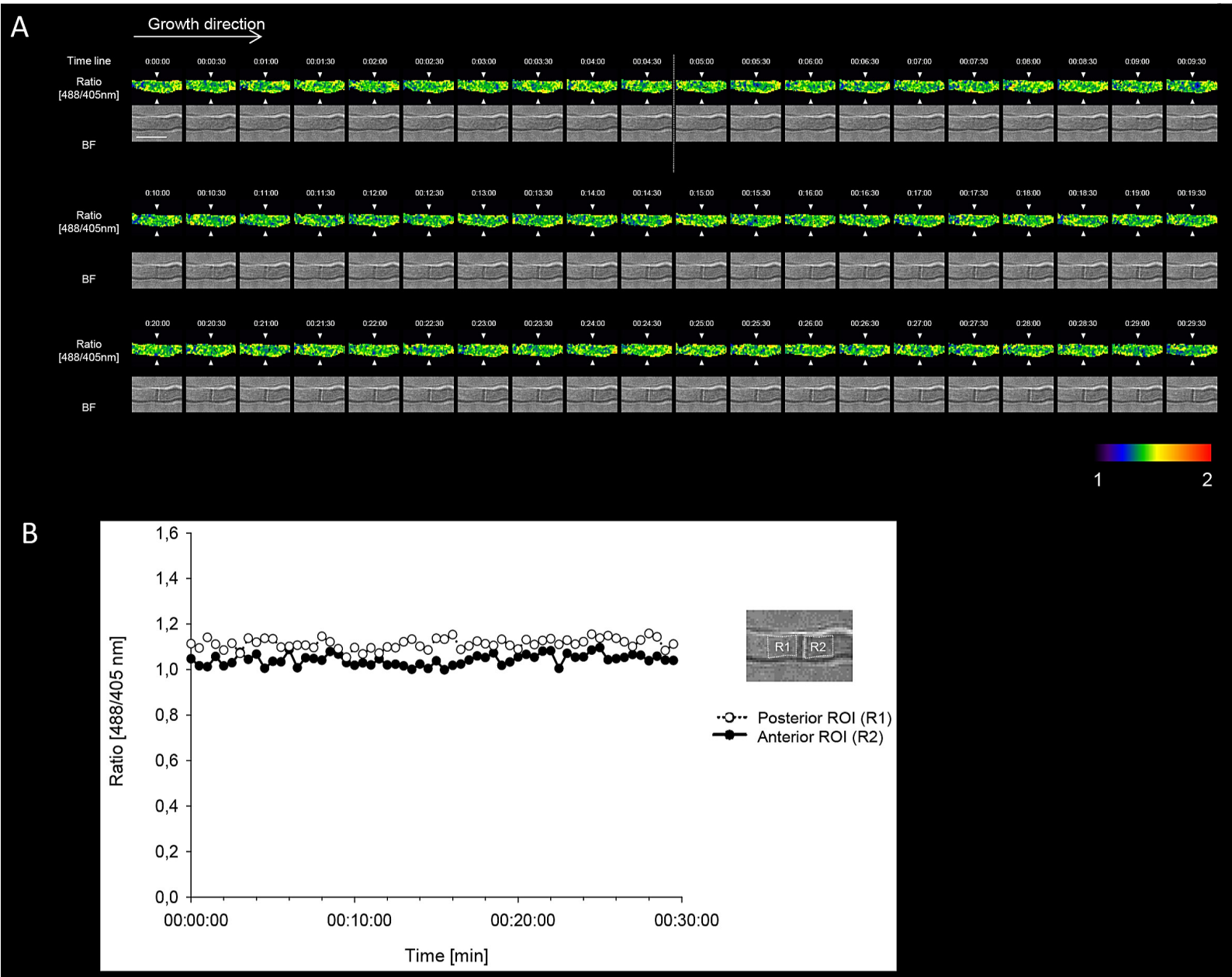

Figure S10. Ratiometric time course assay of septum formation. A. Timing of H_2O_2 fluctuations (represented by the ratio [488/405 nm]) during septum formation in hypha expressing SypHer. The dotted vertical line represents the initiation of septum formation. Scale bar: 5 µm. **B.** Timing of H_2O_2 fluctuations during septum formation. Ratio [488/405 nm] calculated from pixel intensities measurements over time in two regions of interest (ROI R1, covering the posterior part and ROI R2, covering the anterior part of the hypha) marked in a confocal laser scanning time series.

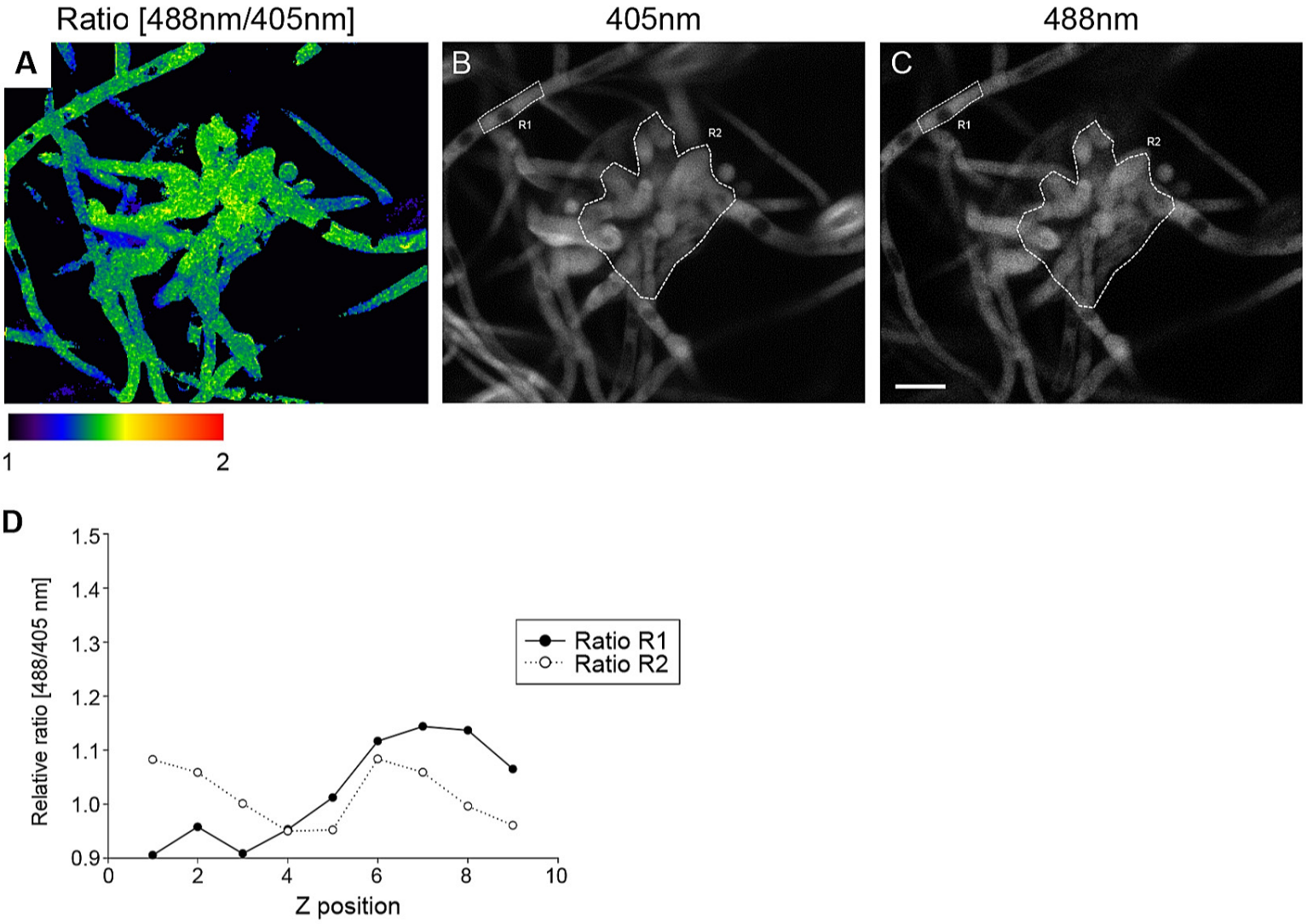

Figure S11. Ratiometric assay of infection cushion formation in hypha expressing SypHer. A-C. Average intensity projections of a tiny infection cushion developed on a wheat palea 6 days post inoculation. **A.** Ratio [488/405 nm]. **B.** Fluorescence excited at 405 nm. **C.** Fluorescence excited at 488 nm. **D.** Pixel intensities were measured over nine z-positions in two regions of interest (ROI R1, covering a part of a runner hypha and ROI R2, covering a part of an infection cushion) marked in the confocal laser scanning z-series shown in A and B. Analysis of ten different infection structures gave similar results. Scale bar 10 µm.

Primer used for amplification of HyPer and SypHer (*Bam*HI enzyme recognition sites introduced to the primers are underlined)		
Sequence (5'→ 3')	**Description**	**Number**
GGATCCGGTACCATGGAGATGGCAAGCCCA GCAGGGCGAGACGATGT	Forward primer HyPer/SypHer	A1
GGATCCGCTTTTAAACCGCCTGTT	Reverse primer HyPer/ SypHer	A2

Video legends

Video S1. Fluorescence time course assay of the fungal response to external H_2O_2. Time lapse video of germinating conidiospore. H_2O_2 (50 mM) supplementation is marked by a red arrow. HyPer-2-fluorescence was recorded after excitation at 405 nm (middle) and 488 nm (left), respectively, and a ratio was calculated (right). Mycelia were raised in a flow chamber (see figure S5) and imaged using confocal laser scanning microscopy.

Video S2. Fluorescence time course assay of fungal tip growth. Time lapse video of hyphal growth. HyPer-2-fluorescence was recorded after excitation at 405 nm and 488 nm, respectively, and a ratio was calculated. Mycelia were raised in a flow chamber (see figure S5) and imaged using confocal laser scanning microscopy.

Video S3. Fluorescence time course assay of nuclear divisions. Time lapse video of nuclear divisions. HyPer-2-fluorescence was recorded after excitation at 405 nm and 488 nm, respectively, and a ratio was calculated. Mycelia were raised in a flow chamber (see figure S5) and imaged using confocal laser scanning microscopy.

Video S4. Fluorescence time course assay of septum formation. Time lapse video of septum formation. HyPer-2-fluorescence was recorded after excitation with 405 nm and 488 nm, respectively, and a ratio was calculated. Mycelia were raised in a flow chamber (see figure S5) and imaged using confocal laser scanning microscopy.