

**A**  $\beta$ '-COP

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**B**  $\gamma$ -COP

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**C**  $\delta$ -COP

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**Fig. S1.** Nucleotide sequences of *N. benthamiana*  $\beta$ '-,  $\gamma$ -, and  $\delta$ -COP genes. The protein coding regions of the corresponding cDNAs are shown.

**A**  $\beta'$ -COP

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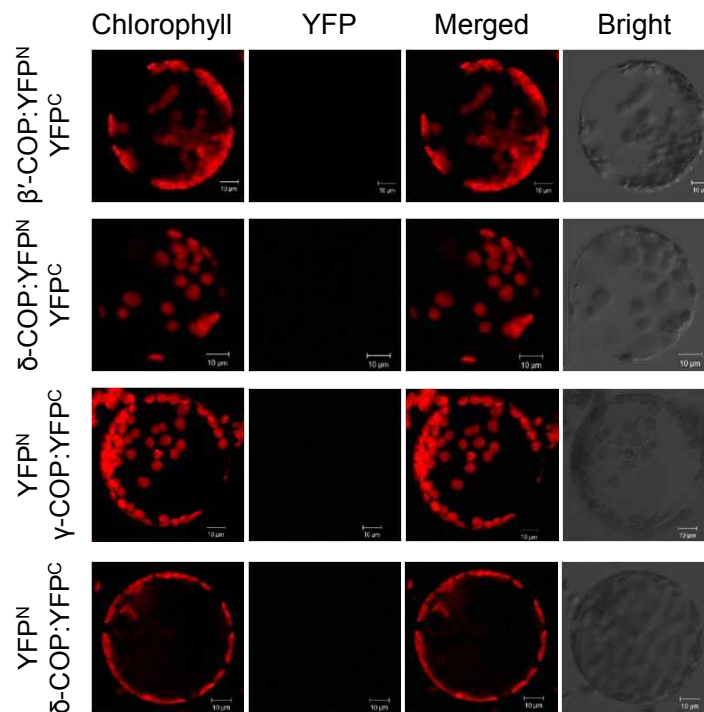
**B**  $\gamma$ -COP

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**C**  $\delta$ -COP

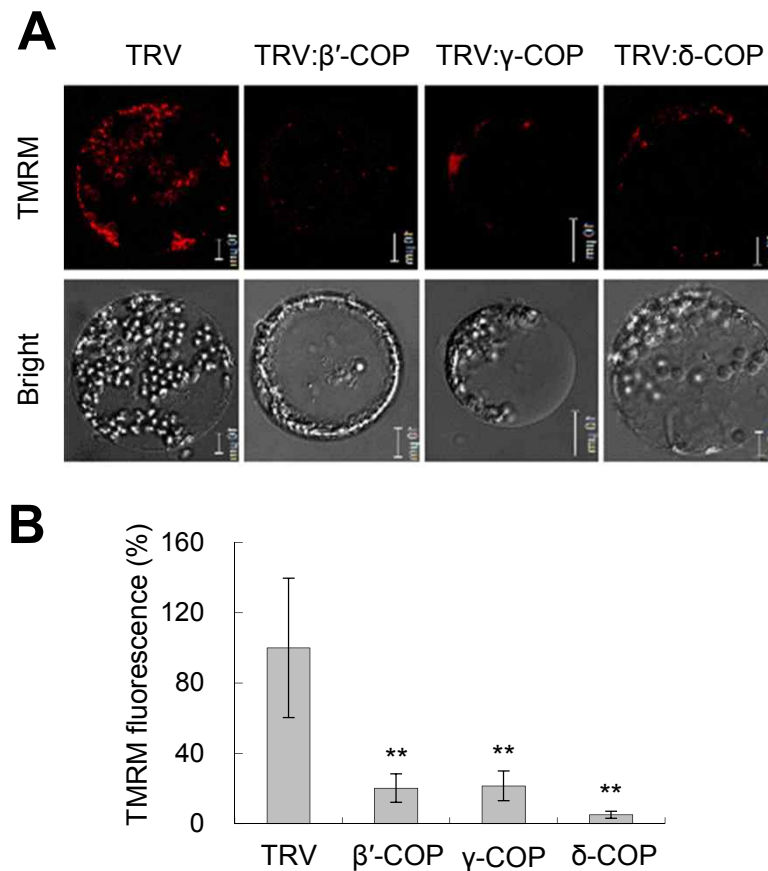
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**Fig. S2.** Amino acid sequences of *N. benthamiana*  $\beta'$ -,  $\gamma$ -, and  $\delta$ -COP proteins.



**Fig. S3.** BiFC controls.

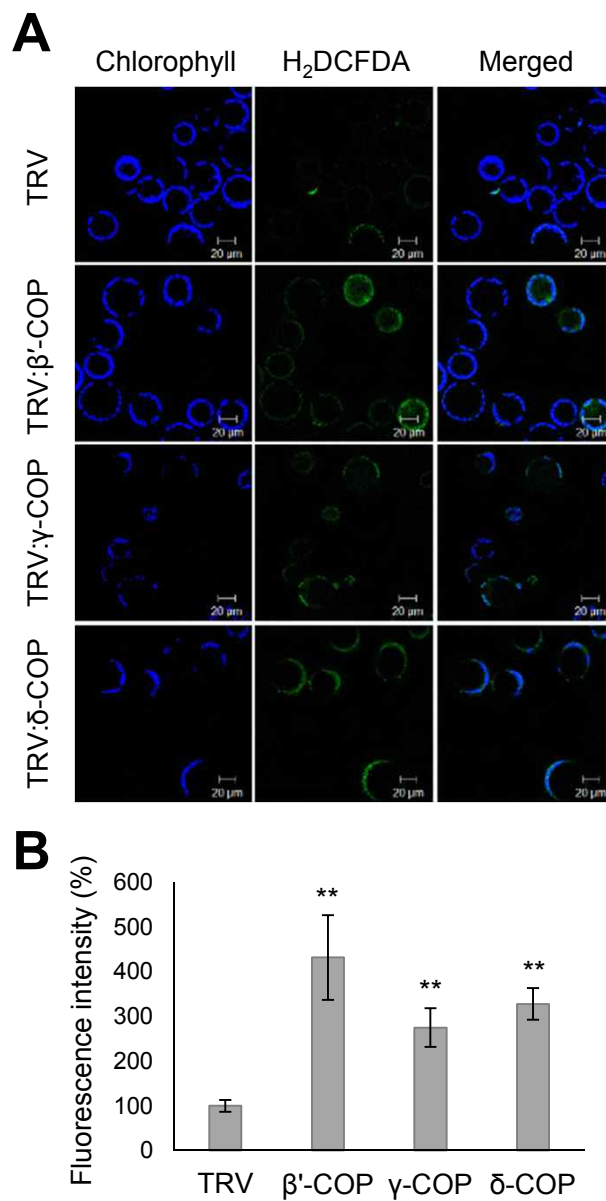
$\beta'$ -COP:YFP<sup>N</sup> and YFP<sup>C</sup>,  $\delta$ -COP:YFP<sup>C</sup> and YFP<sup>C</sup>, YFP<sup>N</sup> and  $\gamma$ -COP:YFP<sup>C</sup>, and YFP<sup>N</sup> and  $\delta$ -COP:YFP<sup>C</sup> were expressed together in *N. benthamiana* leaves via agroinfiltration, and the YFP signal in leaf protoplasts was observed by confocal microscopy. YFP fluorescence was not observed in any of these combinations of gene expression.



**Fig. S4.** Mitochondrial membrane potential in leaf protoplasts of  $\beta'$ -COP,  $\gamma$ -COP, and  $\delta$ -COP VIGS plants.

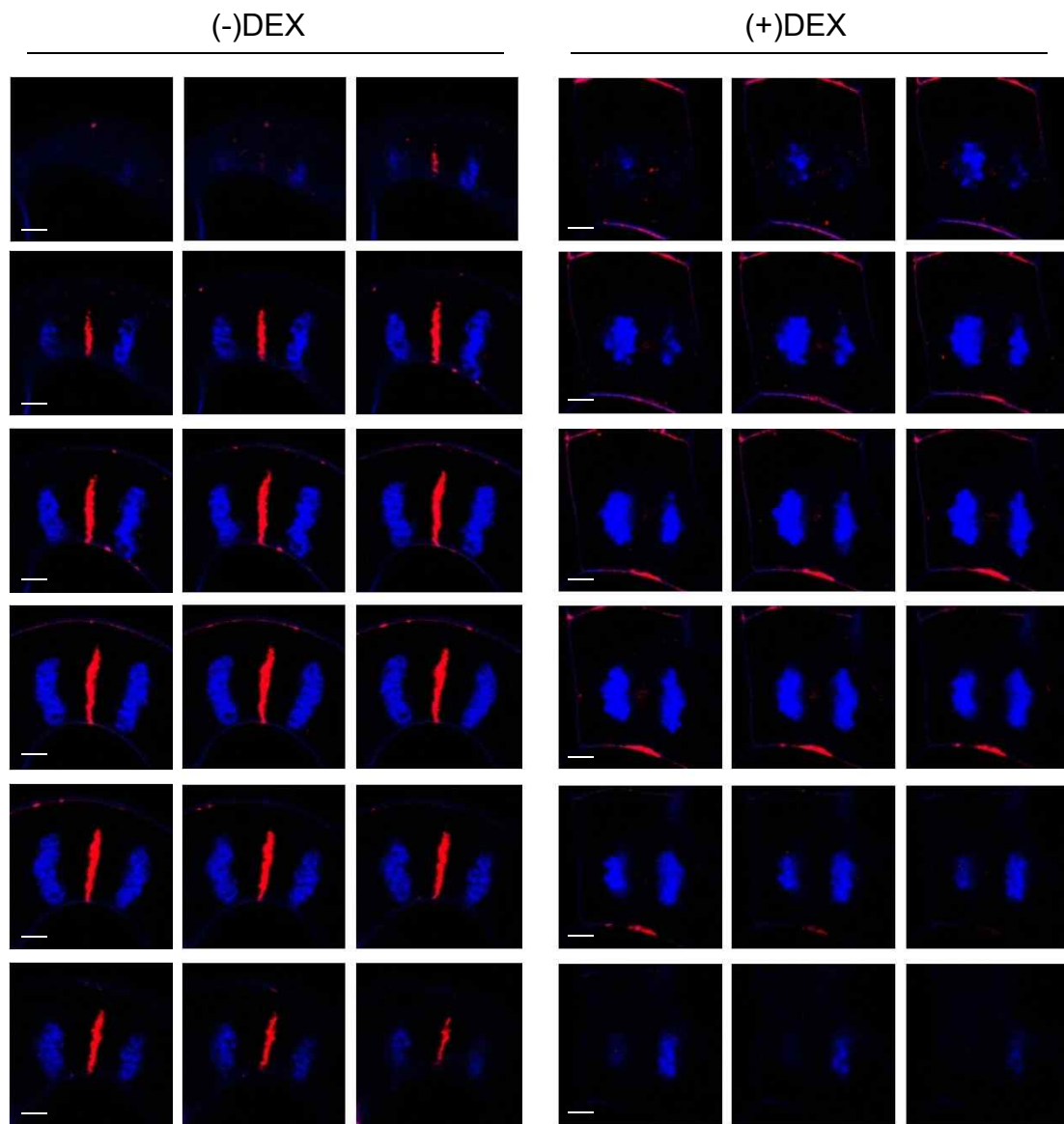
(A) Tetramethylrhodamine methyl ester (TMRM) staining of TRV, TRV: $\beta'$ -COP, TRV: $\gamma$ -COP, and TRV: $\delta$ -COP VIGS plants at 15 DAI. Scale bars = 10  $\mu$ m.

(B) TMRM fluorescence of protoplasts from the VIGS lines was quantified by pixel intensity. Data points represent means  $\pm$  SD of 30 individual protoplasts. Asterisks denote statistical significance of the differences between COPI VIGS and TRV control samples: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ .



**Fig. S5.** H<sub>2</sub>DCFDA staining to detect reactive oxygen species (ROS).

Cellular ROS levels of TRV control, TRV:β'-COP, TRV:γ-COP, and TRV:δ-COP were detected (A) and quantified (B) by H<sub>2</sub>DCFDA fluorescence levels at 15 DAI. Chlorophyll autofluorescence is pseudo-colored blue. Data points represent means ± SD of 30 individual protoplasts. Asterisks denote statistical significance of the differences between COPI VIGS and TRV control samples: \*, P ≤ 0.05; \*\*, P ≤ 0.01. Scale bars = 20 μm.



**Fig. S6.** Visualization of the newly forming cell plate during cytokinesis. The DEX-inducible  $\beta'$ -COP RNAi BY-2 cells were treated with ethanol (-DEX) or 15  $\mu$ M DEX (+DEX) and stained with DAPI (blue) and FM4-64 (red) for visualization of the nuclei and the cell plate, respectively. Confocal microscopy revealed images of consecutive serial sections of a BY-2 cell undergoing cytokinesis. Scale bars = 10  $\mu$ m.