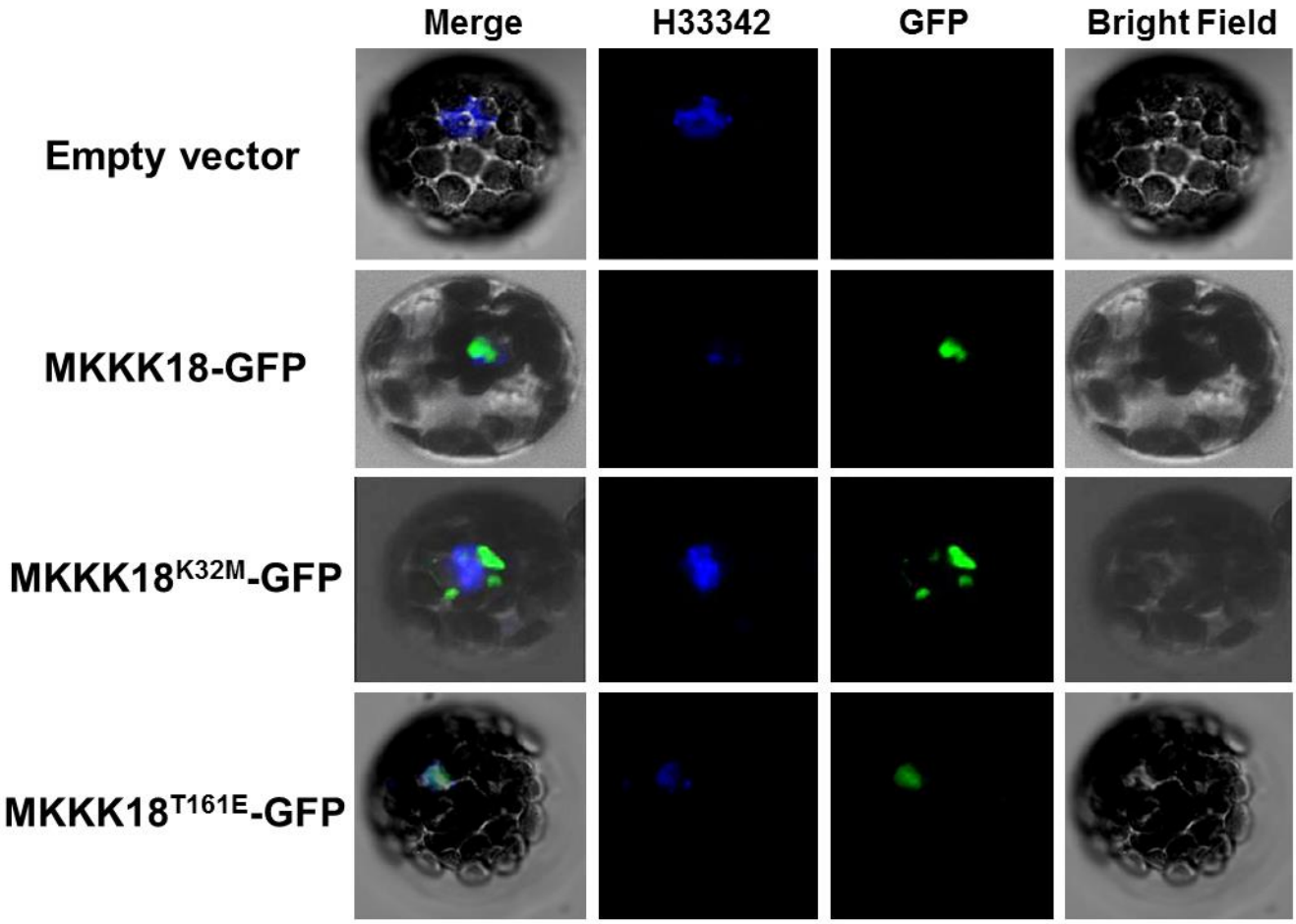
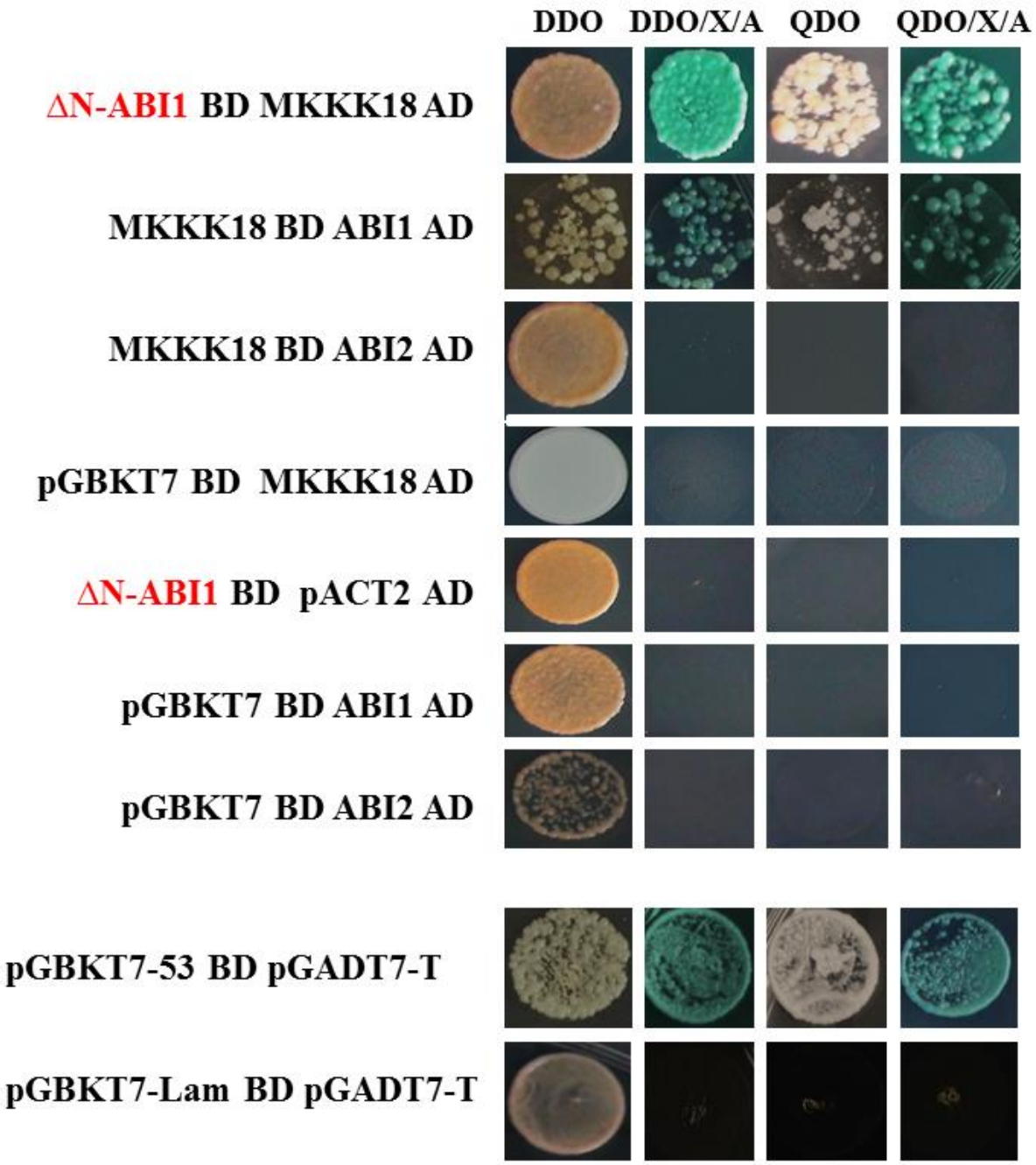


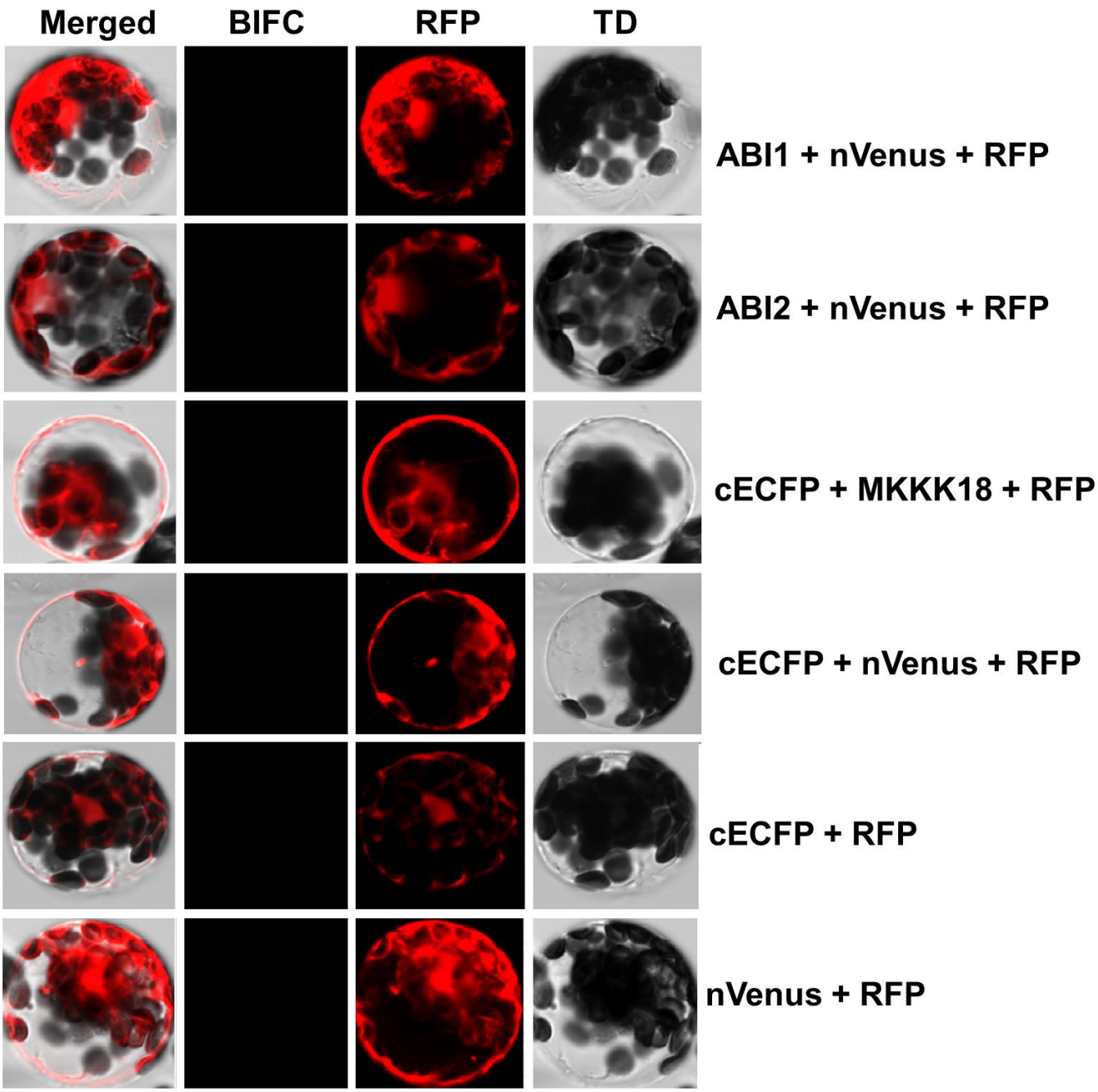
Supplementary Figure S1. Subcellular localization of the MKKK18 protein in the *abi1td* protoplasts.

Microscopy images show nuclear localization of the MKKK18-GFP fusions in protoplasts isolated from leaves of the *abi1td* mutant. Hoechst H33342 was employed to stain the nucleus. Empty pEarlygate 103 was used as negative control.



Supplementary Figure S2. Yeast two-hybrid analysis of the interaction between MKKK18 and ABI1/2 PP2Cs.

Diploid yeast colonies were grown on double (DDO-SD medium without Leu and Trp) or quadruple selective medium (QDO-SD medium without Leu, Trp, His, or Ade) with or without supplemented X-alpha-Gal and *aureobasidin* The bait (MKKK18) did not autoactivate the reporter genes in yeast. Interactions between p53 or Lam and T proteins were used as positive control and negative control, respectively. Abbreviations: BD binding domain, AD-activation domain, ΔN-ABI1 – the N-terminal deletion of ABI1.



Supplementary Figure S3.Negative controls in BiFC experiment.

The negative controls in BiFC analysis were conducted by cotransfecting free ECFP or nVenus fragments together with complementary full length proteins that resulted in no detectable signals. RFP was used as a transformation control.

Supplementary Methods S1. BiFC vector construction

Expression cassettes from pSITEII-4C1 and pSITEII-7N1 (Martin et al., 2009) were PCR amplified using the primers CasF (5'-TAGCTGTTTGCCAACCGGTCAAC-3'), and to avoid double digest of the PCR product with NotI, we used the primer CasPspR (5'-ATAGGGCCCCGTCACTGGATTTTGGTT-3') which changed the NotI site for PspOMI at the 3'-end. PCR products were digested with AgeI and PspOMI (Thermo Scientific). The cassette from pSITEII-4C1 was cloned into the vector pAUX3169 (Goderis et al., 2002) and digested with AgeI and NotI (Thermo Scientific), and the cassette from pSITEII-7N1 was cloned into the vector pAUX3132 (Goderis et al., 2002), giving pSAT3-Venus-DEST and pSAT5-DEST-Dendra2 (nomenclature consistent with Tzfira et al., 2005), respectively. Sequence coding for the C-terminal part of eCFP (aa 155-239) was PCR amplified with the primers CpolFseF (5'-AATGGCCGGCCATGGACAAGCAGAAGA-3') and CpolSpeR (5'-CCCGACTAGTCTTGTACAGCTCGTC-3') using pSITE-1CA (Chakrabarty et al., 2007) as the matrix. Primers NpolFseF (5'-ATTGGCCGGCCATGGTGAGCAAGGGC-3') and NpolSpeR (5'-ATTACTAGTCTCGATGTTGTGGCG-3') were used to amplify sequences coding for the N-terminal part of Cerulean and Venus (aa 1-173) from pSITEII-2C1 and pSITEII-4C1 (Martin et al., 2009), respectively. All PCR products were digested using a restriction pair FseI-SpeI (NEB) and subcloned into pSAT3-Venus-DEST and pSAT5-DEST-Dendra2, replacing Venus and Dendra2. Constructed Gateway®-compatible vectors for N- (pSAT3-cCFP-DEST, pSAT3-nVenus-DEST, pSAT3-nCerulean-DEST) and C-terminal (pSAT5-DEST-cCFP, pSAT5-DEST-nVenus, pSAT5-DEST-nCerulean) protein fusions were used in this study.

Literature

Goderis, I. and Bolle, M.De. (2002) A set of modular plant transformation vectors allowing flexible insertion of up to six expression units. *Plant Mol. Biol.* 50: 17–27.

Tzfira, T., Tian, G.W., Lacroix, B., Vyas, S., Li, J., Leitner-Dagan, Y. et al. (2005) pSAT vectors: a modular series of plasmids for autofluorescent protein tagging and expression of multiple genes in plants. *Plant Mol. Biol.* 57: 503–516.

Chakrabarty, R., Banerjee, R., Chung, S.M., Farman, M., Citovsky, V., Hogenhout, S. et al. (2007) pSITE vectors for stable integration or transient expression of autofluorescent protein fusions in plants: probing *Nicotiana benthamiana*-virus interactions. *Mol. Plant Mic. Inter.* 20: 740–750.

Martin, K., Kopperud, K., Chakrabarty, R., Banerjee, R., Brooks, R. and Goodin, M.M. (2009) Transient expression in *Nicotiana benthamiana* fluorescent marker lines provides enhanced definition of protein localization, movement and interactions in planta. *Plant J.* 59: 150–62.

Supplemental Table S1. Primers used in the study

Name	Primer sequence 5'-3'	Description
MEK18F	CTTCTTGGGTTGGAGTACATTCAT	For *mkkk18-2* genotyping
GKatTDNA	ATATTGACCATCATACTCATTGC	For *mkkk18-2* genotyping
M3K18 LP	TTACCGGGTCGGATATTTAGG	For *mkkk18-1* genotyping
M3K18 RP	GAATGACTCATCAACATGGCC	For *mkkk18-1* genotyping
LBb1.3	ATTTTGCCGATTTCGGAAC	For *mkkk18-1* genotyping
qMK18Fb	TGCGCATTGCGACATTAAGG	qPCR analysis
qMK18Rb	AACCCACCGCCCATATATCACT	qPCR analysis
18S-29For	the same as in Ludwików et al., 2009	qPCR analysis
18S-102Rev	the same as in Ludwików et al., 2009	qPCR analysis
RAB18For	the same as in Ludwików et al., 2009	qPCR analysis
RAB18Rev	the same as in Ludwików et al., 2009	qPCR analysis
RD29BFor	the same as in Fujii et al., 2007	qPCR analysis
RD29BRev	the same as in Fujii et al., 2007	qPCR analysis
GtwMK18F	CACCATGAATTGGACTAGAGGAAAAAC	Cloning into pENTR/sd/d TOPO vector, Gateway primers
GwMK18R	CTAATTCCGTCGAACCGTGATCC	Cloning into pENTR/sd/d TOPO vector, Gateway primers
GMK18R-S	ATTCCGTCGAACCGTGATCC	Cloning into pENTR/sd/d TOPO vector, Gateway primers
M3k18K32MFor	CACTCGCCGTAA**TG**TCCGCCGAGT	Mutagenic primers, K32M substitution in ATP-binding domain
M3k18K32MRev	ACTCGGCGGA**CA**TTACGGCGAGTG	Mutagenic primers, K32M substitution in ATP-binding domain

MK18T161EFa	GGTTGAACCGGAAATA**GAG**GAACCGGTTAGAGGAAC	Mutagenic primers, T161E substitution in kinase domain
MK18T161ERa	GTTCCTCTAACCGGTTC**CTC**TATTTCCGGTTCAACC	Mutagenic primers, T161E substitution in kinase domain
MKKK18fEcoRI	GAATTCATGAATTGGACTAGAGGA	Yeast two hybrid analysis
MAPKKK18rSacI	GAGCTCCTAATTCCGTCGAACC	Yeast two hybrid analysis
pMKKK18FHindIII	AAGCTTCAATAAGTTTCTTAATTAGCA	For construction of MKKK18 promoter-GUS vector
pMKKK18RBH	GGATCCTTGGAGAATGATACTAAAA	For construction of MKKK18 promoter-GUS vector

Literature

Fujii, H., Verslues, P.E. and Zhu, J.K. (2007) Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in *Arabidopsis* . *Plant Cell* 19: 485–494.

Ludwikow, A., Kierzek, D., Gallois, P., Zeef, L., and Sadowski, J. (2009) Gene expression profiling of ozone-treated Arabidopsis *abi1td* insertional mutant: protein phosphatase 2C ABI1 modulates biosynthesis ratio of ABA and ethylene. *Planta.* 230: 1003-1017.