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Supplementary Material

Studies of cytokinin receptor–phosphotransmitter interaction provide evidences for the initiation of cytokinin signalling in the endoplasmic reticulum

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Table S1. Sequences of primers used for PCR

Primer name	Primer sequence
F AHK3/XbaI *R AHK3/Cfr9I*	*5*`-ACGTCTAGAATGAGTCTGTTCCATG-3’ *5*`- ATCCCGGGTGATTCTGTATCTGAAG-3’
F AHK4/XbaI *R AHK4/Cfr9I*	*5*`- ACGTCTAGAATGAGAAGAGATTTTGTG-3’ *5*`- ATCCCGGGCGACGAAGGTGAGAT-3’
F AHP1/BcuI *R AHP1/XhoI*	*5*`-GCCACTAGTATGGATTTGGTTCAG-3` *5*`-CTCGAGAGATCCACCACCTCCACCAAATCCGAGTTCGAC-3`
F *AHP2/BcuI* R *AHP2/XhoI*	5`-GCCACTAGTATGGACGCTCTCAT-3` 5`-GCCCTCGAGGTTAATATCCACTTGA-3`
F *AHP3/BcuI* R *AHP3/XhoI*	5`-GCCACTAGTATGGACACACTCATT-3` 5`-ATCTCGAGAGATCCACCACCTCCACCTATATCCACTTGAGG-3`
F AHP1/NcoI R *AHP1/BamHI*	*5*`-TAACCATGGGGTGGAGGTGGTGGATCTATGGATTTGGTTCAG-3` 5`-AAGGATCCTCAAAATCCGAGTTCGACG-3`
F *AHP3/NcoI* R *AHP3/BamHI*	5`-TAACCATGGGGTGGAGGTGGTGGATCTATGGACACACTCATT-3` 5`-AGGGATCCTTATATATCCACTTGAGGG-3`

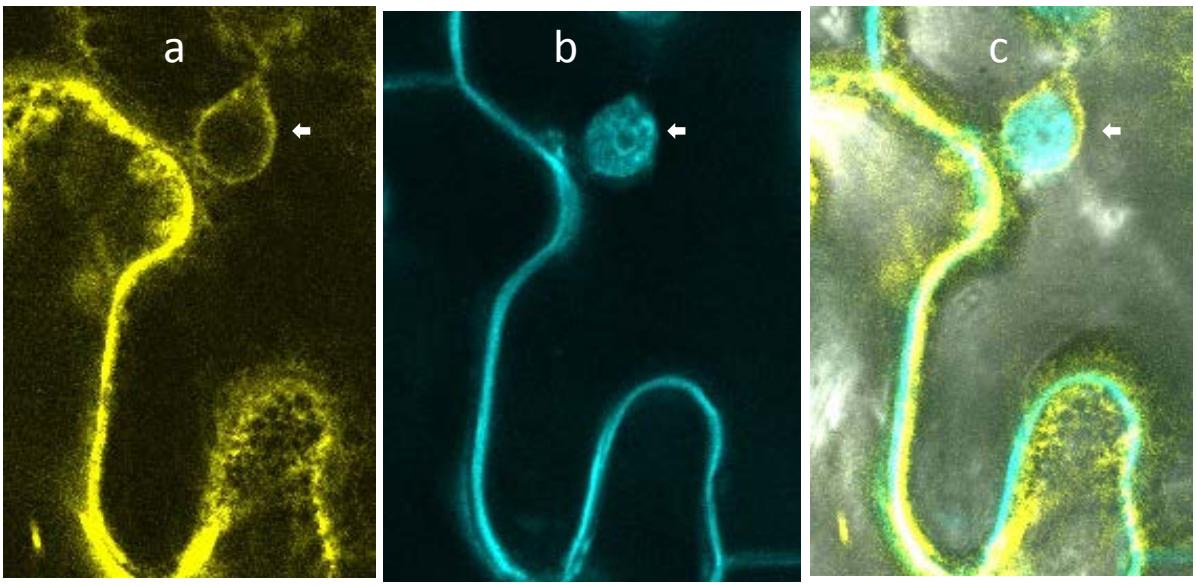

Fig. S1. Interaction pattern of AHK3 and AHP2 shown by BiFC in *N. benthamiana* leaves. (a) Fluorescence signal indicating AHK3 × AHP2 interaction. (b) DAPI staining. (c) Merged pictures of the sections shown in (a) and (b). White arrows indicate the nucleus. The nucleoli is clearly seen in (b) and (c). Apart nuclei, DAPI is known to stain also cell walls (b) (Jordan *et al*. *Plant Mol. Biol.*, 2007, **65**, 163–175; Juntawong *et al*. *The Plant J.*, 2013, **74**, 1016–1028). Note that BiFC staining and DAPI staining do not overlap.

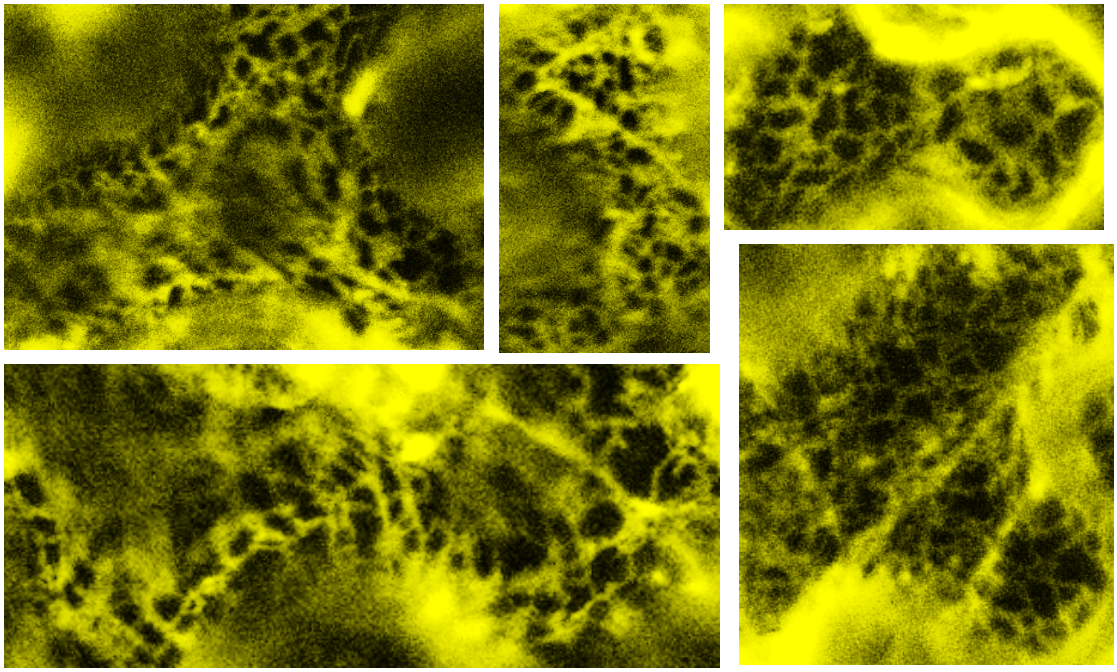

Fig. S2. Interaction AHK4 × AHP3 shown by BiFC in *N. benthamiana* leaves. Fluorescence signals indicating interaction mark the ER network.

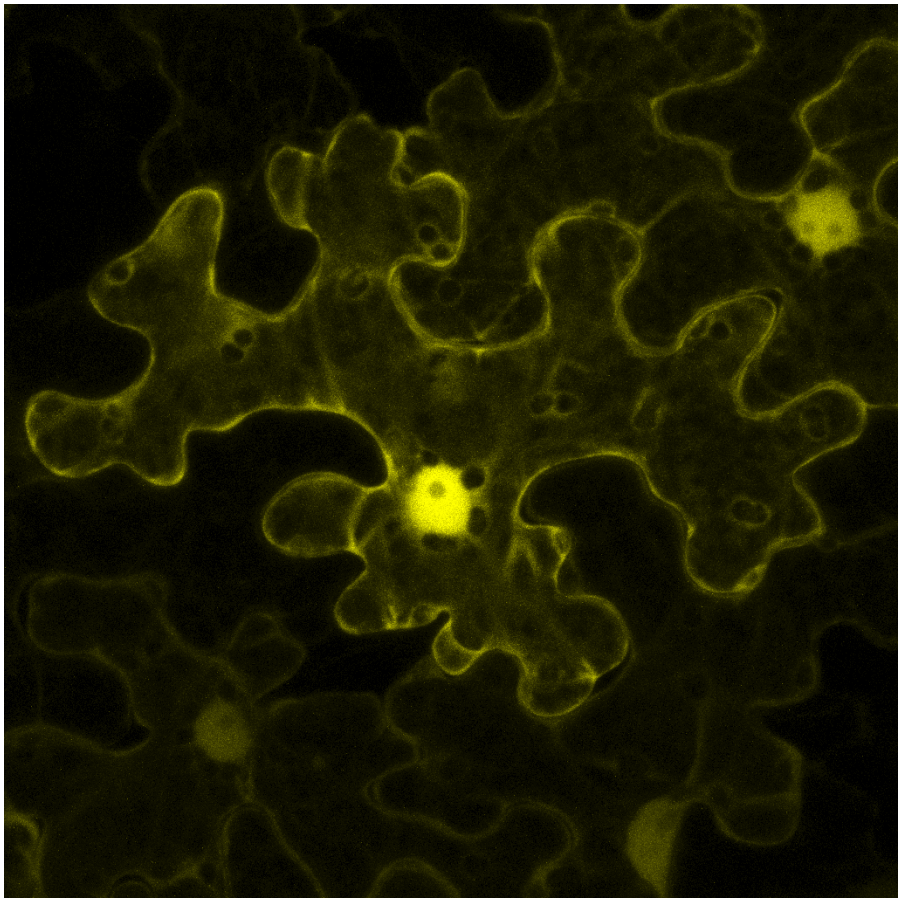

Fig. S3. An example of a fluorescent nucleus labelled by phosphotransmitter interaction (AHP2 × AHP2, BiFC) where nucleolus is clearly seen.

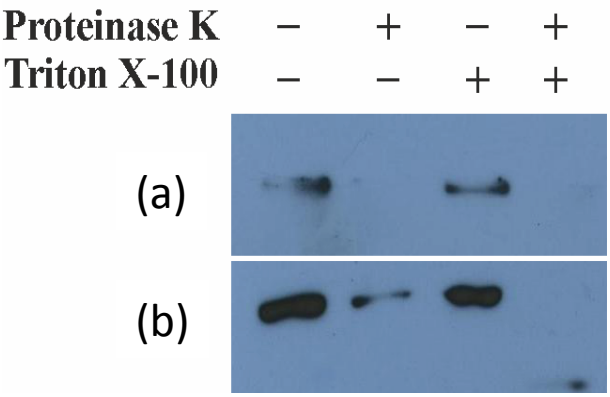

Fig. S4. Determination of AHK3 orientation by a protease protection assay. Microsomes from tobacco leaves expressing the *AHK3-Myc* gene were treated with proteinase K in the presence or absence of Triton X100. The Myc-tag was fused to the C-terminus of AHK3. Microsomal proteins were solubilized, resolved by SDS-PAGE, and subjected to immunoblotting using (a) anti-Myc-tag monoclonal antibodies, or (b) rabbit polyclonal antibodies against the BiP marker of the ER lumen. The disappearance of the signal derived from the detection of the Myc-tag and the preservation of the BiP signal (second column) indicates the localization of these two markers at opposite sides of the membrane. The lowering of BiP content in (b) in the absence of Triton X100 may be due to different reasons, the same trend was observed in the original protocol (Ma *et al*. 2006).