TMK1-based auxin signaling regulates abscisic acid responses via phosphorylating ABI1/2 in *Arabidopsis*

Jie Yang[a,b,c], Hang He[b], Yuming He[b], Qiaozhen Zheng[b], Qingzhong Li[a], Xin Feng[b], Pengcheng Wang[a], Guocheng Qin[a], Yangtao Gu[b], Ping Wu[d], Chao Peng[d], Shilei Sun[b], Yi Zhang[b], Mingzhang Wen[b,e], Rong Chen[a], Yang Zhao[a], and Tongda Xu[b,1]

[a]Shanghai Center for Plant Stress Biology, Center for Excellence in Molecular Plant Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201602, People's Republic of China; [b]FAFU-UCR Joint Center, Horticulture and Metabolic Biology Center, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou 350002, People's Republic of China; [c]University of Chinese Academy Sciences, Beijing 100864, People's Republic of China; [d]National Facility for Protein Science in Shanghai, Zhangjiang Lab, Shanghai Advanced Research Institute, Chinese Academy of Science, Shanghai 201210, People's Republic of China; and [e]Frontiers Science Center for Synthetic Biology (Ministry of Education), Tianjin University, Tianjin 300072, People's Republic of China

Edited by Natasha V. Raikhel, Center for Plant Cell Biology, Riverside, CA, and approved May 3, 2021 (received for review February 8, 2021)

Differential concentrations of phytohormone trigger distinct outputs, which provides a mechanism for the plasticity of plant development and an adaptation strategy among plants to changing environments. However, the underlying mechanisms of the differential responses remain unclear. Here we report that a high concentration of auxin, distinct from the effect of low auxin concentration, enhances abscisic acid (ABA) responses in *Arabidopsis thaliana*, which partially relies on TRANS-MEMBERANE KINASE 1 (TMK1), a key regulator in auxin signaling. We show that high auxin and TMK1 play essential and positive roles in ABA signaling through regulating ABA INSENSITIVE 1 and 2 (ABI1/2), two negative regulators of the ABA pathway. TMK1 inhibits the phosphatase activity of ABI2 by direct phosphorylation of threonine 321 (T321), a conserved phosphorylation site in ABI2 proteins, whose phosphorylation status is important for both auxin and ABA responses. This TMK1-dependent auxin signaling in the regulation of ABA responses provides a possible mechanism underlying the high auxin responses in plants and an alternative mechanism involved in the coordination between auxin and ABA signaling.

auxin | TMK1 | ABA | ABI1/2

Auxin has been reported to regulate nearly all aspects of the development of *Arabidopsis thaliana*, and is therefore considered to be a growth hormone. However, at a relative high concentration, auxin begins to inhibit growth (1, 2), which is similar to the effects of abscisic acid (ABA) but distinct from the effects of a low concentration of auxin. Auxin has also been used to enhance the stress tolerance of agricultural crops (3). However, the underlying mechanism for the effects of high levels of auxin in plant development is still poorly understood. ABA plays an essential role in multiple stress responses such as drought and salinity (4), and is considered as a stress hormone. ABA signaling is also required for plant development, especially in the parts of the plant where auxin accumulates such as the quiescent center in the root (5, 6). Recent work has shown that auxin also participates in salinity and drought stress responses through miR393 and INDOLE-3-ACETIC ACID INDUCIBLE (Aux/IAA) (7, 8), suggesting auxin and ABA have overlapping functions in plants. A previous study showed that auxin controls seed dormancy through ABA INSENSITIVE 3 (ABI3)-mediated ABA signaling in an AUXIN RESPONSE FACTOR (ARF)-dependent manner (9). Meanwhile, ABA regulates auxin-responsive genes through the direct interaction between its receptor, PYL, and MYB transcription factors (10, 11). In *Arabidopsis*, ABA INSENSITIVE 4 (ABI4) participates in cross-talk between ABA and auxin and is involved in *Arabidopsis* lateral root development, primary root growth, seed germination, and flowering time (12–14). Together, these findings suggest a complicated interplay between auxin and ABA in modulating plant development.

The core ABA signaling pathway involves the sensing of ABA by PYR/PYL/RCAR receptors (15–17), the inhibition of type 2C phosphatases (PP2Cs) (negative regulators) (18, 19), and the release of SNF1-related protein kinase 2 (SnRK2s) (positive regulators) (20). In the absence of ABA, PP2Cs directly interact with SnRK2s and block their kinase activities by dephosphorylation, thereby inhibiting ABA signaling. ABA binding causes conformational changes of its receptors that generate a favorable interaction surface for the catalytic site of PP2Cs, which inhibits their enzymatic activity and results in the activation of SnRK2s to mediate downstream ABA responses (21–23). In ABA signaling, PP2Cs are composed of nine members, among which ABI1 and ABI2 play major roles (24). Recent work demonstrates that the ubiquitin ligases PUB12/13 and RGLG1/5 regulate ABI1 and ABI2 through ubiquitination-mediated protein degradation (25, 26), and a WD40 protein ABT interacts with the PYLs and PP2C proteins to hamper the inhibition of ABI1/2 by ABA-bound PYR1/PYL4 (27), which further controls ABA signaling in plants. Furthermore, EAR1 and Feronia-ROP11 are able to enhance ABI1/2 activity, suggesting that within the PP2C family, ABI1/2 function as one of the regulatory hubs in ABA signaling (28, 29).

TRANS-MEMBRANE KINASEs (TMKs) belong to a family of leucine rich-repeat receptor-like kinases that have been reported to be key regulators in both development and auxin signaling in *Arabidopsis*. Mutations in multiple TMKs cause severe growth defects at different developmental stages in plants (30). Recent work

Significance

The complex and diverse functions of the phytohormone auxin rely on its concentration. A high concentration of auxin has distinct effects on plant development and environmental adaptation in comparison with a low level of auxin. In this study, we show that a high concentration of auxin is capable of stimulating ABA responses partially through a TMK1-based mechanism. This involves the phosphorylation of ABI1/2, two negative regulators of ABA signaling, and following the activation of downstream ABA responses. Our work reveals the interplay between the phytohormone auxin and ABA, and provides a basis for interpreting differential phytohormone responses during plant development.

Author contributions: J.Y., R.C., and T.X. designed research; J.Y., H.H., Y.H., Q.Z., Q.L., X.F., P. Wang, G.Q., Y.G., P. Wu, C.P., S.S., Y. Zhang, and R.C. performed research; Y. Zhao contributed new reagents/analytic tools; J.Y. and T.X. analyzed data; and J.Y., M.W., and T.X. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence may be addressed. Email: tdxu@sibs.ac.cn.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2102544118/-/DCSupplemental.

Published June 7, 2021.

has also shown that TMKs mediate both transcriptional and nontranscriptional auxin signaling in *Arabidopsis* (30–34). Here, we report that TMK1 mediates high auxin-enhanced ABA signaling through regulating ABI1/2. This suggests that TMKs might be good candidates for coordinating auxin signaling with other signaling cascades in plant development and possible environmental adaptation, and that TMKs might mediate differential auxin responses by phosphorylating different downstream components.

Results

High Concentration of Auxin Enhances ABA Responses via TMK1. To elucidate the molecular connections between auxin and ABA signaling, we took advantage of typical ABA-related developmental processes as research systems, such as germination. Interestingly, the delayed cotyledon greening during germination, which is ABA dependent, was altered in auxin-deficient mutants. Additionally, the increased auxin in the dominant auxin biosynthesis *yuc1-D* mutant enhanced the ABA responses, while the decreased auxin in the defective auxin biosynthesis *wei8-3tar2-1* mutant reduced it significantly (*SI Appendix*, Fig. S1 *A* and *B*). When we applied yucasin to inhibit endogenous auxin biosynthesis, the inhibition of cotyledon greening with 0.5 μM ABA was reduced by yucasin in a dosage-dependent manner with saturation at 10 μM (Fig. 1*A*). Resupplied auxin (IAA) with a concentration higher than 50 nM was able to restore the ABA responses that were suppressed by yucasin. However, at a concentration of up to 2 μM, auxin itself showed no obvious effect on cotyledon greening (Fig. 1*B* and *SI Appendix*, Fig. S2 *A–C*). Consistent with the cotyledon greening assay, 100 nM IAA but not 10 nM IAA was able to enhance the expression of ABA-responsive genes, while both 10 nM and 100 nM IAA were able to trigger the auxin-induced gene expression (Fig. 1 *C* and *D*). This clearly suggested that a relatively high concentration of auxin plus ABA had a synergetic effect on cotyledon greening. To better understand the mechanism of how auxin enhanced ABA responses, we analyzed the defective mutants in both TMK- and TIR1-based auxin signaling pathways. With regard to the TMK-mediated auxin signaling, we found that the ratio of cotyledon greening in the seedlings treated with ABA plus IAA vs. ABA was 0.53 in the *tmk1-1* mutant compared with 0.17 in wild-type plants, which suggested that *tmk1-1* has reduced sensitivity to auxin. This reduced synergetic effect of auxin on ABA responses in *tmk1-1* was able to be complemented by a genomic *TMK1* fragment (Fig. 1*E* and *G*). Similar to the effects during cotyledon greening, the enhanced expression of ABA-responsive genes by auxin was also diminished in the *tmk1-1* mutant and restored in the complemented lines (*SI Appendix*, Fig. S3 *A–D*). This phenotype was further confirmed in two additional mutant alleles, *tmk1-2* and *tmk1-3*, while other *tmk* mutants did not show obvious defects; and the *tmk4-1* mutant even showed slightly enhanced sensitivities to ABA (*SI Appendix*, Fig. S4 *A* and *B*), suggesting the specific role of TMK1 in auxin-enhanced ABA responses.

By using a TMK1 promoter-driven GUS reporter and the *pTMK1:TMK1-GFP* transgenic line, we found that *TMK1* was highly expressed in germinating seedlings and stomata cells (*SI Appendix*, Fig. S5), suggesting the possible functional roles of TMK1 at these developmental stages. Thus, we tested the role of TMK1 in stomata closure, which was previously reported as a typical ABA response (35). Similar to the effects on cotyledon greening, 2 μM IAA was able to enhance the effects of ABA during stomata closure, which was blocked in *tmk1-1* and rescued in the complemented lines (*SI Appendix*, Fig. S6); this is distinct from the effect of auxin on stomata opening in the dark (36). All these data collectively demonstrate that TMK1 participates in cross-talk between auxin and ABA signaling. Furthermore, in order to exclude a possible effect of ABA synthesis, we quantitated ABA levels in the *tmk1-1* mutant. There was no significant difference in ABA levels in the *tmk1-1* mutant compared to the wild-type plant, even when both were treated with salt, which can strongly induce ABA synthesis (*SI Appendix*, Fig. S7). These data suggest that in *Arabidopsis*, TMK1 is not involved in ABA production, but rather controls ABA signaling.

We next examined TIR1-based auxin signaling and found that the auxin-enhanced ABA responses during the cotyledon greening process were also reduced in the *tir1-1afb2-3afb3-4* mutant, which was consistent with a previous report (9) (*SI Appendix*, Fig. S8 *A* and *B*). However, distinct from the *tmk1* mutant, the *tir1-1afb2-3afb3-4* showed normal responses in stomata closure (*SI Appendix*, Fig. S6), suggesting that TIRs and TMK-based auxin signaling might coordinate to regulate certain ABA responses.

Auxin and TMK1 Regulate ABA Signaling through ABI1/2. The well-established ABA signaling pathway in *Arabidopsis* relies on the perception of ABA by its PYR/PYLs/RCAR receptors followed by repression of PP2C phosphatases to enable SnRK2s-dependent gene transcription (37). To elucidate how TMK1 regulates ABA signaling, we screened a yeast two-hybrid (Y2H) cDNA library, using the TMK1 kinase domain (TMK1-KD) as a "bait." Among the candidate interactors of TMK1, we identified ABI2, a key molecule in ABA signaling. A subsequent yeast two-hybrid assay revealed that the TMK1 kinase domain interacted with ABI1 and ABI2 but not with other subfamily members of PP2C in ABA signaling (Fig. 2*A* and *SI Appendix*, Fig. S9 *A* and *C*). This interaction between TMK1-KD and ABI1/ABI2 proteins was further validated by a pull-down assay (Fig. 2*B*). TMK1-HA proteins were also able to coimmunoprecipitate with ABI1/2-Flag but not with HAB1-Flag proteins from protoplasts (Fig. 2*C* and *SI Appendix*, Fig. S9*B*). Previous work revealed that ABI1/2 functions redundantly in the negative regulation of ABA responses in *Arabidopsis* (28, 38). Consistent with these observations, the *abi1-2abi2-2* double mutant showed enhanced ABA responses (Fig. 2*D*). To further test whether TMK1 affected ABA signaling through ABI1/2 phosphatases, we generated a *tmk1-1abi1-2abi2-2* triple mutant and found that the *tmk1-1abi1-2abi2-2* mutant showed enhanced ABA response, which was similar to the *abi1-2abi2-2* mutant but opposite from the ABA responses of the *tmk1-1* mutant (Fig. 2 *E* and *F*). This result suggests that the regulation of ABA signaling by TMK1 is dependent on ABI1/2.

To further illustrate whether ABI1/2 could be the control nodes between TMK1-mediated auxin signaling and ABA signaling, we analyzed the effect of auxin on ABA responses in different ABA signaling mutants. The results showed that the mutation of the PYR/PYL/RCAR family reduced ABA responses but did not alter the effect of auxin. However, the mutation of ABI1/2 and their downstream SnRK2s family members abolished the effect of auxin on ABA responses in the cotyledon greening assay (*SI Appendix*, Fig. S10 *A* and *B*). These results clearly suggest that the auxin regulation happens at the ABI1/2 step of the signaling pathway. Similarly, auxin and ABA treatment in the *tmk1-1abi1-2abi2-2* triple mutant resulted in similar phenotypes to the *abi1-2abi2-2* mutant (Fig. 2 *D* and *F*). These data reveal the role of ABI1/2 as the molecular connection between TMK1-based auxin signaling and ABA signaling.

TMK1 Inhibits ABI2 Phosphatase Activity via Phosphorylation. To gain insight into the mechanism of how TMK1 regulates ABA signaling through ABI1/2, we tested whether the kinase activity of TMK1 was required during this process. The kinase inactive TMK1 with a point mutation at amino acid 616 (from Lys to Gln) was unable to complement the defect of high auxin-enhanced ABA responses in the *tmk1-1* mutant (Fig. 3 *A–C* and *SI Appendix*, Fig. S6). This suggests that auxin and TMK1 regulate ABA signaling by the phosphorylation of its substrates. Furthermore, the in vitro kinase assay revealed the direct phosphorylation of ABI2 by the TMK1 kinase domain (Fig. 3*D*), which indicates that TMK1 might regulate ABI2 through direct phosphorylation.

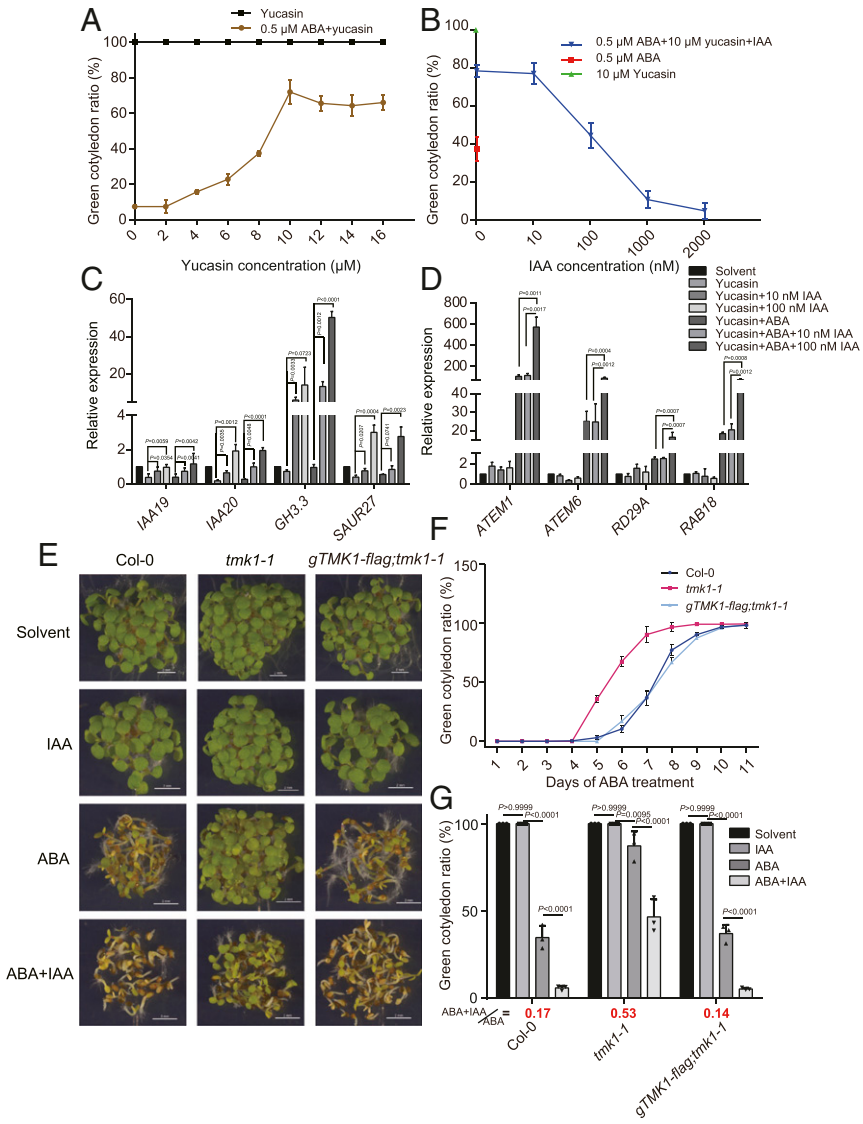

Fig. 1. TMK1 is involved in the high auxin-stimulated ABA responses. (*A*) Quantification of the cotyledon greening ratio of 6-d-old wild-type seedlings treated with different concentrations of yucasin together with or without 0.5 μM ABA. The data are means ± SD ($n = 3$). (*B*) Quantification of the cotyledon greening ratio of 7-d-old wild-type seedlings treated with different concentrations of IAA together with both 10 μM yucasin and 0.5 μM ABA. The data are means ± SD ($n = 3$). (*C* and *D*) Expression of auxin-responsive genes in germinating seedlings treated with 10 nM IAA or 100 nM IAA together with 10 μM yucasin and/or 20 μM ABA (*C*). Expression of ABA-responsive genes in germinating seedlings treated with 10 nM IAA or 100 nM IAA together with 10 μM yucasin and/or 20 μM ABA (*D*). Three biological replicates were performed with consistent results. The figures show results from one representative biological repeat. ($n = 3$, two-tailed Student's *t* test). (*E*–*G*) Cotyledon greening phenotype of Col-0, *tmk1-1*, and the complemented line in response to 2 μM IAA and 0.5 μM ABA. Representative figures at day 7 are shown in *E*. The germination greening ratios under 0.5 μM ABA treatment during the continuous 11 d were quantified in *F*. Data show the means ± SD (Col-0 vs. *tmk1-1:* $P < 0.0001$, Col-0 vs. *gTMK1-flag;tmk1-1:* $P = 0.4634$; $n = 3$ two-way ANOVA). The cotyledon greening ratios on day 7 are quantified in *G*. The change ratio of ABA + IAA vs. ABA indicate the auxin sensitivity of ABA responses. Data show the means ± SD ($n = 3$, two-way ANOVA). Approximately 80 seedlings were analyzed in each replicate. (Scale bars, 2 mm.)

ABI1/2 phosphatases are key mediators in the transduction of ABA signals to the downstream SnRK2s-based transcriptional machinery (37). To determine whether TMK1 affects the function of ABI1/2 in transducing ABA signals, we tested the effect of ABA on the kinase activity of SnRK2s. We used antibodies against the phosphorylated peptide "SVLHSQPK-*p*S-TVGTP," which is essential for the activation of SnRK2s (39–41). The anti-phosphorylation antibodies recognized the activation of SnRK2.6 and SnRK2.2/2.3 upon ABA treatment (41, 42), which was similar in Col-0, *tmk1-1*, and *tir1-1afb2-3afb3-4* mutants. However, with auxin treatment, the activation of SnRK2s by ABA was significantly reduced in *tmk1-1* but only slightly reduced in *tir1-1afb2-3afb3-4* in comparison with Col-0 (Fig. 3*E*). These results suggest that TMK1 positively regulates auxin and ABA-induced activation of SnRK2s in vivo.

Next, we assayed whether TMK1 could affect the assembly of the PYR/PYLs/RCAR-ABI1/ABI2-SnRK2s protein complexes. However, the presence of the TMK1 kinase domain affected neither the binding of PYR1 to ABI1/ABI2 nor the binding of ABI1/ABI2 and SnRK2.2 (*SI Appendix*, Fig. S11 *A* and *B*). Then, we analyzed whether the phosphorylation of ABI2 by TMK1 might directly affect its enzymatic activity using an in vitro phosphatase assay. We found that an adenosine triphosphate (ATP)-triggered phosphorylation reaction by TMK1-KD but not the inactive TMK1^{K616E}-KD significantly reduced the phosphatase activity of ABI2 (Fig. 3*F* and *SI Appendix*, Fig. S12). This partially explains

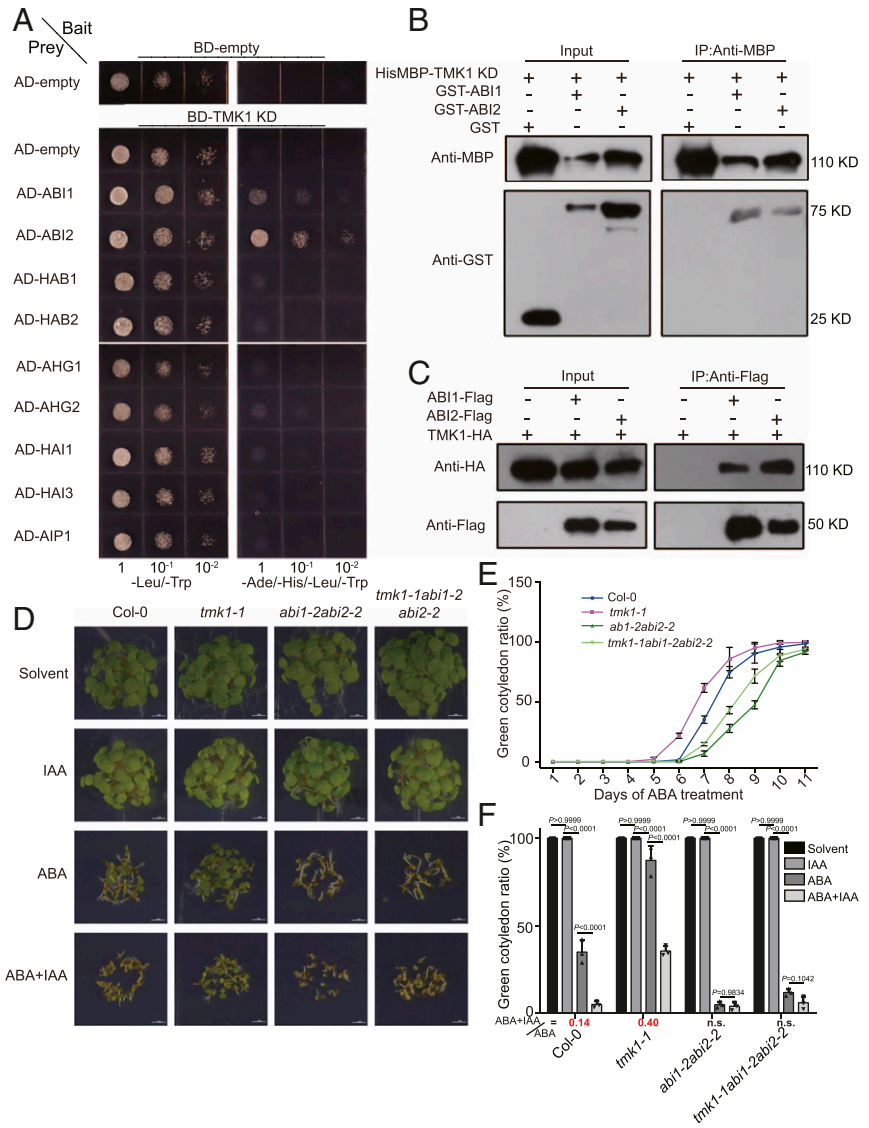

Fig. 2. TMK1 physically and genetically interacts with ABI1, ABI2. (*A*) Yeast two-hybrid assay between the kinase domain of TMK1 and PP2C sub-A family members. DNA binding domain (BD) and activation domain (AD) were used as empty controls. (*B*) In vitro pull-down of *Escherichia coli* expressed HisMBP-fused TMK1 kinase domain and GST-fused ABI1, ABI2 proteins. (*C*) Coimmunoprecipitation between TMK1 and ABI1, ABI2 by coexpressing *35S:TMK1-HA* and *35S:ABI1-flag* or *35S:ABI2-flag* in *Arabidopsis* protoplasts. (*D–F*) Cotyledon greening phenotype of Col-0 and *tmk1-1*, *abi2-2abi1-2*, and *tmk1-1abi1-2abi2-2* mutants in response to 2 μM IAA and 0.5 μM ABA. Representative figures on day 7 are shown in *D*. The greening ratio under 0.5 μM ABA treatment during the continuous 11 d was quantified in *E*. The greening ratios under 0.5 μM ABA and 2 μM IAA treatment on day 7 were quantified in *F*. The change ratio of ABA + IAA vs. ABA indicate the sensitivity of auxin in ABA responses. Data show the means ± SD ($n = 3$, two-way ANOVA). Approximately 80 seedlings were analyzed in each replicate. (Scale bars, 2 mm.)

how TMK1 regulates the ABA-dependent activation of SnRK2s and the various downstream ABA responses through ABI1/2.

Auxin and TMK1 Regulate ABI2 Activity by Phosphorylation at Thr-321. To characterize the regulatory mechanism of ABI1/2, we first tested whether auxin could activate TMK1 to phosphorylate ABI2. Our gel-shift assay indicated that auxin could trigger the phosphorylation on TMK1 in vivo, while yucasin decreased TMK1 phosphorylation (*SI Appendix*, Fig. S13*A*). Next, we used the immunoprecipitated TMK1 proteins from seedlings, treated with or without auxin, as kinases to phosphorylate ABI2 proteins in a semisynthetic reaction assay with ATP-γ-S (43). We found that auxin could enhance the phosphorylation on ABI2 proteins by TMK1 (*SI Appendix*, Fig. S13*B*). Next, we purified ABI2 proteins from plant cells and detected the phosphorylation sites of ABI2 using mass spectrometric analysis. We found a few TMK1-induced phosphorylation sites, including threonine 321 (T321) in the phosphatase catalytic domain of ABI2 (*SI Appendix*, Fig. S14 *A* and *B*). Further quantitative mass spectrometry analysis showed that T321 phosphorylation level decreased dramatically in the *tmk1-1* mutant compared with wild type, suggesting that TMK1 phosphorylates ABI2 at the T321 site in vivo (Fig. 4*A* and *SI Appendix*, Fig. S14*C*). By sequence alignment, we found that this T321 phosphorylation site was conserved in ABI1 of *Arabidopsis* and its orthologs from other species, and that TMK1 was also conserved in these species (*SI Appendix*, Fig. S15). These results indicate that the regulation at the T321 site in ABI2 proteins is a possible fundamental mechanism for TMK1-mediated ABA responses in plants.

To verify the function of this specific phosphorylation site, we mutated the T321 residue to alanine (T321A) to mimic the

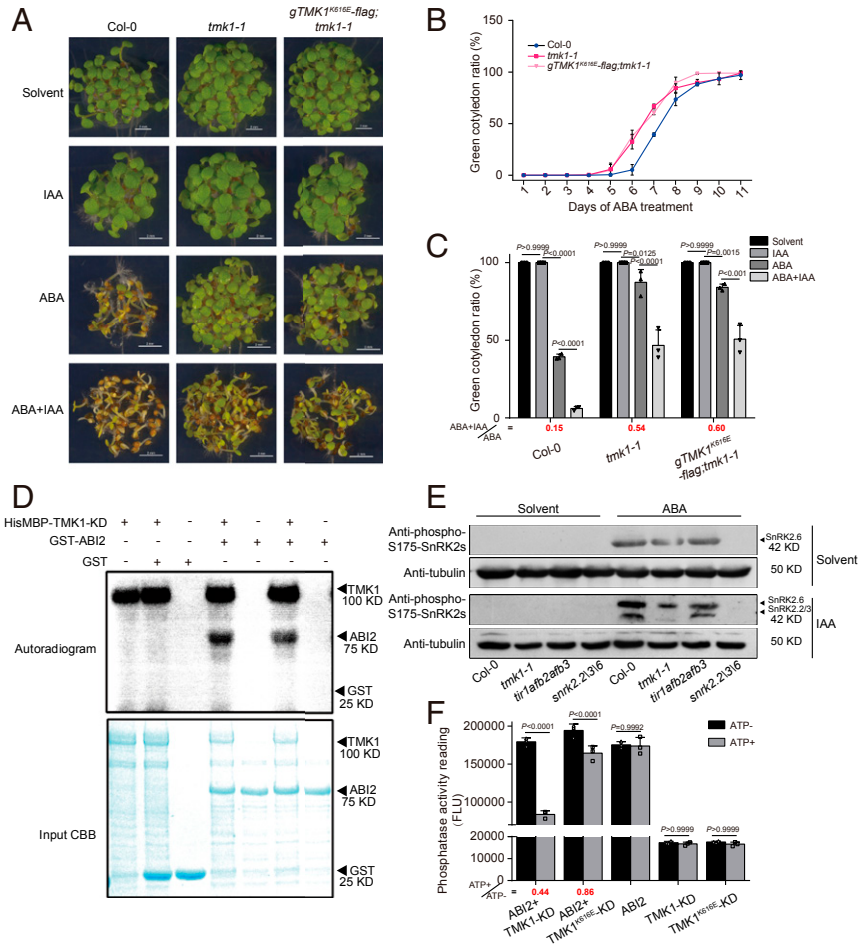

Fig. 3. TMK1 inhibits ABI2 phosphatase activity via phosphorylation. (*A–C*) Cotyledon greening phenotype of Col-0, *tmk1-1*, and the complemented lines by the kinase-inactive variant TMK1^{K616E} in response to 2 μM IAA and 0.5 μM ABA. Representative images of cotyledon greening on day 7 are shown in *A*. The greening ratios under 0.5 μM ABA treatment during the continuous 11 d were quantified in *B*. Data show the means ± SD (*tmk1-1* vs. Col-0: $P < 0.0001$, *tmk1-1* vs. *gTMK1^{K616E}-flag;tmk1-1*: $P = 0.1239$; $n = 3$, two-way ANOVA). The greening ratios under 0.5 μM ABA and 2 μM IAA treatment at day 7 were quantified in *C*. The change ratio of ABA + IAA vs. ABA indicates the sensitivity to auxin in ABA responses. Data show the means ± SD ($n = 3$, two-way ANOVA). Approximately 80 seedlings per genotype were measured in each replicate. (Scale bars, 2 mm.) (*D*) TMK1 phosphorylates ABI2 in vitro. (*Top*) Autoradiogram showing phosphorylation of HisMBP-TMK1-KD and GST-ABI2 (recombinant proteins expressed in *E. coli* strain BL21) that were incubated in an in vitro kinase buffer with radioactive isotopes. (*Bottom*) Input protein visualized by Coomassie brilliant blue (CBB) staining. (*E*) ABA and ABA together with auxin induce autophosphorylation of SnRK2s in 9-d-old seedlings. Anti-phospho-S175-SnRK2s antibody was used to detect autophosphorylation of SnRK2s. For auxin and ABA treatment, we pretreated the seedlings with 2 μM IAA for 1 h and then applied ABA to activate the SnRK2s. β-Tubulin was used as a loading control. The experiments were performed two times with consistent results. (*F*) Via phosphorylation, TMK1 kinase, but not the kinase dead variant, inhibits the phosphatase activity of ABI2 in vitro. ATP^{+} indicates the phosphorylation reaction between TMK1-KD/TMK1^{K616E}-KD and ABI2 with ATP, and ATP^{-} indicates the abolished reaction without ATP. The FLU number denotes the activity of ABI2 (*Materials and Methods*). Bars are means ± SD ($n = 3$, two-way ANOVA). Each replicate was independently measured.

nonphosphorylation state of the protein and to aspartic acid (T321D) to mimic the phosphorylation state. By the same phosphatase activity assay, ABI2^{T321A} showed similar activity as wild-type ABI2, while the activity of ABI2^{T321D} was significantly reduced (Fig. 4*B*). Furthermore, the TMK1 kinase domain was no longer capable of reducing the phosphatase activity of either ABI2^{T321A} or ABI2^{T321D} (Fig. 4*C*), suggesting TMK1 suppresses ABI2 through the T321 site. Together, these results support our hypothesis that auxin triggers TMK1 to inhibit ABI2 activity through direct phosphorylation at the T321 residue.

To further verify the physiological function of the T321 phosphorylation site in plants, we transformed 35S promoter driven *ABI2*, *ABI2^{T321A}*, and *ABI2^{T321D}* into the *abi1-2abi2-2* double mutant. We found that both *ABI2* and the nonphosphorylated form *ABI2^{T321A}*, but not the constitutively phosphorylated form *ABI2^{T321D}*, could rescue the hypersensitive ABA responses in the *abi1-2abi2-2* mutant. *ABI2^{T321A}* was able to rescue the phenotype better than wild-type *ABI2*. Unlike wild-type *ABI2*, neither *ABI2^{T321A}* nor *ABI2^{T321D}* could restore the synergistic effect of a high concentration of auxin on ABA-mediated responses, including downstream gene transcriptions in the *abi1-2abi2-2* mutant (Fig. 4 *D–F* and *SI Appendix*, Fig. S16). This suggests that the regulation of this phosphorylation site on ABI2 is required for ABI2 function in ABA signaling, especially in response to auxin in plants. Taken together, our findings reveal a role for TMK1 and a clear mechanism involved in the coordination between auxin and ABA signaling in plants.

Discussion

The Complex Roles of Auxin and ABA in Plants. Auxin plays concentration-dependent and complex roles in plants (1, 2, 31) and, at the same time, participates in multiple stress responses (8, 44–46). Synthetic auxin has also been applied to agricultural crops to enhance stress tolerance

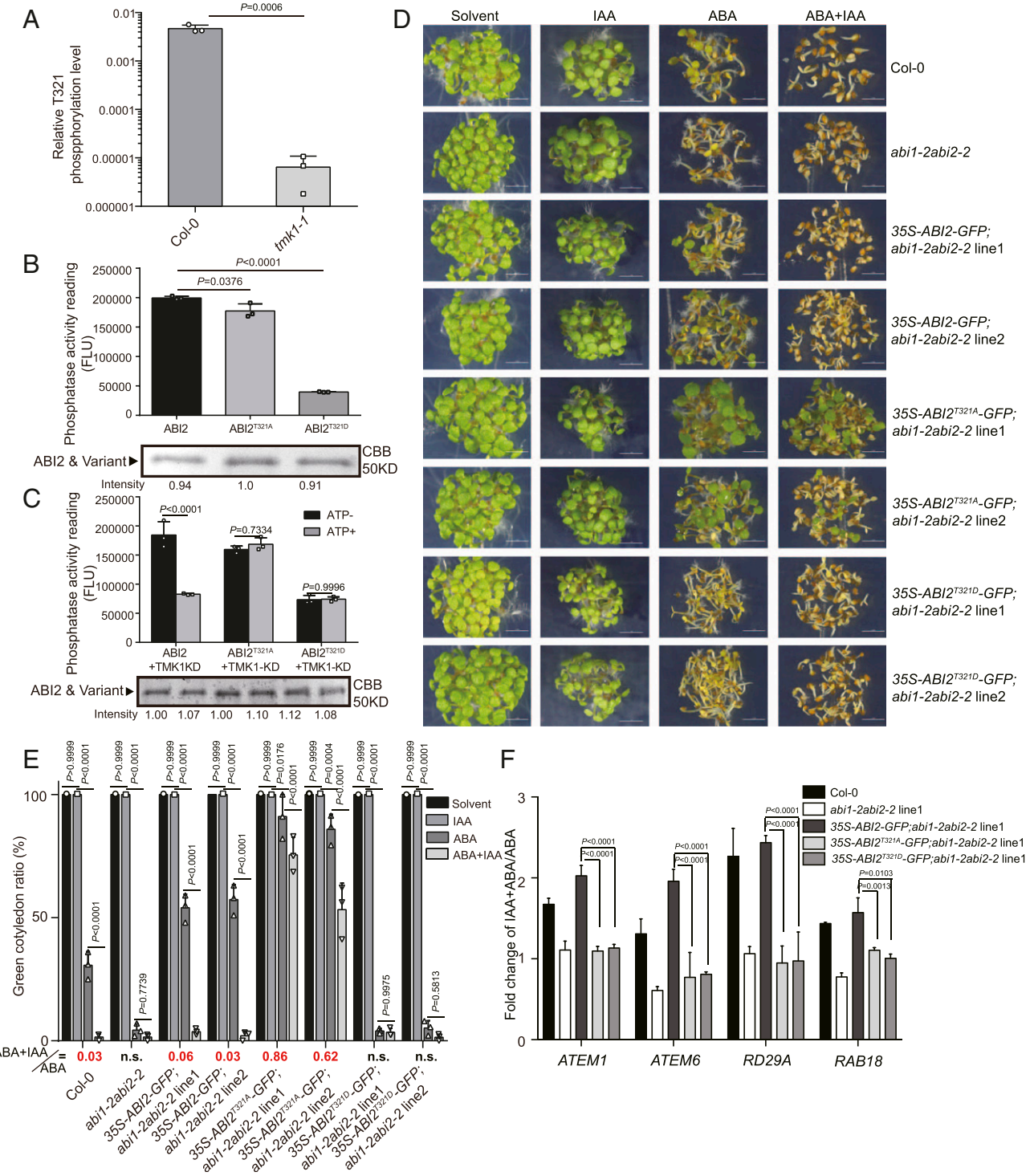

Fig. 4. TMK1 phosphorylates T321 of ABI2, which is required for both auxin and ABA responses. (*A*) Three independent biological replicates of a quantitative mass spectrometric analysis showing the T321 phosphorylation level of ABI2 decreased in *tmk1-1* compared with Col-0. ABI2-Flag proteins were immunoprecipitated from both Col-0 and *tmk1-1* protoplasts treated with 20 μM ABA and 2 μM IAA for 30 min and then analyzed by mass spectrometry ($n = 3$, two-tailed Student's t test). A corresponding nonphosphorylated peptide was used as a standard. (*B*) Phosphatase activity of *E. coli*-purified His-ABI2, His-ABI2^{T321A}, and His-ABI2^{T321D}. (*C*) TMK1-KD cannot inhibit the phosphatase activity of the ABI2 variants, ABI2^{T321A} and ABI2^{T321D}. ATP^{+} indicates the phosphorylation reaction between TMK1-KD and ABI2, ABI2^{T321A} and ABI2^{T321D} with ATP. ATP^{-} indicates the abolished reaction without ATP. The FLU number denotes the activity of ABI2 (*Materials and Methods*). Bars are means ± SD ($n = 3$, two-way ANOVA). Loading shows protein amounts of ABI2 and variants using Coomassie bright blue staining. (*D* and *E*) Cotyledon greening phenotype of Col-0, *abi1-2abi2-2*, and the ABI2-GFP, ABI2^{T321A}-GFP and ABI2^{T321D}-GFP complemented lines treated with 0.5 μM ABA or 0.5 μM ABA together with 2 μM IAA for 7 d. Representative images of greening cotyledon on day 7 are shown in *D*. Greening ratios on day 7 were quantified in *E*. The change ratio of ABA + IAA vs. ABA indicates the sensitivity to auxin in ABA responses. Data show the means ± SD ($n = 3$, two-way ANOVA). Approximately 80 seedlings for each genotype were measured in each replicate. (Scale bars, 2 mm.) (*F*) T321 phosphorylation of ABI2 is required for auxin-enhanced expression of ABA-responsive genes. The relative gene expression ratio of auxin + ABA vs. ABA represent auxin induction of ABA-responsive genes. Three biological replicates were performed for the experiment ($n = 3$, two-way ANOVA).

(3, 47), which is similar to the effect of ABA. ABA has been reported to regulate multiple developmental processes, including vegetative growth and reproduction (48), and quiescent center maintenance at the root tip (5, 6, 49). However, the interplay between auxin and ABA is quite complex, which is both concentration and context dependent. For example, excessive auxin levels promote the differentiation of distal stem cell (DSC), which is opposite to ABA, but the optimal auxin accumulation at the root tip is also required for the maintenance of root DSC identity, which is consistent with ABA effects (50–52). Both auxin and ABA play positive roles in the acquisition of embryogenic competence during somatic embryo initiation (53, 54), suggesting the coordination of these two hormones in cell fate transition during certain developmental processes. Here we report that a high concentration of auxin takes advantage of ABA signaling to regulate plant development in a TMK1-dependent manner. A low concentration of auxin does not trigger ABA signaling, while a high concentration of auxin starts to enhance ABA responses in a TMK1-dependent way (*SI Appendix*, Fig. S17). It has also been reported that the TIR1/AFB-mediated canonical auxin signaling pathway is required for ABA responses during seed germination (9). This suggests that differential concentrations of auxin might coordinate TIR1- and TMK1- based pathways to regulate these complicated developmental processes, which might explain the distinct roles of auxin in plant development. The nature and the underlying mechanisms of the cross-talk between these two pathways and also among different hormones remain an interesting direction for future investigations. Furthermore, the mechanism of the effects of high levels of auxin in the full repertoire of the developmental processes and those ABA-regulated stress responses should also be studied.

Multiple Roles of TMK Family Proteins in Plants. Receptor-like kinases (RLKs) connect extracellular signals and cellular responses and normally coordinate different processes by controlling specific signaling cascades. It is well documented that RLKs participate in multiple signaling pathways by perceiving various signals.

Previous work suggests that TMKs are key mediators in auxin signaling that activate Rho GTPases, which control the cytoskeleton (33). In addition, recent work demonstrates that auxin accumulation at the concave side of the apical hook stimulates TMK1 cleavage, which then results in cytosolic and nuclear translocation to regulate gene transcription via stabilizing two noncanonical Aux/IAA proteins (31). Moreover, TMK4 has been reported to regulate BR-mediated plant development (55) and functions specifically in negative regulation of auxin biosynthesis (34). These results provide hints of the complex roles of different TMKs, at least in auxin signaling. Here, we report that TMK1-mediated auxin signaling is also required for ABA responses. Given the essential roles of ABA in plant stress responses, TMK-mediated signaling may also be important for stress adaptations in plant. Our results again suggest that TMKs may act as integrators that mediate multiple signaling pathways under different conditions. The potential roles of the TMK family in other hormone signaling and stress responses will be interesting to explore in the future.

Materials and Methods

Plant Materials and Growth Conditions. All plant materials on Murashige & Skoog (MS) media (Phytotechnology, Cat. No. M519) were grown in plant growth chambers (PERVICALAR-66L3) with long day conditions (16 h light/8 h dark) at 22 °C, unless indicated otherwise. The seeds were sown on 1/2 MS medium containing 1% (wt/vol) sucrose and 0.8% (wt/vol) agar after sterilization with 75% ethanol for 15 min, incubated at 4 °C for 48 h for stratification, and transferred to the plant growth chamber. All the T-DNA insertion lines used in this study were in Col-0 background, and Col-0 was used as wild type.

The mutants *tmk1-1* (SALK_016360), *tmk1-2* (SAIL_812_G09), *tmk1-3* (SALK_008771), *tmk4-1* (GABI_348E01), *abi1-2* (SALK_072009), *abi2-2* (SALK_015166), *pyr1pyl1pyl2pyl4*, *snrk2.2snrk2.3snrk2.6* and *tir1-1afb2-3afb3-4* were used in this study. The complemented lines *gTMK1-flag;tmk1-1*, and *gTMK1^{K616E}-flag;tmk1-1* were described previously (31). The *abi1-2abi2-2* double and *tmk1-1abi1-2abi2-2* triple mutants were generated by crossing. The primers for mutant characterization are provided in *SI Appendix*, Table S2.

To create *ABI2* and *ABI2^{T321A}*, *ABI2^{T321D}* overexpression transgenic lines in *abi1-2abi2-2* backgrounds, coding sequences of *ABI2* were inserted into pDonor-Zeo with BP clonase, which will catalyze the recombination of AttB and AttPP site, and the *ABI2^{T321A}*, *ABI2^{T321D}* were constructed by transgene Fast Mutagenesis Kit (Transgene, Cat. No. FM111-01) and then the fragments were recombined into the pGWB505 gateway destination vector with LR clonase, which will catalyze the recombination of AttL and AttR site. The constructs were introduced into *abi1-2abi2-2* through *Agrobacterium*-mediated transformation to generate the *35S-ABI2-GFP;abi1-2abi2-2*, *35S-ABI2^{T321A}-GFP;abi1-2abi2-2*, and *35S-ABI2^{T321D}-GFP;abi1-2abi2-2*. The expression of ABI2 and ABI2^{T321A}, ABI2^{T321D} were checked by Western blot with GFP antibody (Transgene, Cat. No. HT801-01). The primers for point mutations are provided in *SI Appendix*, Table S2.

Phenotype Analysis. For the seed germination assay, ~80 seeds were sown on 1/2 MS medium with 0.8% agar containing 0 or 0.5 μM ABA (Sigma, Cat. No. A1049) with/without 0 to 16 μM yucasin (Sigma, Cat. No. L321451) or 0 to 2 μM IAA (Sigma, Cat. No. I3750). The plates were transferred from 4 °C to the plant growth chamber, where they were grown for 11 d to observe seed germination phenotypes. The cotyledon greening ratios were quantified every day and photographs were taken on day 7. For the stomata closure assay, epidermal strips were peeled from 3-wk-old seedlings and a small brush was used to remove the mesophyll cells. The leaves were treated with 0 or 20 μM ABA together with 2 μM IAA in the stomata opening buffer (10 mM MES-KOH pH 6.15, 10 mM KCl, 50 mM $CaCl_2$) for 1 h. The photographs were taken under the microscope (Leica DM6B-460292). The width and length of stomata pores were measured by ImageJ 1.52a. Approximately 100 stomata cells were analyzed in each replicate.

Yeast Two-Hybrid Assay. The TMK1-KD, TMK2-KD, TMK1^{K616E}-KD, and nine members from the group A PP2C subfamily were cloned from *Arabidopsis* cDNA and inserted into the pDonor-Zeo vector by BP reaction. The TMK1-KD fragment was recombined into the pGWBKT7 gateway destination vector via LR reaction and used as bait. PP2Cs were recombined into the pGWADT7 gateway destination vector via LR reaction and used as prey. Yeast two-hybrid assays were performed in the golden yeast strain. The yeast transformation was carried out by the Frozen-EZ Yeast Transformation II Kit (ZYMO RESEARCH, Cat. No. T2001). The transformed yeast was grown on synthetic dropout (SD) base (−Trp −Leu −Ade −His) medium (Clontech, Cat. No. 630411).

The Coimmunoprecipitation Assay. The coimmunoprecipitation assay was performed by using transient expression in protoplasts from *Arabidopsis* mesophyll cells, which was described previously (31, 56). The protoplasts were isolated and transformed at room temperature (25 °C to 28 °C), and then incubated at 22 °C for 14 h under light conditions.

The full-length coding sequences (CDSs) of *TMK1*, *ABI1*, *ABI2*, and *HAB1* were cloned into the HBT vector, which is driven by the 35S promoter and fused with a HA or Flag tag. A total of 80 μg *35S:TMK1-HA* plasmids were cotransformed into 1 mL protoplasts (2×10^5) along with 100 μg *35S:ABI1-Flag* or *35S:ABI2-Flag* plasmids. Total proteins were extracted from protoplasts by extraction buffer (100 mM NaCl, 50 mM Na-phosphate pH 7.4, 0.1% Nonidet P-40, 1 mM dithiothreitol (DTT) and 1× protease inhibitor mixture) and then centrifuged at 13,000 rpm for 30 min. The supernatant fractions were incubated with anti-Flag–Agarose Affinity Gel (Sigma, Cat. No. A2220) at 4 °C for 3 h. The agarose beads were washed three times with wash buffer (100 mM NaCl, 50 mM Na-phosphate pH 7.4, 1 mM DTT and 1× protease inhibitor mixture) for 2 min each time and then boiled for 10 min in 5 × sodium dodecyl sulfate (SDS) loading buffer. After centrifugation at 12,000 × *g* for 2 min, the supernatants were analyzed by Western blotting with Flag antibody (Abmart, Cat. No. M20008) and HA antibody (Abmart, Cat. No. M20003).

Protein Pull-Down Assay. The pull-down assay was performed as described (28) with some modifications. The corresponding CDSs of *TMK1-KD* (588-942aa) and *ABI1*, *ABI2* were cloned into the pDu vector (His-MBP tag at the N terminus) and the pGEX4T-2 vector (with GST tag), respectively. HisMBP-TMK1-KD proteins were purified by Ni Sepharose (Qiagen, Cat. No. 30230). The GST, ABI1-GST or ABI2-GST proteins were purified by GST Sepharose (Sigma, Cat. No. G4510). For *SnRK2.2* and *PYR1*, the CDS was cloned into the

PET-14a vector with His tag, and the proteins were purified by Ni Sepharose (Qiagen, Cat. No. 30230).

For the pull-down assay between TMK1 and ABI1, ABI2, the purified HisMBP-TMK1-KD proteins were incubated with amylase resin (NEB, Cat. No. E8021L) in pull-down buffer (200 mM NaCl, 50 mM Tris·HCl pH 8.0, 1 mM phenylmethylsulfonyl fluoride (PMSF)) at 4 °C for 2 h. After centrifugation at 1,000 × *g* for 3 min, the supernatants were discarded and the beads were mixed with 2 μg GST, ABI1-GST, or ABI2-GST proteins in fresh binding buffer at 4 °C for 2 h. The resin was washed three times by pull-down buffer for 2 min each time and boiled for 10 min in 5 × SDS loading buffer. After centrifugation at 12,000 × *g* for 2 min, the supernatants were used for Western blotting by MBP antibody (Abmart, Cat. No. M20051) and GST antibody (Abmart, Cat. No. M20007).

Pull-down assays between ABI1, ABI2, and PYR1 or between ABI1, ABI2, and SnRK2.2 were conducted by a similar procedure as above, except that 100 μM ABA was applied in the ABI1, ABI2, and PYR1 reaction.

In Vitro Kinase Assay. A total of 1 μg HisMBP-TMK1-KD (588 to 942 amino acids [aa]) protein and 1 μg GST or ABI2-GST were mixed in the reaction buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 1 mM DTT with 3 μCi γ 32p ATP and 30 μM ATP). After incubation at 25 °C for 45 min at 900 rpm in a Thermo shaker (DLAB HCM100-Pro), the reaction was stopped by adding 5 × SDS loading buffer and boiling at 95 °C for 10 min. Radioactive signals from HisMBP-TMK1-KD and ABI2-GST proteins were detected by the Typhoon Imaging System (GE Healthcare TYPHOON FLA9500).

SnRK2s Activation Assay. The 9-d-old seedlings that were first treated with or without 2 μM IAA for 30 min then with 50 μM ABA or solvent for 30 min, were used to extract proteins by extraction buffer [5 mM ethylene diamine tetraacetic acid, 5 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid, 2 mM DTT, 25 mM NaF, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20% glycerol, 1 mM PMSF, 1× protease inhibitor mixture, and 50 mM HEPES-KOH, pH 7.5]. Proteins (10 μg/lane) were separated on 10% SDS–polyacrylamide gel electrophoresis gel and Western blot was performed using the polyclonal anti-phosphorylation antibodies for Ser175 in SnRK2.6 that were described previously (41). The membranes were incubated at 4 °C overnight in TBS + Tween 20 (TBST) with 5% skim milk containing 1:5,000 diluted anti-pS175 or anti–β-tubulin antibodies. After washing three times (10 min each time) with TBST buffer, the membranes were incubated for 2 h at room temperature in TBST containing 1:5,000 diluted goat anti-rabbit horseradish peroxidase (HRP)-conjugated antibodies.

Measurement of PP2C Activity. PP2C activity was measured by a ProFluor Ser/Thr PPase Assay Kit (Promega, Cat. No. V1260). The CDS of *ABI2* and *ABI2 T321A*, *ABI2 T321D* was cloned into PET-14a with His tag, the corresponding CDSs of *TMK1-KD* (588 to 942 aa) and *TMK1 ^{K616E}-KD* were cloned into the pDu vector (His-MBP tag at N terminus) and the proteins were purified by Ni Sepharose. To measure phosphatase activity of ABI2 after reaction with TMK1-KD, 2 μg ABI2 were combined with TMK1-KD and TMK1^{K616E}-KD in the kinase reaction buffer (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 1 mM DTT) with or without 50 μM ATP at 25 °C for 45 min, incubated at 900 rpm in a Thermo shaker (DLAB HCM100-Pro), and then the total protein was used for PP2C activity measurement.

To measure phosphatase activity, the proteins were mixed with 25 μL peptide solution and incubated at room temperature for 30 min. The reactions were stopped by adding 25 μL protease solution and incubating for 90 min at room temperature (25 °C). Then 25 μL stabilizer solution was added to the reaction system, the R110 fluorescence signals (S/T PPase R110 substrate) were detected by an excitation wavelength of 485 nm and an emission wavelength of 530 nm, and the 7-amino-4-methylcoumarin fluorescence signals (control for compounds that may inhibit the protease reaction) were detected by an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The fluorescence intensity (FLU) number of R110 fluorescence signals denoted the activity of PP2C.

Statistical Analyses. All box plots and bar graphs were generated by GraphPad Prism 7.0 software. Statistical significances based on the Student's *t* test and one-way or two-way ANOVA were determined with GraphPad Prism 7.0 (GraphPad Software, http://www.graphpad.com/).

Gel shift, liquid chromatography–tandem mass spectrometry, in vitro kinase assay with ATP-γ-S, quantification of salt-induced ABA biosynthesis, GUS staining and imaging, qRT-PCR analyses, and primers together with an ortholog list (*SI Appendix*, Tables S1 and S2) are provided in *SI Appendix, Materials and Methods*.

Data Availability. All study data are included in the article and/or *SI Appendix*.

ACKNOWLEDGMENTS. This work was supported by the National Key R&D Program of China (2016YFA0503200), the National Natural Science Foundation of China (grants 31422008 and 31870256), and start-up funds from Shanghai Center for Plant Stress Biology and Fujian Agriculture and Forestry University (to T.X.).

1. K. V. Thimann, Hormones and the analysis of growth. *Plant Physiol.* **13**, 437–449 (1938).
2. B. O. Bargmann *et al*., A map of cell type-specific auxin responses. *Mol. Syst. Biol.* **9**, 688 (2013).
3. Y. L. Hsueh, C. H. Lou, Effects of 2,4-D on seed germination and respiration. *Science* **105**, 283–285 (1947).
4. K. Nakashima, K. Yamaguchi-Shinozaki, ABA signaling in stress-response and seed development. *Plant Cell Rep.* **32**, 959–970 (2013).
5. H. Zhang *et al*., ABA promotes quiescence of the quiescent centre and suppresses stem cell differentiation in the Arabidopsis primary root meristem. *Plant J.* **64**, 764–774 (2010).
6. W. Han, H. Zhang, M. H. Wang, Fluridone affects quiescent centre division in the Arabidopsis thaliana root stem cell niche. *BMB Rep.* **43**, 813–817 (2010).
7. M. J. Iglesias *et al*., MiR393 regulation of auxin signaling and redox-related components during acclimation to salinity in Arabidopsis. *PLoS One* **9**, e107678 (2014).
8. M. Salehin *et al*., Auxin-sensitive Aux/IAA proteins mediate drought tolerance in Arabidopsis by regulating glucosinolate levels. *Nat. Commun.* **10**, 4021 (2019).
9. X. Liu *et al*., Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 15485–15490 (2013).
10. L. Xing, Y. Zhao, J. Gao, C. Xiang, J. K. Zhu, The ABA receptor PYL9 together with PYL8 plays an important role in regulating lateral root growth. *Sci. Rep.* **6**, 27177 (2016).
11. Y. Zhao *et al*., The ABA receptor PYL8 promotes lateral root growth by enhancing MYB77-dependent transcription of auxin-responsive genes. *Sci. Signal.* **7**, ra53 (2014).
12. D. Shkolnik-Inbar, D. Bar-Zvi, ABI4 mediates abscisic acid and cytokinin inhibition of lateral root formation by reducing polar auxin transport in Arabidopsis. *Plant Cell* **22**, 3560–3573 (2010).
13. J. He *et al*., DEXH box RNA helicase-mediated mitochondrial reactive oxygen species production in Arabidopsis mediates crosstalk between abscisic acid and auxin signaling. *Plant Cell* **24**, 1815–1833 (2012).
14. S. Yu, C. Ligang, Z. Liping, Y. Diqiu, Overexpression of OsWRKY72 gene interferes in the abscisic acid signal and auxin transport pathway of Arabidopsis. *J. Biosci.* **35**, 459–471 (2010).
15. J. Guo, X. Yang, D. J. Weston, J. G. Chen, Abscisic acid receptors: Past, present and future. *J. Integr. Plant Biol.* **53**, 469–479 (2011).
16. S. Y. Park *et al*., Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**, 1068–1071 (2009).
17. Y. Ma *et al*., Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **324**, 1064–1068 (2009).
18. T. Umezawa *et al*., Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 17588–17593 (2009).
19. W. Yang, W. Zhang, X. Wang, Post-translational control of ABA signalling: The roles of protein phosphorylation and ubiquitination. *Plant Biotechnol. J.* **15**, 4–14 (2016).
20. A. Kulik, I. Wawer, E. Krzywińska, M. Bucholc, G. Dobrowolska, SnRK2 protein kinases–Key regulators of plant response to abiotic stresses. *OMICS* **15**, 859–872 (2011).
21. L. M. Ng, K. Melcher, B. T. Teh, H. E. Xu, Abscisic acid perception and signaling: Structural mechanisms and applications. *Acta Pharmacol. Sin.* **35**, 567–584 (2014).
22. T. Umezawa *et al*., Molecular basis of the core regulatory network in ABA responses: Sensing, signaling and transport. *Plant Cell Physiol.* **51**, 1821–1839 (2010).
23. F. F. Soon *et al*., Molecular mimicry regulates ABA signaling by SnRK2 kinases and PP2C phosphatases. *Science* **335**, 85–88 (2012).
24. A. Schweighofer, H. Hirt, I. Meskiene, Plant PP2C phosphatases: Emerging functions in stress signaling. *Trends Plant Sci.* **9**, 236–243 (2004).
25. L. Kong *et al*., Degradation of the ABA co-receptor ABI1 by PUB12/13 U-box E3 ligases. *Nat. Commun.* **6**, 8630 (2015).
26. Q. Wu *et al*., Ubiquitin ligases RGLG1 and RGLG5 regulate abscisic acid signaling by controlling the turnover of phosphatase PP2CA. *Plant Cell* **28**, 2178–2196 (2016).
27. Z. Wang *et al*., Counteraction of ABA-mediated inhibition of seed germination and seedling establishment by ABA signaling terminator in Arabidopsis. *Mol. Plant* **13**, 1284–1297 (2020).
28. K. Wang *et al*., EAR1 negatively regulates ABA signaling by enhancing 2C protein phosphatase activity. *Plant Cell* **30**, 815–834 (2018).
29. F. Yu *et al*., FERONIA receptor kinase pathway suppresses abscisic acid signaling in Arabidopsis by activating ABI2 phosphatase. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 14693–14698 (2012).
30. N. Dai, W. Wang, S. E. Patterson, A. B. Bleecker, The TMK subfamily of receptor-like kinases in Arabidopsis display an essential role in growth and a reduced sensitivity to auxin. *PLoS One* **8**, e60990 (2013).
31. M. Cao *et al*., TMK1-mediated auxin signalling regulates differential growth of the apical hook. *Nature* **568**, 240–243 (2019).

32. R. Huang *et al*., Noncanonical auxin signaling regulates cell division pattern during lateral root development. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 21285–21290 (2019).
33. T. Xu *et al*., Cell surface ABP1-TMK auxin-sensing complex activates ROP GTPase signaling. *Science* **343**, 1025–1028 (2014).
34. Q. Wang *et al*., A phosphorylation-based switch controls TAA1-mediated auxin biosynthesis in plants. *Nat. Commun.* **11**, 679 (2020).
35. A. C. Allan, M. D. Fricker, J. L. Ward, M. H. Beale, A. J. Trewavas, Two transduction pathways mediate rapid effects of abscisic acid in Commelina guard cells. *Plant Cell* **6**, 1319–1328 (1994).
36. M. Klein *et al*., The plant multidrug resistance ABC transporter AtMRP5 is involved in guard cell hormonal signalling and water use. *Plant J.* **33**, 119–129 (2003).
37. H. Fujii *et al*., In vitro reconstitution of an abscisic acid signalling pathway. *Nature* **462**, 660–664 (2009).
38. S. Merlot, F. Gosti, D. Guerrier, A. Vavasseur, J. Giraudat, The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. *Plant J.* **25**, 295–303 (2001).
39. F. Vlad *et al*., Phospho-site mapping, genetic and in planta activation studies reveal key aspects of the different phosphorylation mechanisms involved in activation of SnRK2s. *Plant J.* **63**, 778–790 (2010).
40. C. Belin *et al*., Identification of features regulating OST1 kinase activity and OST1 function in guard cells. *Plant Physiol.* **141**, 1316–1327 (2006).
41. Y. Zhao *et al*., Arabidopsis duodecuple mutant of PYL ABA receptors reveals PYL repression of ABA-independent SnRK2 activity. *Cell Rep.* **23**, 3340–3351.e5 (2018).
42. P. Wang, J. K. Zhu, Assessing kinase activity in plants with in-gel kinase assays. *Methods Mol. Biol.* **1363**, 189–197 (2016).
43. J. J. Allen *et al*., A semisynthetic epitope for kinase substrates. *Nat. Methods* **4**, 511–516 (2007).
44. P. Wang *et al*., Phosphatidic acid directly regulates PINOID-dependent phosphorylation and activation of the PIN-FORMED2 auxin efflux transporter in response to salt stress. *Plant Cell* **31**, 250–271 (2019).
45. E. Shani *et al*., Plant stress tolerance requires auxin-sensitive Aux/IAA transcriptional repressors. *Curr. Biol.* **27**, 437–444 (2017).
46. J. H. Hong *et al*., A sacrifice-for-survival mechanism protects root stem cell niche from chilling stress. *Cell* **170**, 102–113.e14 (2017).
47. J. Popko, R. Hänsch, R. R. Mendel, A. Polle, T. Teichmann, The role of abscisic acid and auxin in the response of poplar to abiotic stress. *Plant Biol.* **12**, 242–258 (2010).
48. H. Fujii, J. K. Zhu, Arabidopsis mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction, and stress. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 8380–8385 (2009).
49. Y. Liang, D. M. Mitchell, J. M. Harris, Abscisic acid rescues the root meristem defects of the Medicago truncatula latd mutant. *Dev. Biol.* **304**, 297–307 (2007).
50. Z. Ding, J. Friml, Auxin regulates distal stem cell differentiation in Arabidopsis roots. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 12046–12051 (2010).
51. H. Tian *et al*., WOX5-IAA17 feedback circuit-mediated cellular auxin response is crucial for the patterning of root stem cell niches in Arabidopsis. *Mol. Plant* **7**, 277–289 (2014).
52. R. Wu *et al*., The *6xABRE* synthetic promoter enables the spatiotemporal analysis of ABA-mediated transcriptional regulation. *Plant Physiol.* **177**, 1650–1665 (2018).
53. M. Gaj, Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to Arabidopsis thaliana (L.) Heynh. *Plant Growth Regul.* **43**, 27–47 (2004).
54. A. Kikuchi, N. Sanuki, K. Higashi, T. Koshiba, H. Kamada, Abscisic acid and stress treatment are essential for the acquisition of embryogenic competence by carrot somatic cells. *Planta* **223**, 637–645 (2006).
55. M. H. Kim *et al*., Identification of Arabidopsis BAK1-associating receptor-like kinase 1 (BARK1) and characterization of its gene expression and brassinosteroid-regulated root phenotypes. *Plant Cell Physiol.* **54**, 1620–1634 (2013).
56. S. D. Yoo, Y. H. Cho, J. Sheen, Arabidopsis mesophyll protoplasts: A versatile cell system for transient gene expression analysis. *Nat. Protoc.* **2**, 1565–1572 (2007).