Supplemental Data

Adenosine monophosphate deaminase modulates the activity of BIN2 through hydrogen peroxide-induced oligomerization

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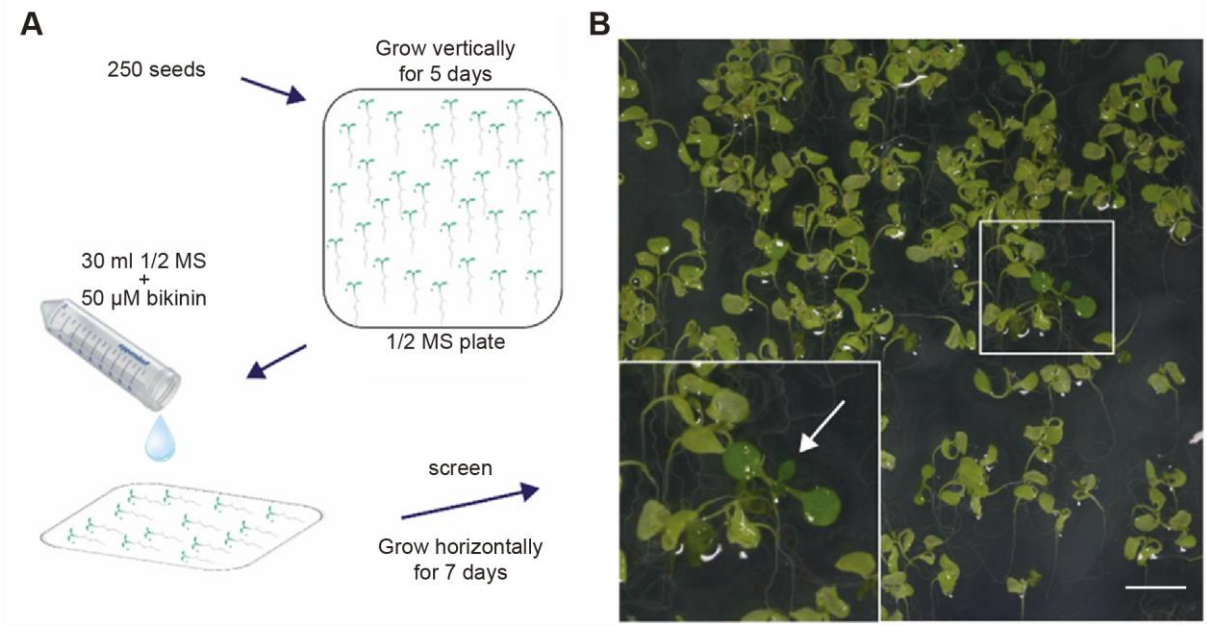
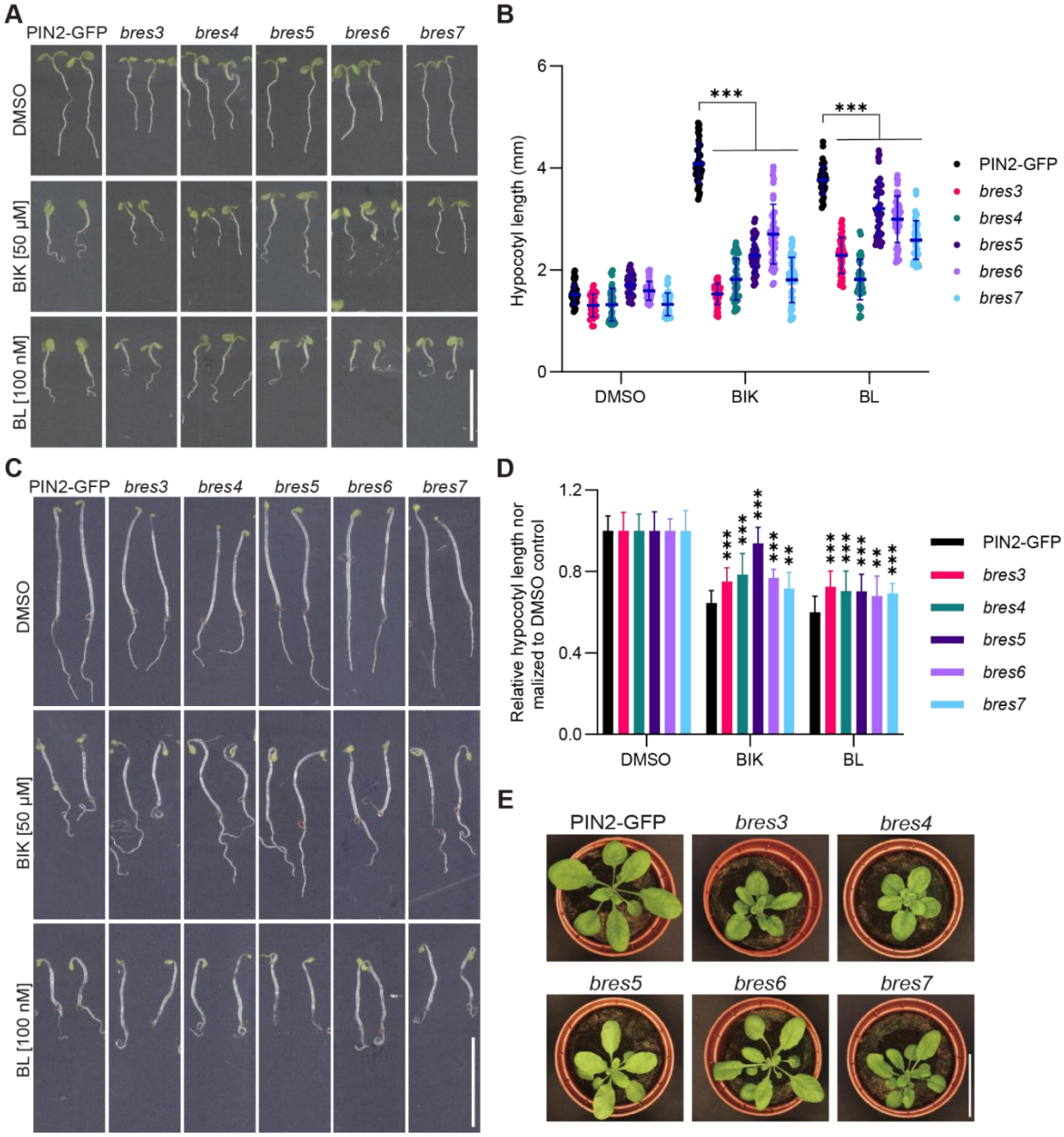
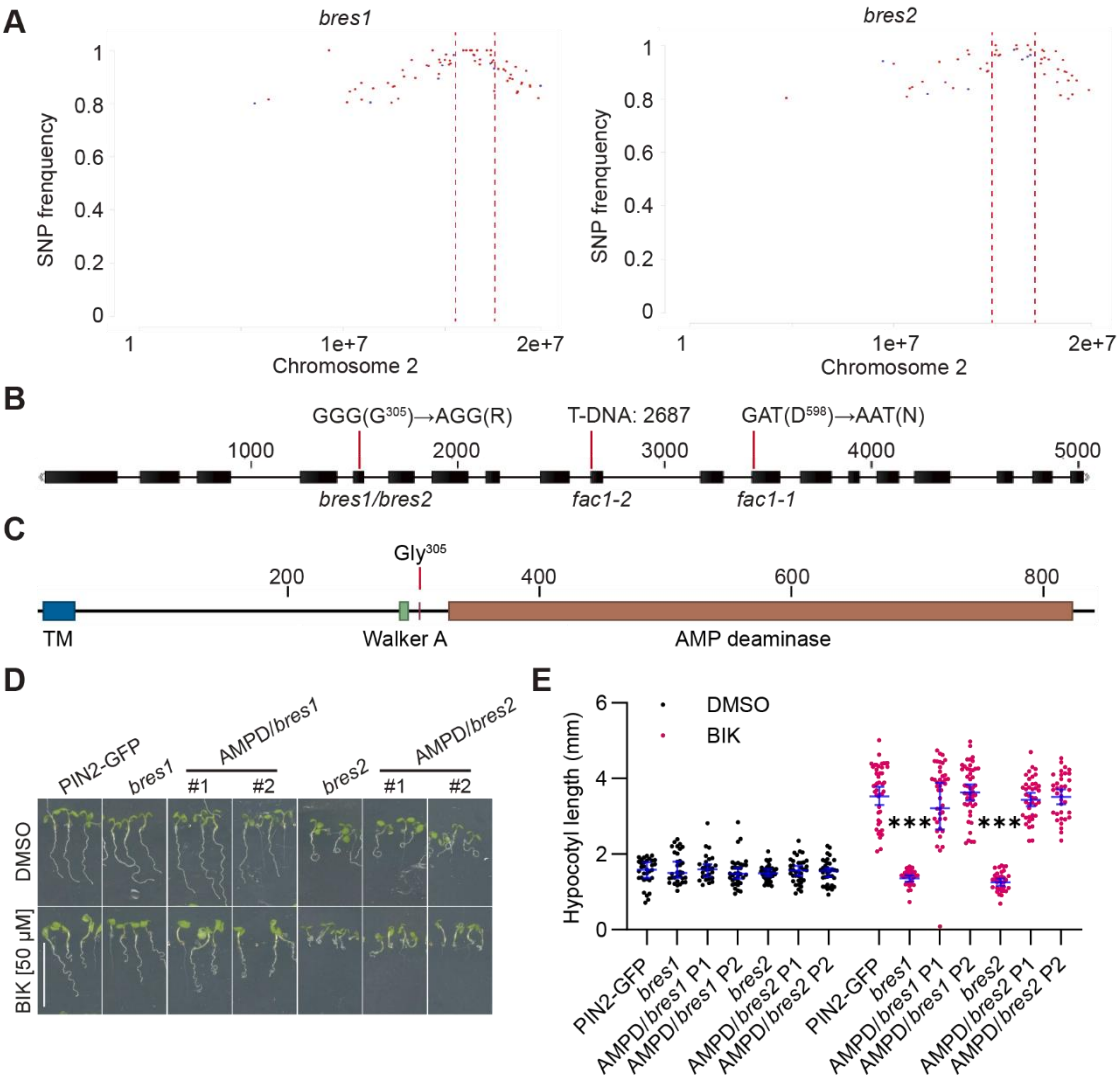


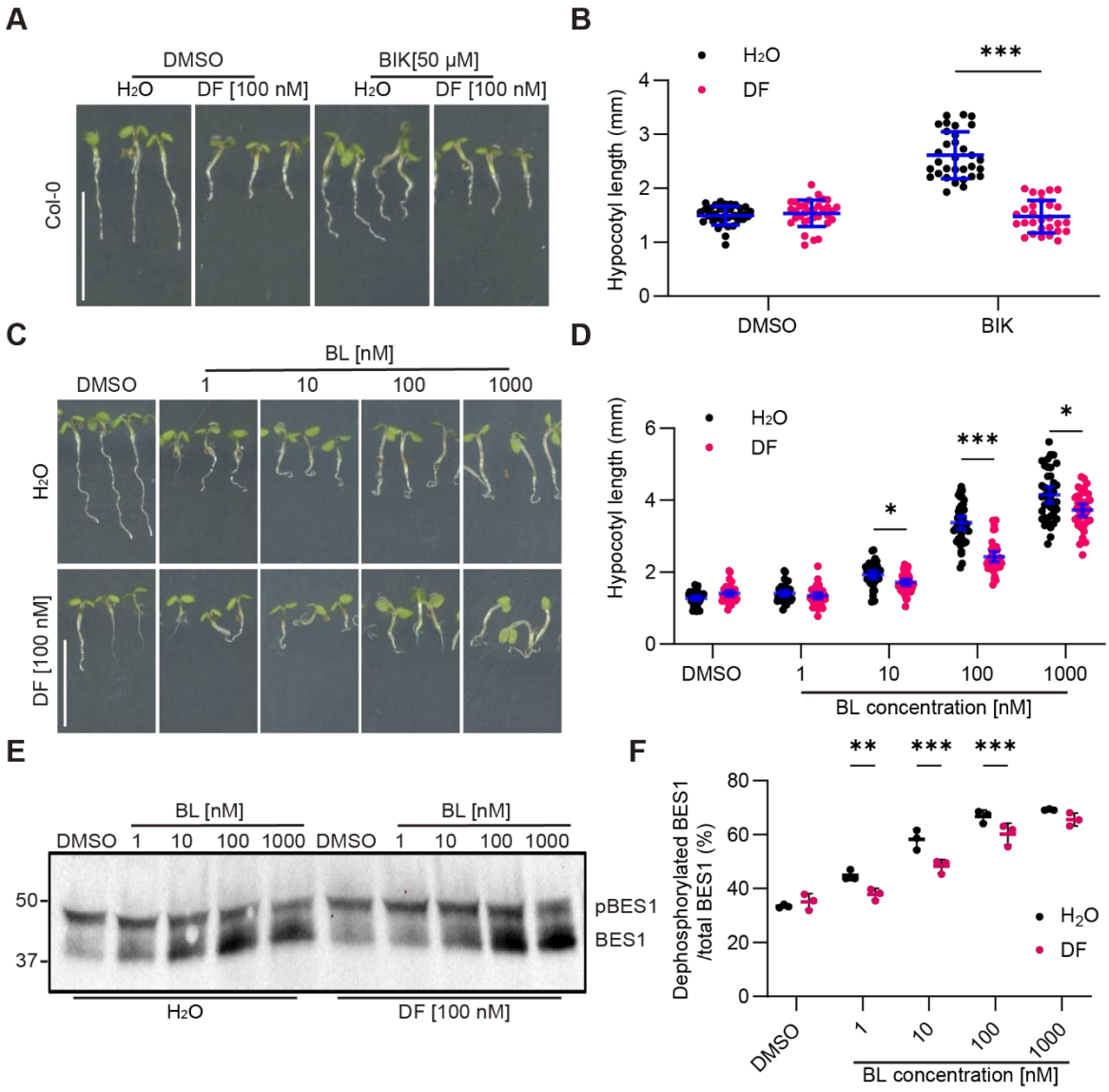
Supplemental Figure S1 Schematic illustration of the forward genetic screen for bikinin-resistant mutants. A, Screening procedure. Seedlings were grown for 5 days vertically on agar plates, transferred horizontally, and overlaid with 30 ml liquid medium supplemented with 50 µM bikinin (BIK). Seedlings were grown additionally for 7 days horizontally. B, Example of a bikinin-resistant mutant. The hypocotyl did not elongate, the cotyledons did not curl and remained green. Scale bar, 1 cm. (Supports Figure 1)



Supplemental Figure S2 Phenotypes of the *bikinin-resistant3* (*bres3)* to *bres7* mutants. A and C, Arabidopsis seedlings of five *bres* mutants and the control (*PIN2p::PIN2-GFP*/Col-0) germinated and grown for 5 days in light (A) and dark (C), on medium supplemented with either 50 µM bikinin (BIK) or 100 nM brassinolide (BL). B and D, Quantification of the hypocotyl lengths of genotypes in (A) and (C). Scatter dot plots show all the individual points with the means and standard errors. *P* values were compared to *PIN2p::PIN2-GFP*/Col-0 plants using the two-way ANOVA with Dunnett's multiple comparisons test, ***$P < 0.001$, **$P < 0.01$; *n*, at least 40 seedlings from three independent experiments. E, Phenotypes of *bres3* to *bres7* mutants and the control, *PIN2p::PIN2-GFP*/Col-0, grown in soil for 4 (top) and 6 (bottom) weeks. All EMS mutants showed variable growth defects. Scale bars, 1 cm (A) and (C), 2 cm (E). (Supports Figure 1)

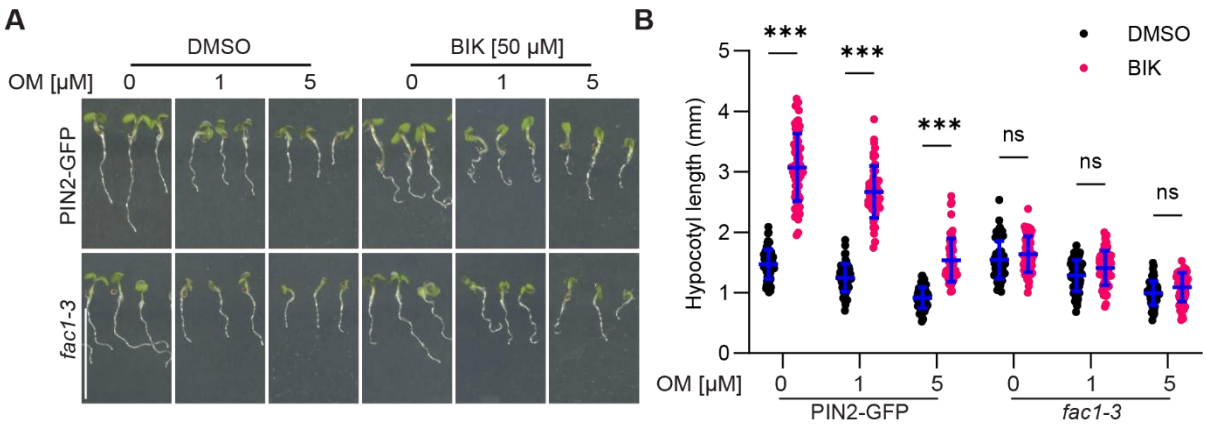


Supplemental Figure S3 *AMPD* rescued *bres1* and *bres2.* A, SHOREmap analysis of *bres1* and *bres2*. The mapping-by-sequencing analysis revealed a genetic linkage to a 3400-kb region on chromosome 2. B, Structure of the *AMPD* gene. The *fac1-1*, *fac1-2*, *bres1,* and *bres2* mutations are indicated. Black boxes indicate the coding exons. G, glycine; R, arginine; D, aspartic acid; N, asparagine. C, Schematic drawing of AMPD protein structure. The transmembrane domain (TM), Walker A motif, adenosine monophosphate (AMP) deaminase domain, and the residue Glycine^{305} (Gly^{305}) are indicated. D, Arabidopsis seedlings of the *PIN2p:PIN2-GFP*/Col-0 parental line, *bres1*, *bres2*, and the two independent transgenic lines *AMPDp::gAMPD*/*bres1* and *AMPDp::gAMPD*/*bres2* were germinated and grown for 5 days on agar medium supplemented with 50 μM bikinin (BIK) or DMSO (mock). E, Quantification of the hypocotyl lengths of seedlings in (D). Scatter dot plots show all the individual points with the means and standard errors. *P* values were compared to *PIN2p::PIN2-GFP*/Col-0 plants using the two-way ANOVA with Dunnett's multiple comparisons test, ***$P < 0.001$. *n*, at least 40 seedlings from two independent experiment. Scale bar, 1 cm (C). (Supports Figure 1)

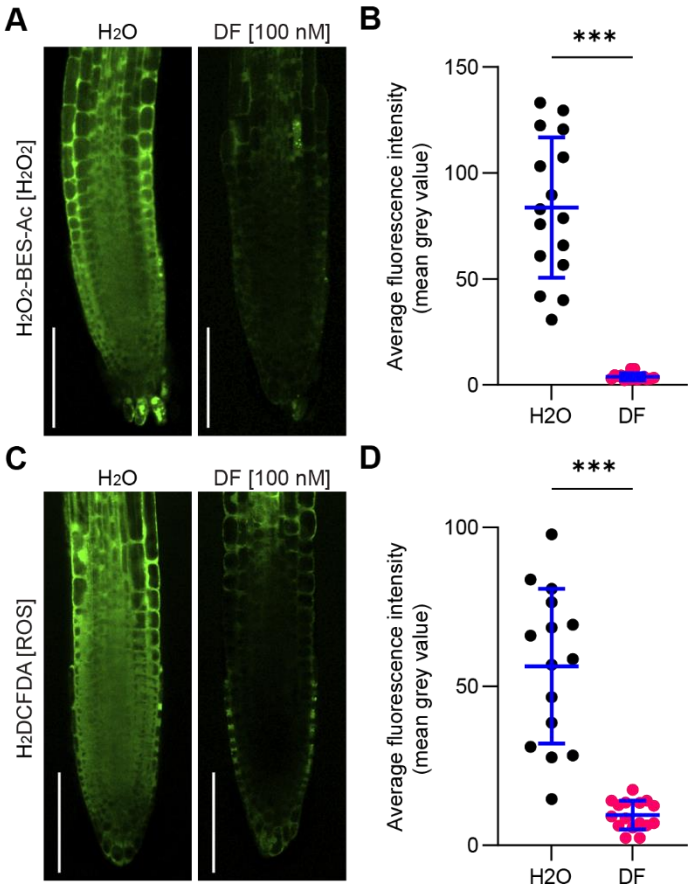


Supplemental Figure S4 Pharmacological inhibition of AMPD reduced the sensitivity of Arabidopsis to bikinin and BL. A, Arabidopsis seedlings of Col-0 germinated and grown for 5 days on agar medium supplemented with 100 nM deaminoformycin (DF) or H_2O (mock for DF) in the presence of 50 μM bikinin (BIK) or DMSO (mock for BIK). B, Quantification of the hypocotyl length of seedlings in (A). *P* values were compared to Col-0 plants treated with H_2O using the two-way ANOVA with Dunnett's multiple comparisons test, ***$P < 0.001$. *n*, at least 30 seedlings from two independent experiments. C, Arabidopsis seedlings of Col-0 germinated and grown for 5 days on medium supplemented with 100 nM deaminoformycin (DF) or H_2O (mock for DF) in the presence of BL (1-1000 nM) or DMSO (mock for BL). D, Quantification of the hypocotyl length of seedlings in (C). *P* values were compared to H_2O control using the two-way ANOVA with Dunnett's multiple comparisons test, *$P < 0.05$, ***$P < 0.001$. *n*, at least 30 seedlings from two independent experiments. E, Immunoblot analysis of BES phosphorylation of seedlings in (C) with a specific anti-BES1 antibody. pBES1, phosphorylated BES1. F, Quantification of the blot in (E). *P* values were compared to H_2O control using the

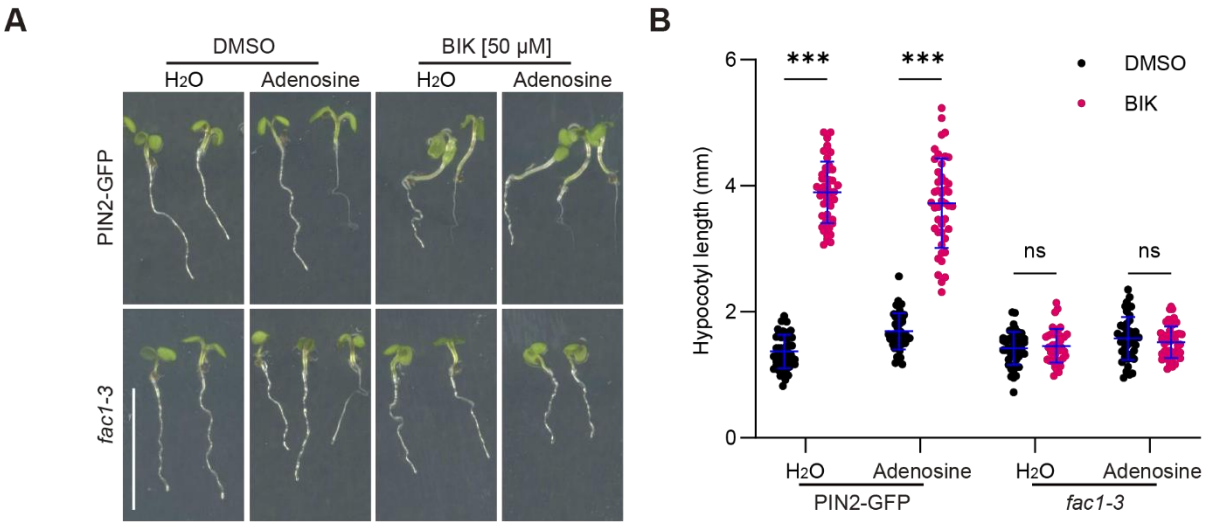
two-way ANOVA with Dunnett's multiple comparisons test, $^{**}P < 0.01$, $^{***}P < 0.001$. *n*, three independent experiments. B, D and F, Scatter dot plots show all the individual points with the means and standard errors. Scale bars, 1 cm (A), (C) and (E). (Supports Figure 1)



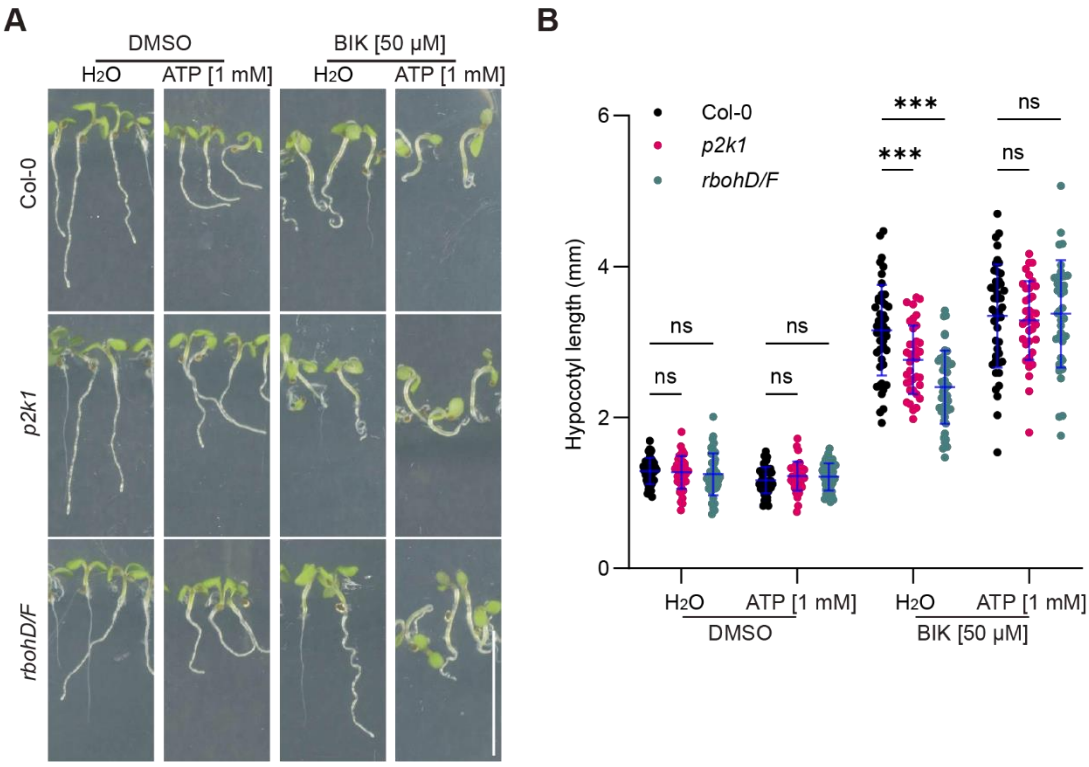
Supplemental Figure S5 Adenosine triphosphate (ATP) production inhibition by oligomycin did not restore the *fac1-3* sensitivity to bikinin. A, Arabidopsis seedlings of *pPIN2::PIN2-GFP*/Col-0 and *fac1-3* germinated and grown for 5 days on agar medium supplemented with 50 µM bikinin (BIK) in the presence of 1 µM or 5 µM oligomycin (OM). DMSO was used as mock for bikinin and oligomycin. B, Quantification of the hypocotyl length of seedlings in (A). Scatter dot plots show all the individual points with the means and standard errors. *P* values were compared to mock for oligomycin using the two-way ANOVA with Dunnett's multiple comparisons test, ***$P < 0.001$. ns, not significant. n = at least 50 seedlings from three independent experiments. Scale bar, 1 cm (A). (Supports Figure 2).



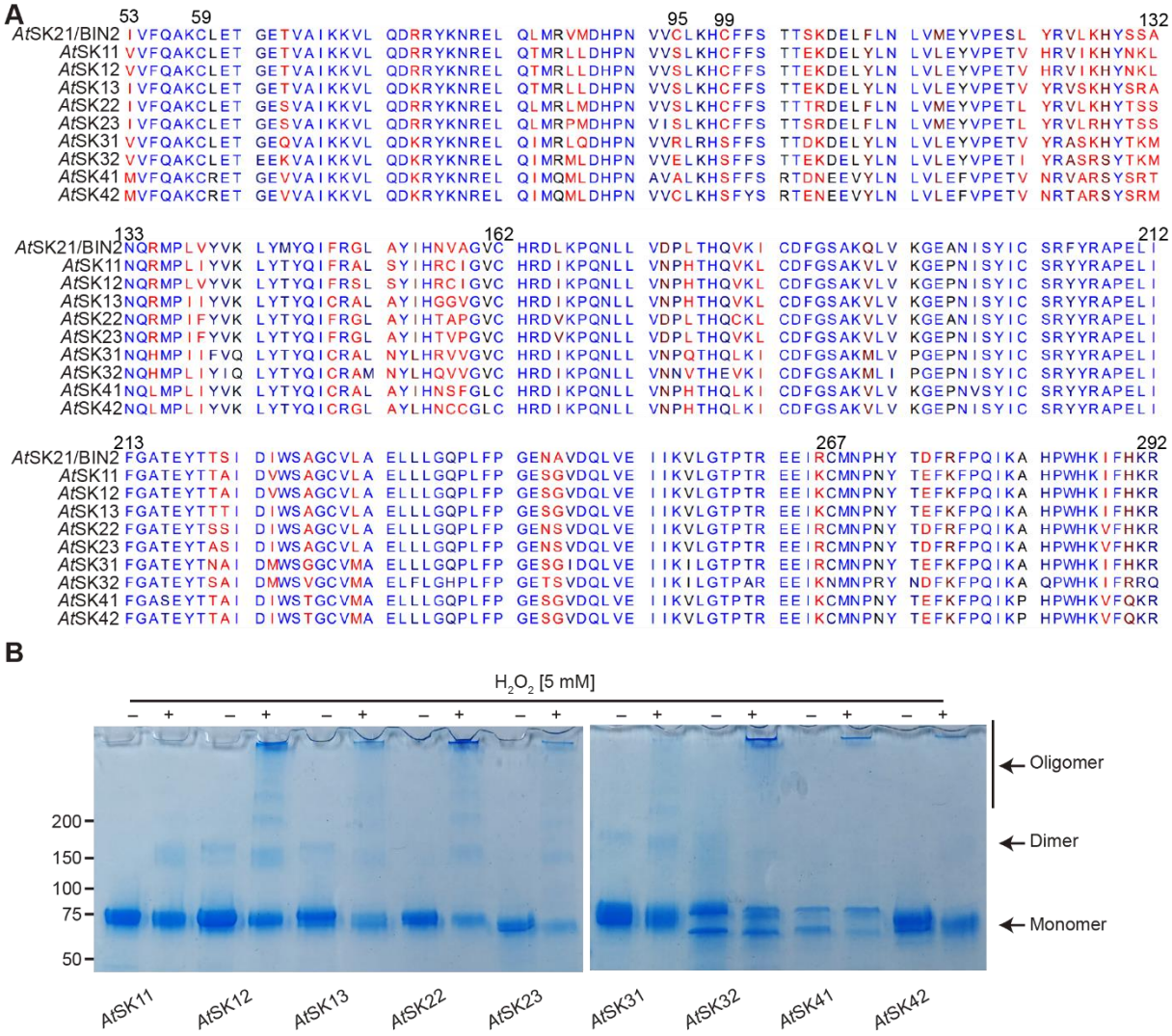
Supplemental Figure S6 Pharmacological inhibition of AMPD reduced ROS levels. A and C, Confocal images of the root tips of 5-day-old Col-0 seedlings stained with the H_2O_2 probe H_2O_2-BES-Ac (A) or the ROS probe 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) (B) for 30 min after treatments with 100 nM deaminoformycin (DF) or H_2O (mock) for 24 h. B and D, Quantification of the fluorescent intensities in the root tips of the seedlings in (A) and (C). Scatter dot plots show all the individual points with the means and standard errors. *n*, at least 15 seedlings from three independent experiments. *P* values were compared to the mock (H_2O) (Student's *t*-test), ***$P < 0.001$. Scale bars, 50 μm (A) and (C). (Supports Figure 2)



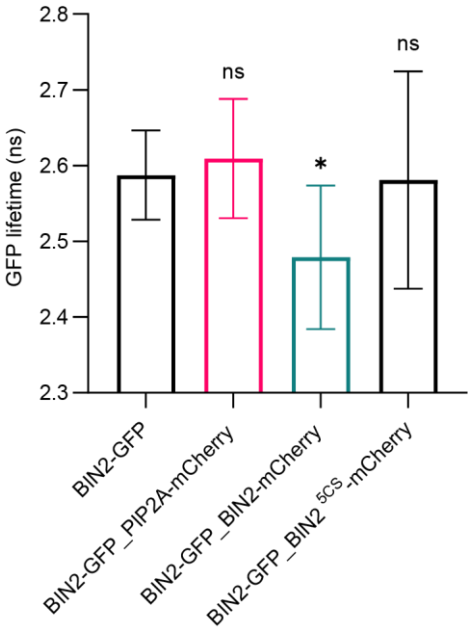
Supplemental Figure S7 Adenosine treatment did not restore the *fac1-3* sensitivity to bikinin. A, Arabidopsis *PIN2p::PIN2-GFP*/Col-0 and *fac1-3* seedlings germinated and grown for 5 days on agar medium supplemented with 0.5 mM adenosine or H_2O (mock) in the presence of 50 µM bikinin (BIK) or DMSO (mock for BIK). B, Quantification of the hypocotyl lengths of seedlings in (A). Scatter dot plots show all the individual points with the means and standard errors. *P* values were compared to H_2O mock using the two-way ANOVA with Dunnett's multiple comparisons test, ***$P < 0.001$, ns, not significant. *n*, at least 40 seedlings from two independent experiments. Scale bar, 1 cm (A). (Supports Figure 3)



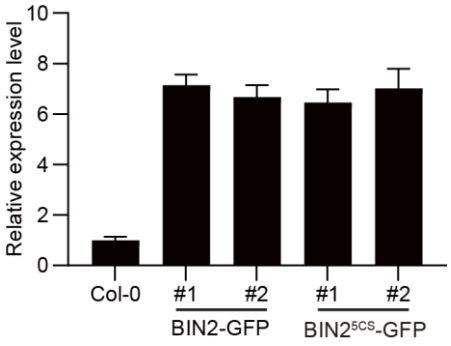
Supplemental Figure S8 *p2k1* and *rbohD/F* mutants are less sensitive to bikinin. A, Arabidopsis seedlings of Col-0*, p2k1,* and *rbohD/F* germinated and grown for 5 days on agar medium supplemented with 1 mM ATP or H_2O (mock for ATP) in the presence of 50 µM bikinin (BIK) or DMSO (mock for BIK). B, Quantification of the hypocotyl length of seedlings in (A). Scatter dot plots show all the individual points with the means and standard errors. *P* values were compared to Col-0 using the two-way ANOVA with Dunnett's multiple comparisons test, ***$P < 0.001$, ns, not significant.. *n*, at least 40 seedlings from two independent experiments. Scale bar, 1 cm (A). (Supports Figure 3)



Supplemental Figure 9 Hydrogen peroxide induced oligomerization of the GSK3-like kinases in *vitro*. A, Alignment of the ten *At*SK proteins (residues 53-292 of BIN2) using CLC Main Workbench (QIAGEN). C, Coomassie Brilliant Blue (CBB)-stained non-reducing SDS-PAGE gel analysis of the oligomerization of nine HIS-SUMO-*At*SKs after treatment with 5 mM H_2O_2 for 30 min. *At*SK, *Arabidopsis thaliana* Shaggy/GSK3-like kinase. (Supports Figure 4)



Supplemental Figure 10 BIN2-GFP interacts with BIN2-mCherry but not BIN2^{5CS}-mCherry. BIN2-GFP lifetime was measured by Fluorescence Resonance Energy Transfer by fluorescence lifetime imaging (FRET-FLIM) in tobacco (*Nicotiana benthamiana*) expressing *35Sp::BIN2-GFP* alone or with *UBQ10p::PIP2A-mCherry*, *35Sp::BIN2-mCherry* and *35Sp*::*BIN2*^{5CS}-*mCherry*, respectively. BIN2-mCherry reduced the lifetime of BIN2-GFP while PLASMA MEMBRANE INTRINSIC PROTEIN 2A (PIP2A) and BIN2^{5CS}-mCherry did not. Error bars indicate SD. *P* values were compared to BIN2-GFP control using one-way ANOVA with Dunnett's multiple comparisons test, **P* < 0.05, ns, not significant. *n*, at least 10 cells. (Supports Figure 4)



Supplemental Figure 11 Real-Time Quantitative Reverse Transcription (qRT)-PCR analysis of BIN2 expression level. Relative transcript levels of *BIN2* normalized to the *ELONGATION FACTOR 1A* gene (*EF1a*, *AT1G07940*) in 12-day-old seedlings of two independent transgenic Arabidopsis lines for *BIN2p::BIN2-GFP/bin2-3* and *BIN2p::BIN2^{C5S}-GFP/bin2-3*, respectively, and Col-0. Error bars indicate SD. (Supports Figure 4)

Supplemental Table S1. Primers used

Primer name	Sequence
Cloning	
AMPD ATTB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGAACCCAATATTTACCAACTT
AMPD ATTB2	GGGGACCACTTTGTACAAGAAAGCTGGGTTTGGAACAACTTCATCAGAGATAAC
AMPD Gibson Forward	ACAGAGAACAGATTGGTGGATCCAATCTAGTCCTTGAGCGTGG
AMPD Gibson Reverse	TCGACTTAAGCATTATGCGGCCGCTTATGGAACAACTTCATCAGAGAT
BIN2 C59S Forward	TTCCAAGCAAAATCTTTGGAGACTGGAGAAACCG
BIN2 C59S Reverse	AGTCTCCAAAGATTTTGCTTGGAAAACGATCCC
BIN2 C162S Reverse	AGATCTCTGTGAGAAACTCCAGCAACATTGTGAA
BIN2 C162S Forward	TTGCTGGAGTTTCTCACAGAGATCTAAAGCCTC
BIN2 C267S Forward	GAAGAAATCCGTTCTATGAATCCACATTACACAGA
BIN2 C267S Reverse	TGTGGATTCATAGAACGGATTTCTTCTCGAGTTG
BIN2 C95S 99S Forward	TCTTTGAAGCATTCCTTCTTTTCGACTACAAGTAAAG
BIN2 C95S 99S Reverse	AAGGAATGCTTCAAAGAAACCACATTCGGATGATCCATC
Gibson AtSK11 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGGCGTCAGTGGGTATAGCTC
Gibson AtSK11 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCTCACAAACCGAGCCAAGGACA C
Gibson AtSK12 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGGCCTCGGTGGGCATAGAGC
Gibson AtSK12 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCTCACAAACTGAGCCACGGACA T
Gibson AtSK13 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGGCTTCTGTGGGAACATTAC C

Gibson AtSK13 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCTTAGAGAGCGAGGAAGGAACATTG
Gibson BIN2 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGGCTGATGATAAGGAGATGC
Gibson BIN2 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCTTAAGTTCCAGATTGATTCAAGA
Gibson AtSK22 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGGCCTCATTACCATTGGG
Gibson AtSK22 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCTTAACTGTTTTGTAATCCTGTG
Gibson AtSK23 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGACTTCGATACCATTGGG
Gibson AtSK23 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCCTAGGGTCCAGCTTGAAATGGA
Gibson AtSK31 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGAATGTGGTGCGGAGATTAAC
Gibson AtSK31 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCTCATTTCCTTGCATGCTCAGG
Gibson AtSK32 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGAACGTGATGCGTCGTCTC
Gibson AtSK32 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCCTAAGAGCTACTTCCCGTTCC
Gibson AtSK41 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGGCATCCTCTGGACTGGGA
Gibson AtSK41 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCTTACGAATGCAAAGCCATGAAG
Gibson AtSK42 F	ATTGAGGCTCACAGAGAACAGATTGGTGGATCCATGGAATCTCATCTGGGAAATG
Gibson AtSK42 R	TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCTTACGAGTGTAATGCCATGAAG
BES1 ATTB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCAATGACGTCTGACGGAGCAAC
BES1 ATTB2	GGGGACCACTTTGTACAAGAAAGCTGGGTAACTATGAGCTTTACCATTTCCAAG
qRT-PCR	
BIN2 qPCR Forward	CACAAAAGGATGCCCCCAGA
BIN2 qPCR Reverse	TTGAAGAGAGGCGGGAAAGG

EF1a qPCR Forward	TGAGCACGCTCTTCTTGCTTTCA
EF1a qPCR Reverse	GGTGGTGGCATCCATCTTGTTACA