Supporting Information

Experimental Section

Materials

TPE-Ph-In was synthesized according to the Ref.[1] The Agar was purchased from PlantMedia™ and culture medium from Sigma-Aldrich. Standard chemicals were purchased from Sigma–Aldrich. Milli-Q water was purified via the Milli-Q Plus System (Millipore Corporation).

Growth Conditions of *Arabidopsis* and Tobacco Seedlings

Arabidopsis (wild type Col-0 and Mito-GFP) and tobacco seeds were inoculated onto the surface of 1/2 MS solid medium, refrigerated at 4°C for two days, then transferred to a light incubator for cultivation until the desired number of days. The formulation of the 1/2 MS growth medium is 2.37 g MS medium solid powder, 8 g agar, and 10 g sucrose dissolved in 1 L Milli-Q water, adjusted to pH 5.8. The plates inoculated with seeds were placed in a plant growth chambers with a 16-h-light/8-h-dark cycle with a constant temperature at 22 ± 2 °C.

Culture of Tobacco BY-2 Cells and Digestion of Cell Walls

Tobacco BY-2 cells were cultured in BY-2 MS medium, which contained 4.3 g/L Murashige and Skoog Basal Salt Mixture, 100 mg/L myo-inositol, 1 mg/L thiamine hydrochloride, 0.2 mg/L 1,2,4-dichlorophenoxyacetic acid, 255 mg/L KH_2PO_4, and 30 g/L sucrose. The pH was adjusted to 5.0 with KOH, and the medium was sterilized by filtration through a 0.22μm filter membrane before use. And BY-2 cells cultured in BY-2 MS medium were shaken at 130 rpm under dark conditions.

For cell wall digestion of BY-2 cells, transfer 10 ml of BY-2 cells into 150 ml falcon tubes, centrifuge at 100g for 2 min at room temperature and remove the supernatant. Transfer enzyme solution into falcon tube containing 3 ml of compact cells to 15 ml in total (The enzyme solution contains 1% (wt/vol) cellulase, 0.05% pectinase and 0.2% driselase dissolved in TEX medium and filtered the with 0.22 μm filter). Invert the tube several times to thoroughly mix the enzyme solution and cells. Incubate cell in a shaker set at 65 rpm at 27 ˚C for 2.5 h. Centrifuge at room temperature for 15 min at 80g with horizontal centrifuge. Insert the pipette tip below the liquid surface of the upper protoplasts, carefully aspirate the lower solution with peristaltic pump until the floating protoplasts approaches the bottom of the tube. Add 35 ml of EP buffer into the protoplasts and mix them gently, and centrifuge again at 80g for 10 min. Repeat the washing Steps twice. The protoplasts were then resuspended in TEX buffer for subsequent staining and imaging procedures. The configuration methods for the EP buffer and TEX buffer followed previous reports,, and the medium was sterilized by filtration through a 0.22 μm filter membrane before use. [2]

Fluorescence Confocal Imaging

Collect 7-day-old *Arabidopsis* and tobacco seedlings from solid medium and stain them by immersing in a 10 μM TPE-Ph-In dilution solution for 10 minutes at room temperature. Before imaging on the confocal microscope, the seedings were washed with 1/2 MS medium for three times. The sample was placed on a slide and cover with 1/2 MS buffer and then imaged using fluorescence microscopes (3i spinning disk confocal microscope and Leica Stellaris 8 confocal microscope). Excitation wavelength was 488 nm for Mito-GFP, 560 nm for TPE-Ph-In. Emission wavelength range was 500-550nm for Mito-GFP, 575-625nm for TPE-Ph-In. Pearson's correlation coefficient and colocalization rate were calculated using Fiji.

For time-lapse confocal imaging of tobacco seedlings, the Leica Stellaris 8 confocal microscope was used. The excitation wavelength was 561 nm for TPE-Ph-In, with a fluorescence intensity of 3.0%. The emission wavelength range was 582-700 nm. For time-lapse confocal imaging of BY-2 cells, the Leica Stellaris 8 confocal microscope

was used. The excitation wavelength was 530 nm for TPE-Ph-In, with a fluorescence intensity of 2.0%. The emission wavelength range was 551-750 nm.

Super-resolution Imaging

Tobacco BY-2 cells were stained by immersing in a 10 μM TPE-Ph-In dilution solution for 10 minutes. Imaging was performed using a Multimodality Structured Illumination Microscopy (NanoInsights-Tech) system equipped with a 561 nm excitation laser and an emission filter of 570-630 nm, matching the spectral properties of TPE-Ph-In. Super-resolution images acquired via SIM enabled precise visualization of the mitochondrial network and its fusion/fission dynamics. Finally, images were processed using ImageJ for image analysis.

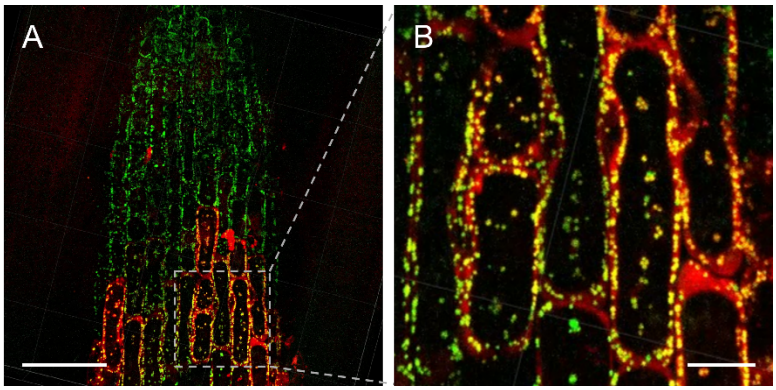

Figure S1 (**A**) Confocal images of *Arabidopsis* seedling expressing Mito-GFP after staining with TPE-Ph-In (10 μM). Green color for Mito-GFP signals, red for TPE Ph-In, and yellow for merged. (**B**) The quadruple-magnified area of the specified section in (**A**). Scale bar in (**A**): 50 μm, scale bar in (**B**): 10 μm.

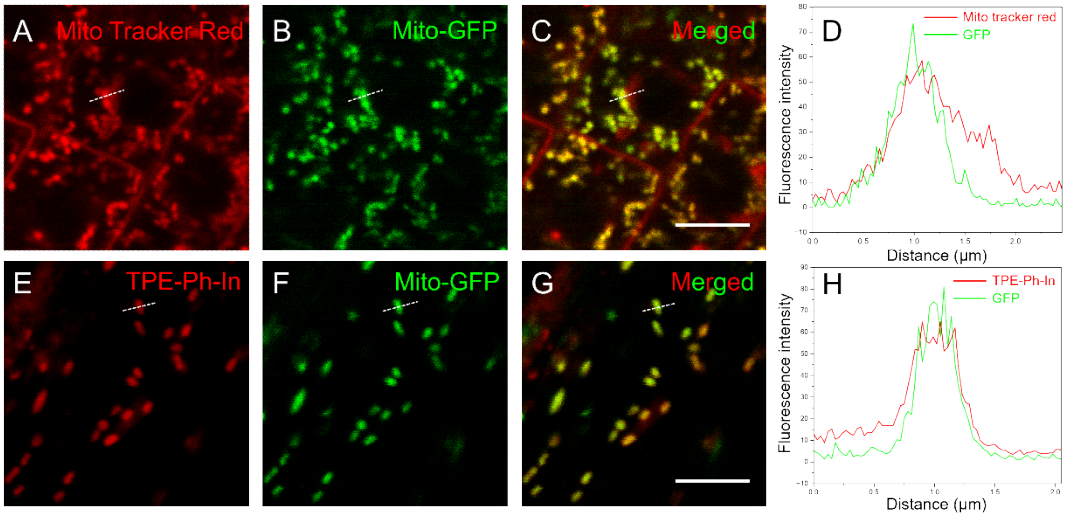

Figure S2 Confocal images of *Arabidopsis* seedling after staining with (**A**) MitoTracker Red (500 nM). (**B**) Mito-GFP, (**C**) overlay of Mito-GFP and MitoTracker Red signals. (**D**) Fluorescence intensity distribution curve of the white line in (**A**) and (**B**). Confocal images of *Arabidopsis* seedling after staining with (**E**) TPE-Ph-In (10 μM). (**F**) Mito-GFP, (**G**) overlay of Mito-GFP and TPE-Ph-In signals. (**H**) Fluorescence intensity distribution curve of the white line in (**E**) and (**F**). Scale bars: 5 μm.

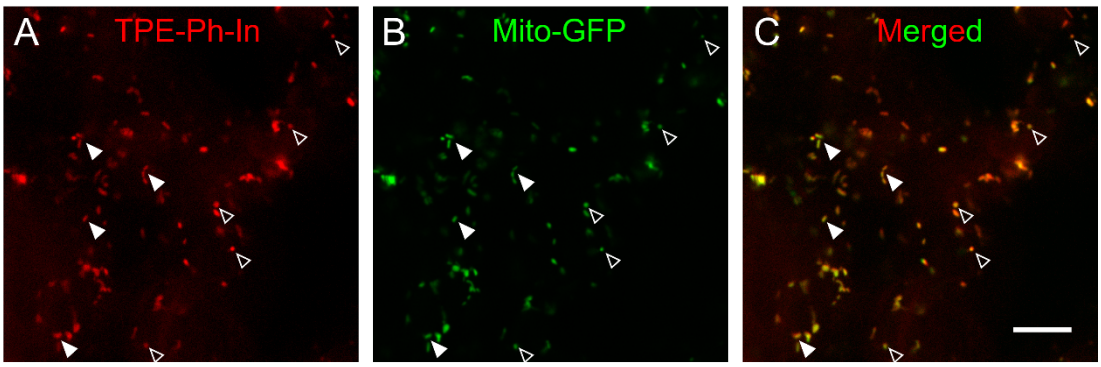

Figure S3 Confocal images of *Arabidopsis* leaf cells staining with (**A**) TPE-Ph-In (10 μM) and (**B**) Mito-GFP, (**C**) overlay of Mito-GFP and TPE-Ph-In signals. Scale bar: 10 μm.

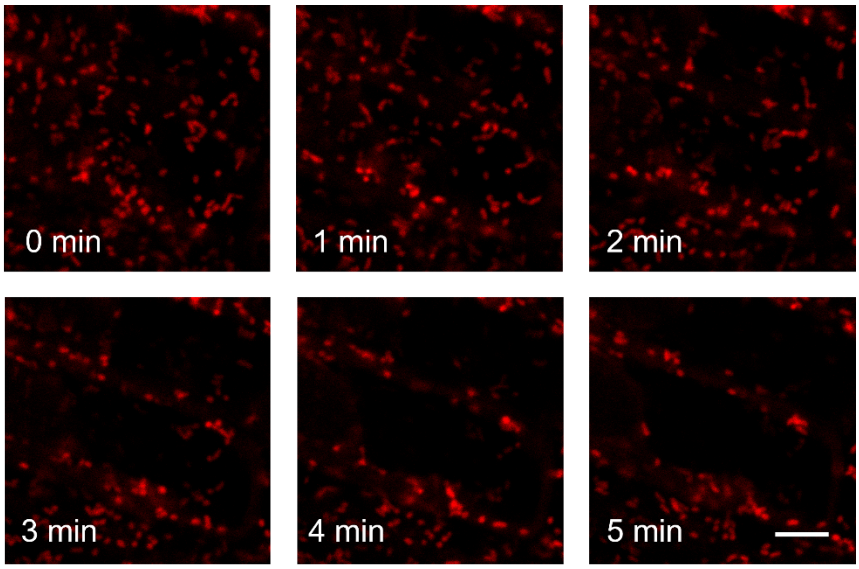

Figure S4 Confocal images of tobacco seedling stained with TPE Ph-In at different time point. Scale bars: 10 μm.

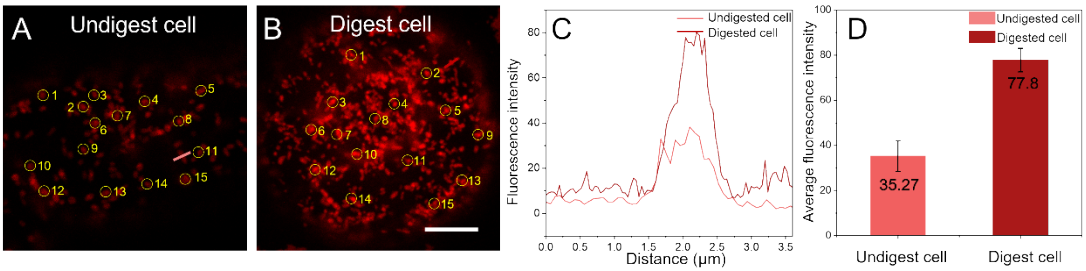

Figure S5 Confocal images of tobacco BY-2 cells stained with TPE-Ph-In without (**A**) and with (**B**) cell wall digestion. (**C**) Fluorescence intensity distribution curve of the pink line in (**A**) and red line in (**B**). (**D**) The average fluorescence intensity of the circled points in (**A**) and (**B**). Scale bar: 10 μm.

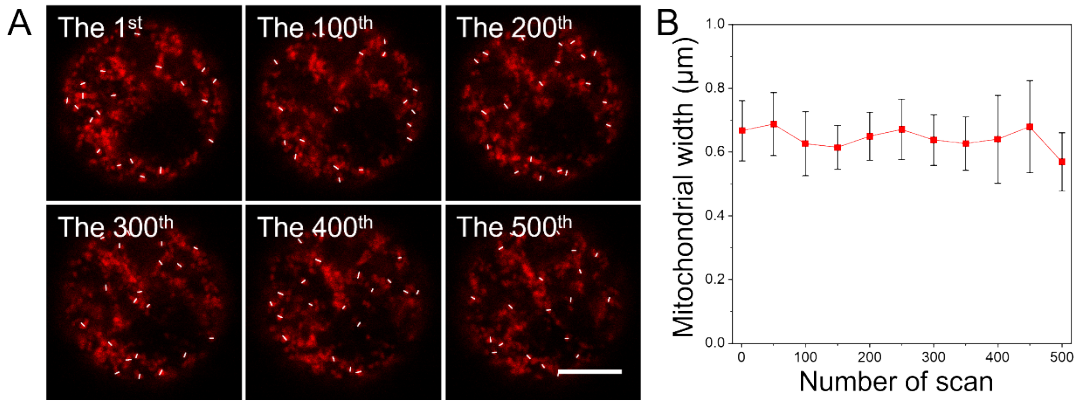

Figure S6 (**A**) The 1st, 100th, 200th, 300th, 400th and 500th scan confocal images continuous exposure under 561 nm excitation on tobacco BY-2 protoplast. (**B**) mitochondrial width fluctuation during continuous exposure. Scale bar: 10 μm.

Reference

1 N. Zhao, S. Chen, Y. Hong and B. Z. Tang, *Chemical Communications*, 2015, **51**, 13599-13602.

2 Y. Miao and L. Jiang, *Nature Protocols*, 2007, **2**, 2348-2353.