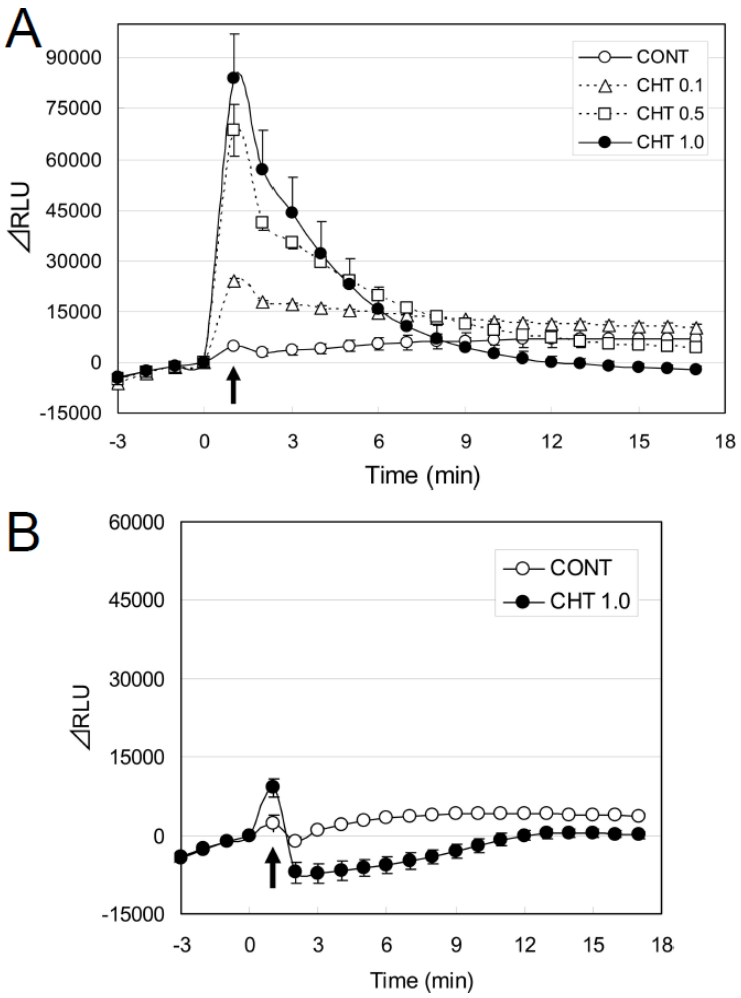

Supplementary Fig. S2. A, Dependence of superoxide ($O_2^{\bullet-}$) production on concentration of chitosan (CHN) (0.1, 0.5 or 1.0 mg ml^{-1}) added to liquid cultures of *Physcomitrella patens*. **B**, Superoxide ($O_2^{\bullet-}$) production in protoplast culture of *P. patens* treated with CHN (1.0 mg ml^{-1}). Error bars indicate standard deviation ($n = 3$ in A; $n = 4$ in B).

For the experiment, protonemal tissue of *P. patens*, ecotype Gransden (Ashton and Cove 1977), was grown in Petri dishes (diameter 9 cm) on a cellophane membrane placed on BCD medium [1 mM $MgSO_4$, 1.85 mM KH_2PO_4 (pH 6.5, adjusted with KOH), 10 mM KNO_3, 45 µM $FeSO_4$, 0.22 µM $CuSO_4$, 0.19 µM ZnSO4, 10 µM H_3BO_4, 0.10 µM Na_2MoO_4, 2 µM $MnCl_2$, 0.23 µM $CoCl_2$, 0.17 µM KI] (Ashton and Cove 1977) supplemented with 1mM $CaCl_2$, 45 µM ethylenediaminetetraacetic acid disodium salt (Na_2-EDTA), and 5 mM ammonium tartrate [$(NH4)_2C_4H_4O_6$] , and solidified with 0.8% agar. The cultures were grown in a growth chamber (Model 3755, Forma Scientific, Marietta, OH, USA) at 23°C (photoperiod 12 h, light intensity 60 µmol $m^{-2}s^{-1}$) and subcultured weekly.

Protoplasts of *P. patens were* prepared according to Schaefer et al. (1991) from 1 week old protonema grown on BCD media supplemented with 1mM $CaCl_2$, 45 µM ethylenediaminetetraacetic acid disodium salt (Na_2-EDTA) and 5 mM ammonium tartrate.

Superoxide ($O_2^{\bullet-}$) production in liquid moss culture medium was measured by chemiluminescence of a luciferin analog MCLA [2-methyl-6-(p-methoxyphenyl)-3,7-dihydroimidazo(1, 2-α)pyrazin-3-one] (Sigma, St. Louis, MO, USA). Pieces of protonemal mats (~5 mm diameter) were placed in the cuvette (Sarstedt No. 68.750; Sarstedt, Nümbrecht, Germany) containing 1.0 ml of modified Y-medium and incubated in the dark at 23°C for 30 min before starting the measurements. Protoplasts (3.2×10^5 ml^{-1}) were suspended in 8.5% mannitol and incubated as above. First, 1 µM MCLA (final concentration) was added to the cuvette for background measurement, followed by addition of CHN. Chemiluminescence was measured for 5s at each time point with Luminoskan TL Plus luminometer (Thermo Labsystems, Waltham, MA, USA). The condition of protoplasts was checked with microscope after all series of measurements.

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