Supplemental material

Liu et al., https://doi.org/10.1083/jcb.201810121

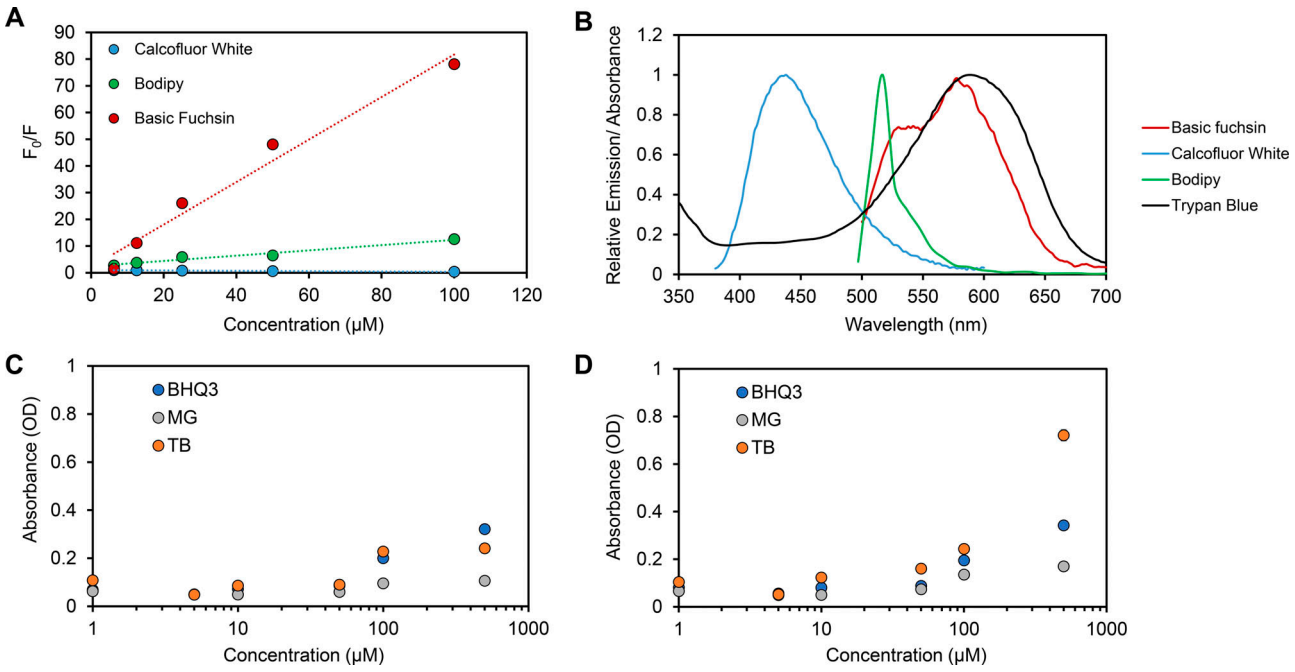

Figure S1. **Quencher characteristics. (A)** Stern-Volmer plots showing the quenching efficiency for the indicated fluorescent dyes by TB in water. **(B)** Comparison of absorption spectrum of TB with the emission spectra of the same three fluorescent dyes. Correlation of quenching efficiency (A) with spectral overlap of quencher absorption and dye emission (B) supports the hypothesis that TB quenching is based on Förster resonance energy transfer. **(C and D)** Concentration-dependent absorbance of BHQ3, MG, and TB at 488 nm (C) and 514 nm (D). It shows a low absorbance at the concentrations (up to 100 µM) that are used in the quenching assays.

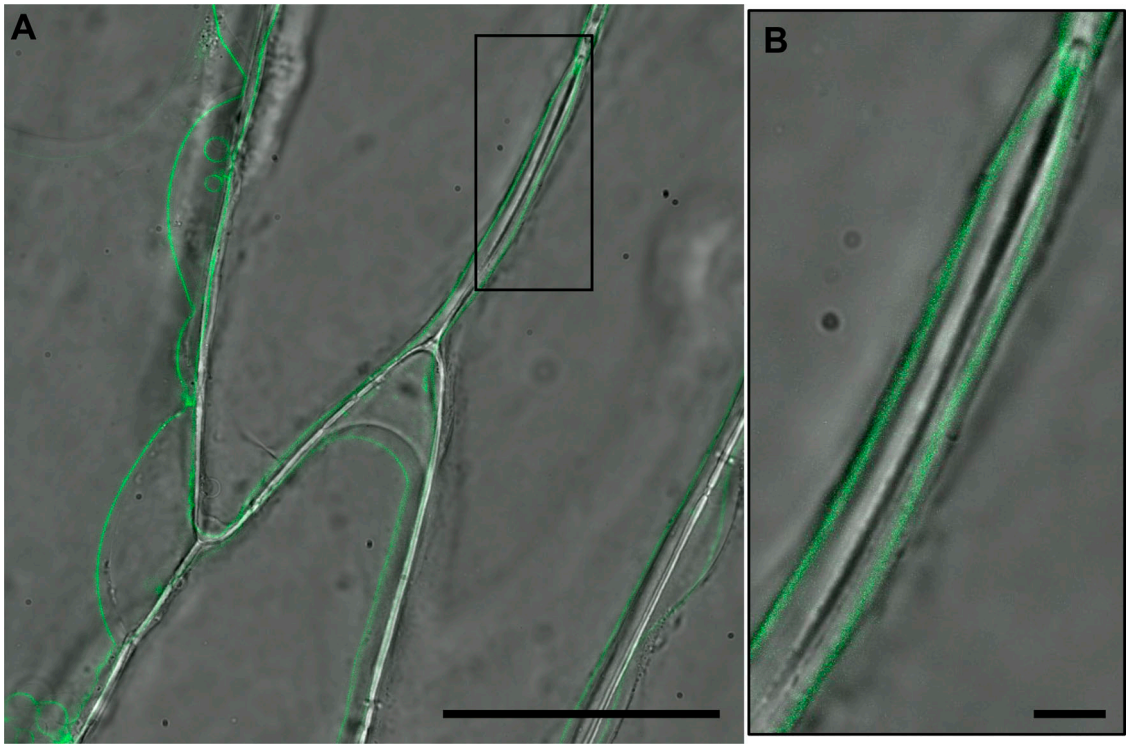

Figure S2. **Plasmolysis of onion epidermis cells stained with FM4-64.** A layer of onion epidermis was stained with 20 µM FM4-64 for 3 min, rinsed with PBS, covered with 30% (wt/vol) sucrose on the slide, and imaged after 5 min of incubation by phase contract and fluorescence microscopy. **(A and B)** Overview (A), magnified area (B) as indicated by black box in A. Bars: 10 µm (A); 1 µm (B).

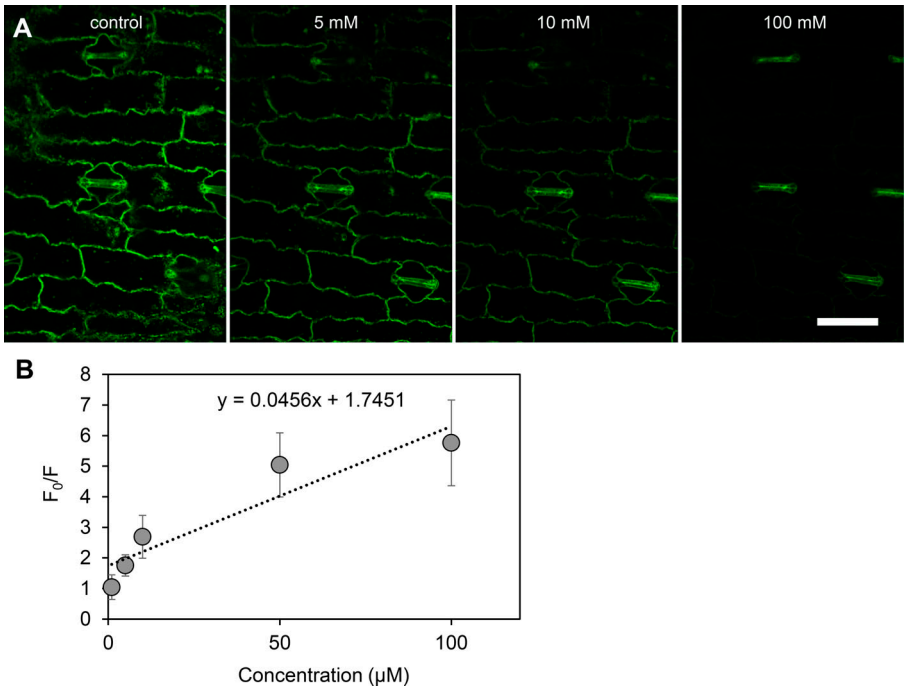

Figure S3. **Application of the quenching assay to maize leaves. (A)** Images of maize leaf epidermis cells labeled with FM4-64 in the absence (control) or presence of TB at the indicated concentration. **(B)** Stern-Volmer plot in which the slope of the regression line indicates quenching efficiency. Error bars indicate standard deviation of the mean (n = 4). Bar, 50 µm.

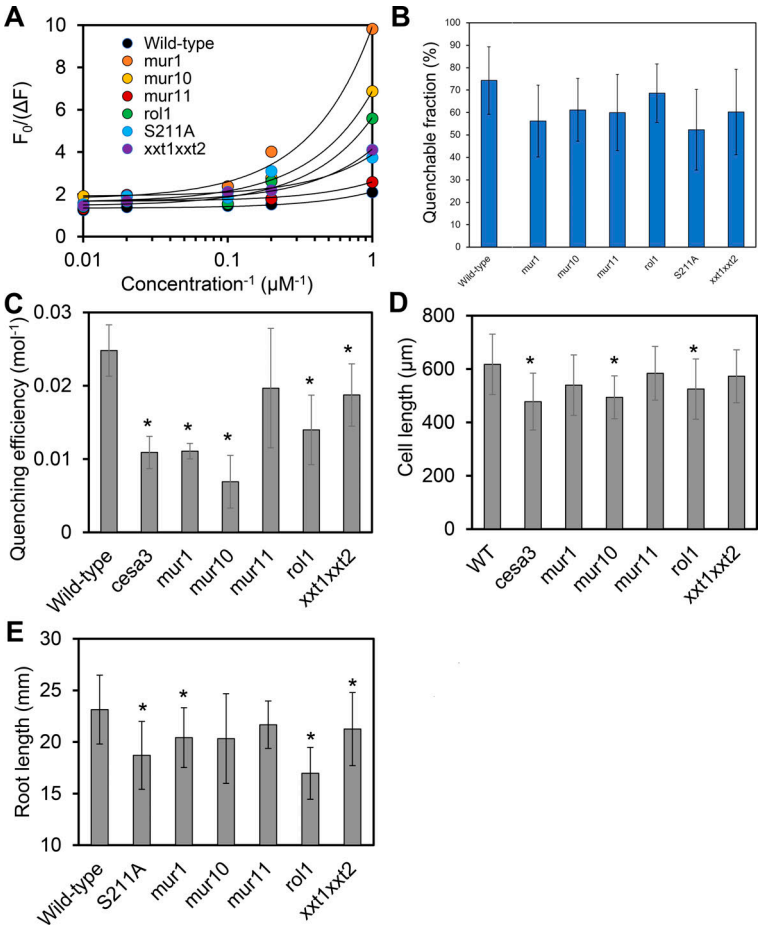

Figure S4. **Quenchable fraction and quenching efficiency in cell wall mutants. (A)** Modified Stern-Volmer plot indicating quenchable fraction in *Arabidopsis* wild-type and cell wall mutant plants. The fraction of FM4-64 fluorescence quenched by TB can be calculated from the y-axis crossing point of regression lines according to Eq. 2. **(B)** Comparison of quenchable fractions indicates limited variation between wild-type and mutant plants. **(C–E)** Quenching efficiency (C), cell length (D), and root length (E) of *Arabidopsis thaliana* wild-type and mutant plants grown under control conditions. Quenching efficiency (C) and cell length (D) were determined for epidermis cells of the elongation zone. All error bars indicate standard deviation of the mean. Asterisks indicate significant difference ($P < 0.05$) from wild type. Number of replicates $n = 4$ (A–C) and $n > 20$ (D and E).

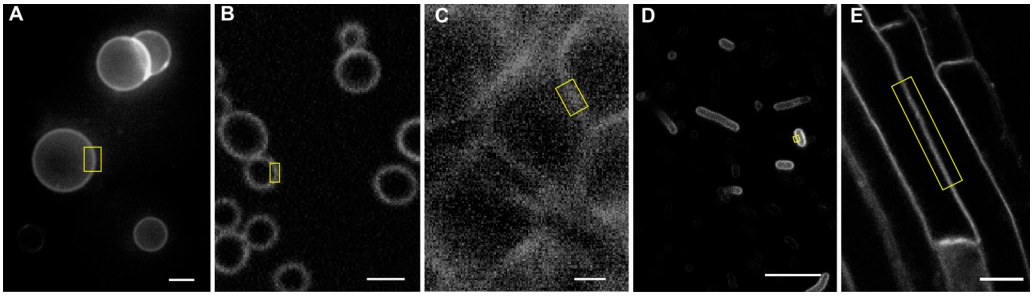

Figure S5. **Quantification of peripheral membrane fluorescence.** Mean fluorescence was measured in an ROI (yellow box) with constant size in each of the experimental systems. **(A)** GUVs, **(B)** *S. cerevisiae*, **(C)** cultured HEK cells, **(D)** *E. coli*, and **(E)** *Arabidopsis* root cells. Bars, 5 μm.