



Supplementary information, Figure S9 *In situ* calibration of BCECF-AM in protoplast vacuoles.

(A) Protoplasts containing vacuolar BCECF in a series of equilibration buffers were imaged from 488 nm (panels in the top row, red) and 458 nm (panels in the second row, green) excitation of BCECF. Ratio images were pseudo-colored to show the pH gradient (panels in the third row). Bright field images were also captured from the same cell (panels in the bottom row). Scale bar = 10 μ m. **(B)** The fluorescence ratios (488/458 nm) were plotted against the pH value of the equilibration buffers to generate a calibration curve.