

New Phytologist Supporting Information

Article title: Abscisic acid promotes jasmonic acid biosynthesis *via* a “SAPK10-bZIP72-AOC” pathway to synergistically inhibit seed germination in rice (*Oryza sativa* L.)

Authors: Yifeng Wang, Yuxuan Hou, Jiehua Qiu, Huimei Wang, Shuang Wang¹, Liqun Tang, Xiaohong Tong, Jian Zhang

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The following Supporting Information is available for this article:

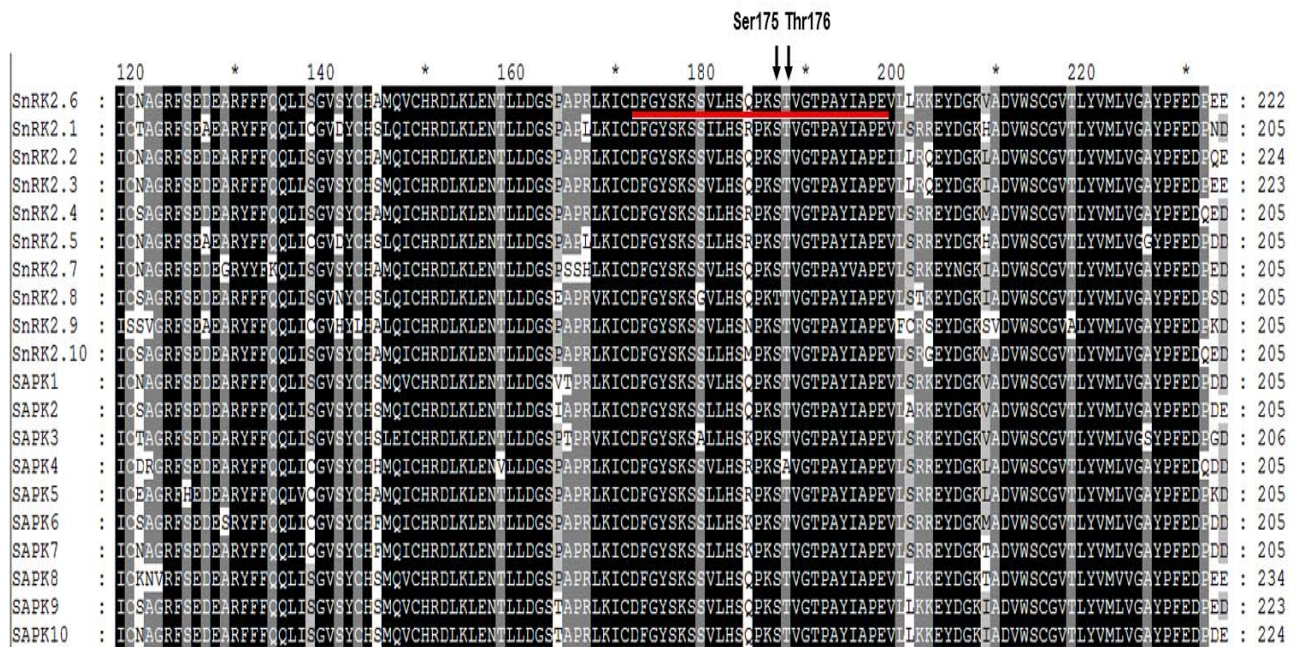


Fig. S1 Sequence alignment of T- loop region of 10 *Arabidopsis* and 10 Rice SnRK2 family members. Arrows indicate the residues of SnRK2.6 (OST1) phosphorylation sites. The putative T- loop predicted for SnRK2.6 is underlined in red.

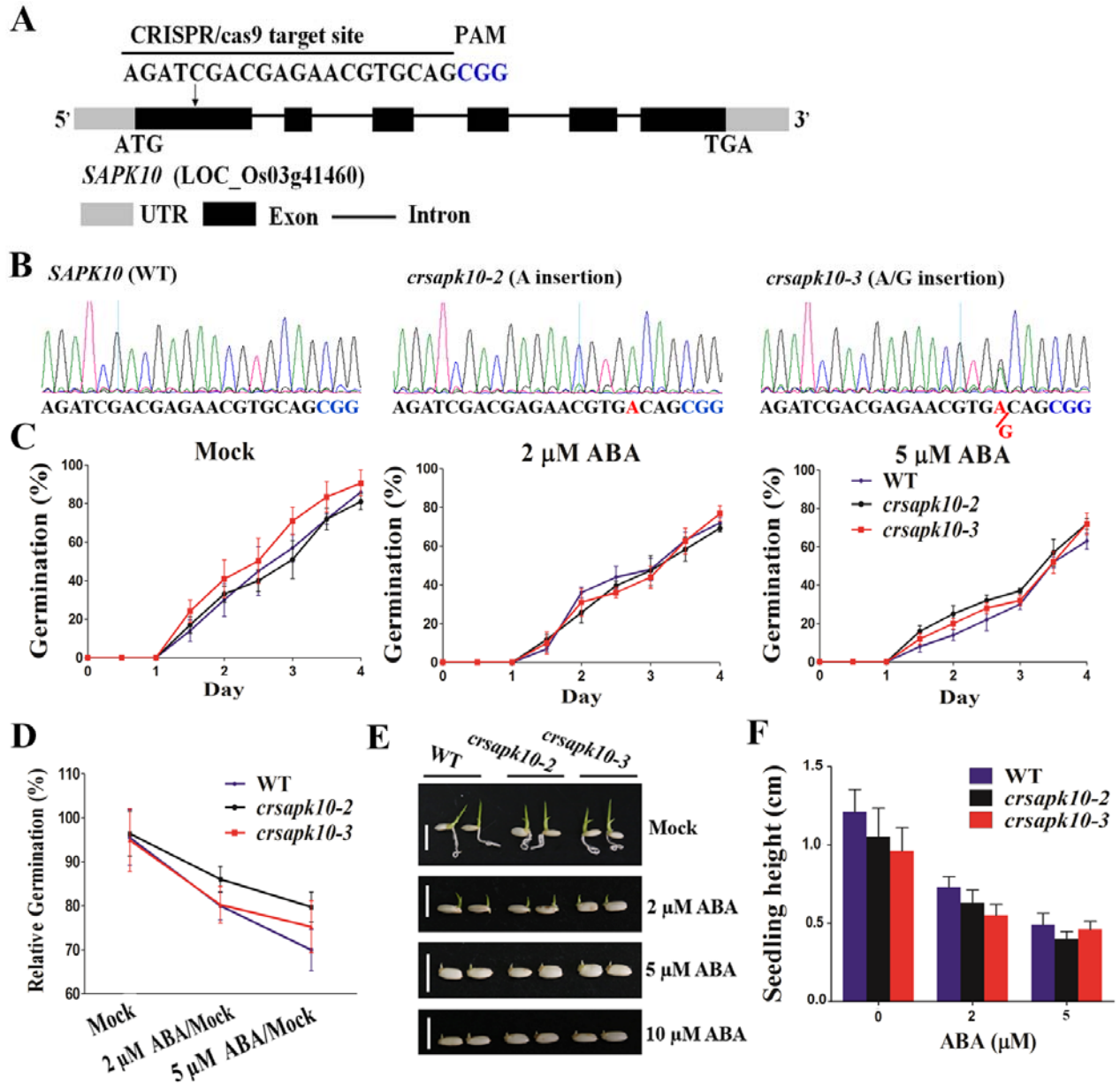


Fig. S2 Molecular characterization of *sapk10* mutant and seed germination in response to ABA. (A) Schematic presentation of the gene structure of *SAPK10* and CRISPR-cas9 editing site. Grey boxes: untranslated regions; Black boxes: exons; black line: intron. PAM: protospacer adjacent motif. (B) Sanger sequencing chromatograph of the CRISPR-cas9 target site in homozygous mutants of *crsapk10*. The letter in red represented the mutant sites. The letter in blue represented the PAM sequence. (C) Germination time courses of WT and *crsapk10* lines grown on half-strength MS medium containing different concentration of ABA (0, 2, 5 μ M), respectively. (D) The relative germination of the WT and *crsapk10*

seeds under ABA treatments were determined after 4 days and expressed as a percentage of those grown on Mock condition. (E) Germination phenotypes of WT and *crsapk10* treated with 0, 2, 5 μ M ABA, respectively. Photographs were taken on day 4. Bar=1cm. (F) Seedling heights of WT and *crsapk10* in accordance to (E). Error bars indicate SD with biological triplicates (n=3, each replicates containing 50 seeds) in (C) and 50 biological replicates (n=50) in (F).

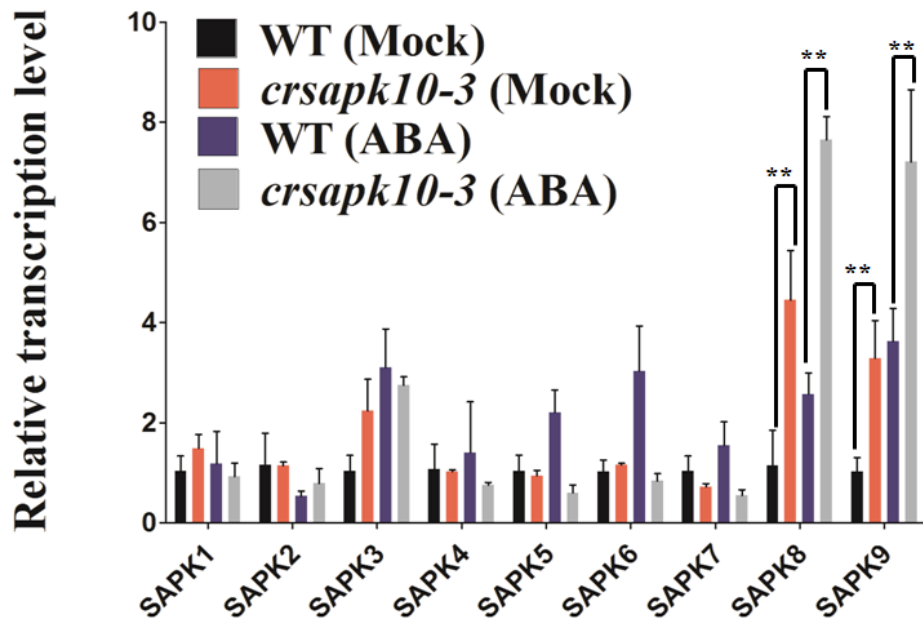


Fig. S3 qRT-PCR analysis for transcript accumulation of nine SnRK2 family members (*SAPK1-SAPK9*) in the seeds of WT or *crsapk10* lines, respectively. Seeds were grown on mock or 10 μ M ABA treated half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t*-test analysis: ** P<0.01.

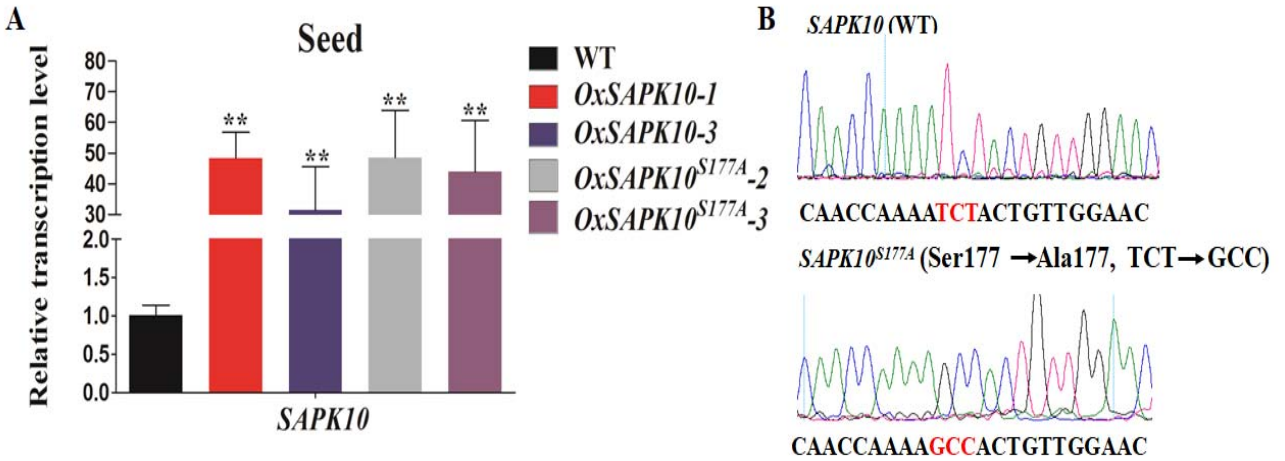


Fig. S4 Molecular characterization of *SAPK10*, *SAPK10^{S177A}* over-expressing transgenic lines. (A) qRT-PCR analysis for transcript accumulation of *SAK10* in the seeds of *OxSAPK10* or *OxSAPK10^{S177A}* transgenic lines, respectively. Seeds were grown on half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t*-test analysis: ** P<0.01. (B) Sanger sequencing chromatograph of the mutated *SAPK10* cDNA in *OxSAPK10^{S177A}* transgenic lines. The letter in red represented the mutant sites.

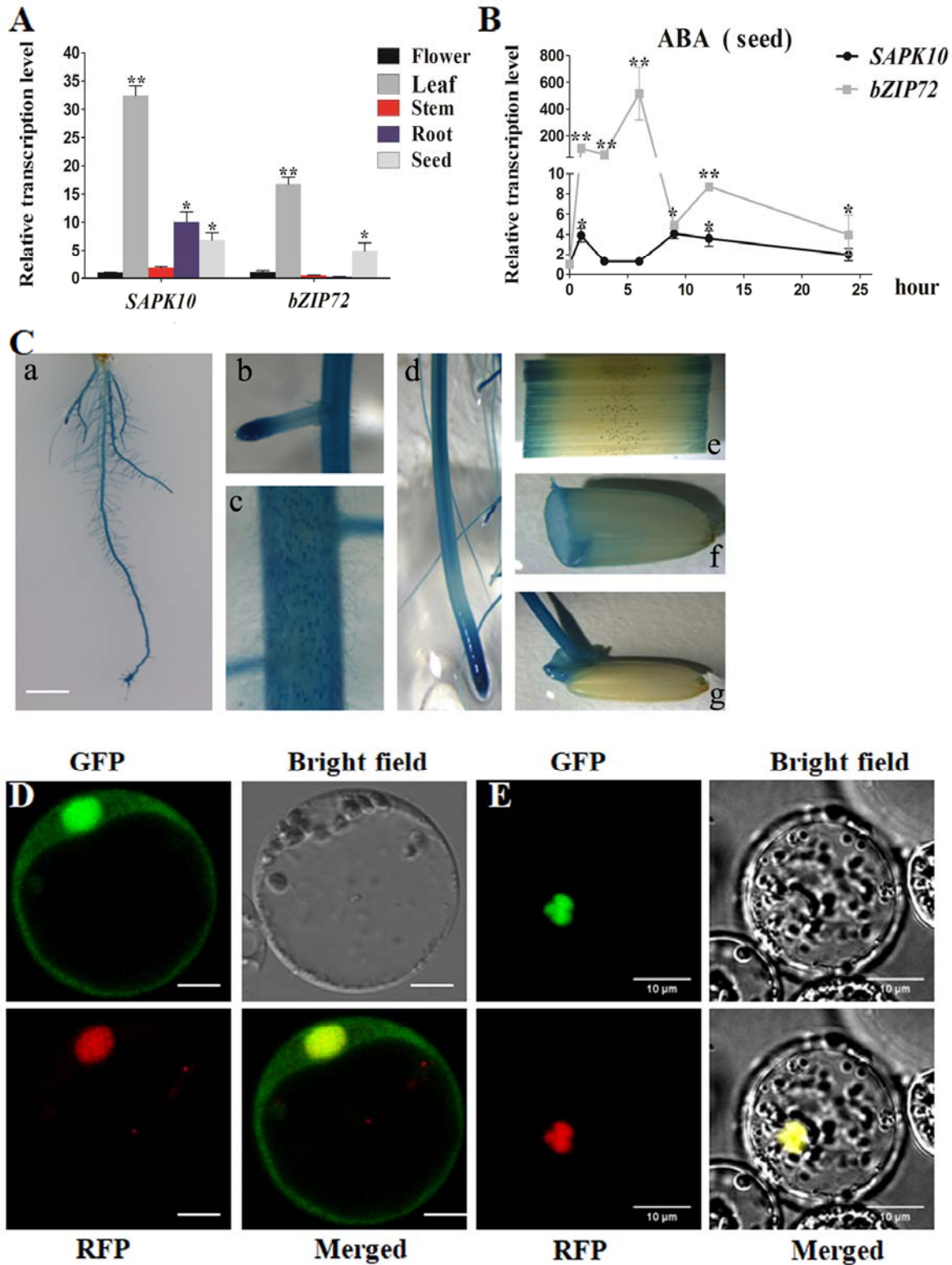


Fig. S5 Expression pattern of *SAPK10* and *bZIP72*. (A) qRT-PCR analysis of *SAPK10* and *bZIP72* transcription level in flower, leaf, stem, root and seed, respectively. (B) qRT-PCR

analysis of *SAPK10* and *bZIP72* during seeds germination grown on half-strength MS medium treated with 10 μ M ABA. (C) GUS staining of the primary root (a), lateral roots (b), root hairs (c), crown root (d), leaf (e), seed (f) and plumule (g) of the transgenic plants harboring the *proSAPK10:GUS* fused gene. Bar =1 cm in (a). (D and E) Subcellular localization of SAPK10 in (D) and bZIP72 in (E). *35S:SAPK10-GFP* or *35S:bZIP72-GFP* was co-transformed with a nuclear marker *35S:D53-mKate* (Zhou *et al.*, 2016) into protoplast. Bar=10 μ m. Error bars indicate SD with biological triplicates (n=3) in (A and B). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t-test* analysis: * P<0.05, ** P<0.01.

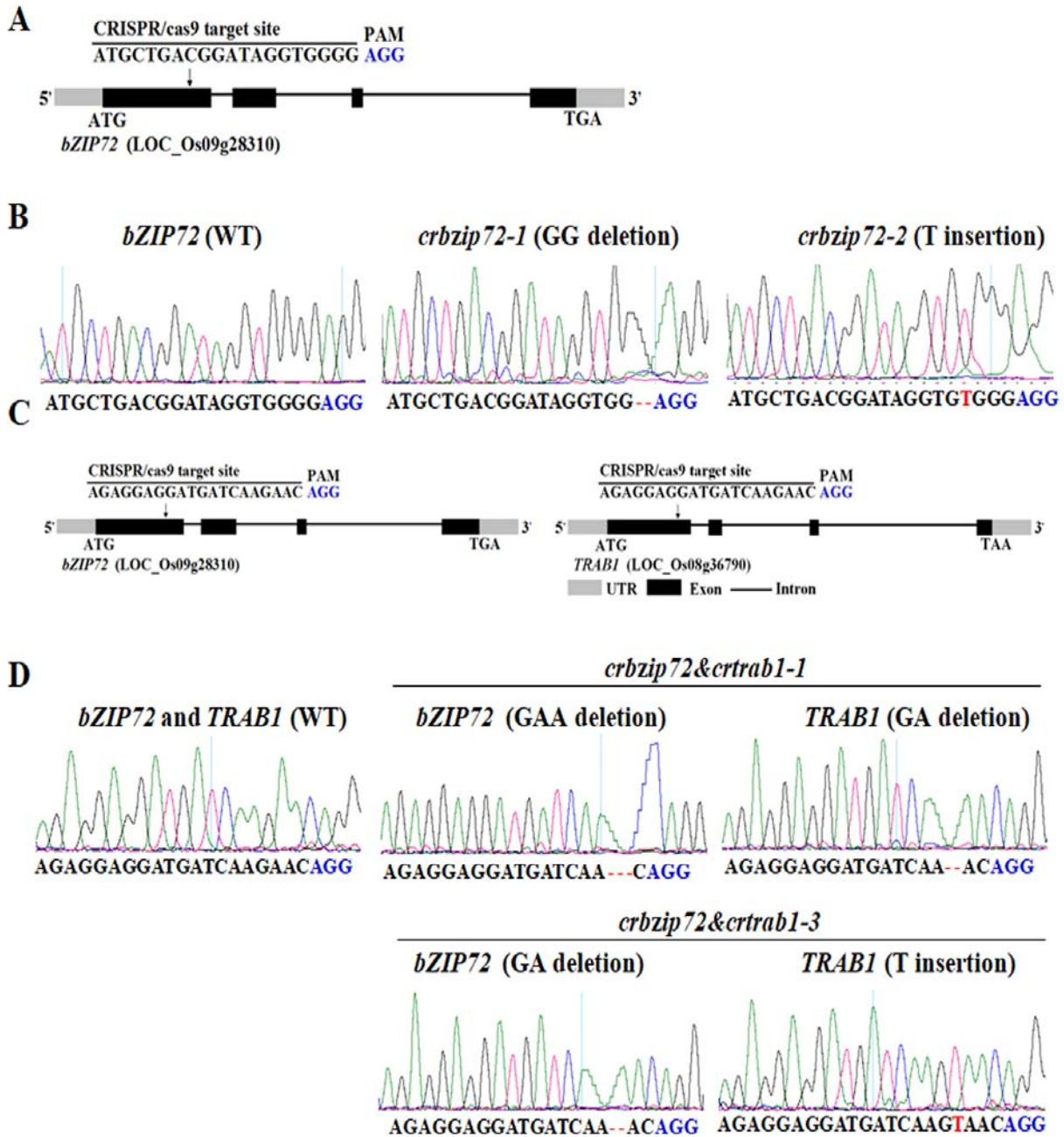


Fig. S6 Molecular characterization of *crbzip72*, *crtrab1* and *crbzip72/crtrab1* mutant. (A) Schematic presentation of the gene structure of *bZIP72* and CRISPR-cas9 editing site. PAM: protospacer adjacent motif. Grey boxes: untranslated regions; Black boxes: exons; black line: intron. (B) Sanger sequencing chromatograph of the CRISPR-cas9 target site in homozygous mutants of *crbzip72*. The letter in red represented the mutant sites. The letter in blue represented the PAM sequence. (C) Schematic presentation of the gene structure of *bZIP72* and *TRAB1*. Both genes shared the same CRISPR-cas9 editing PAM sequence. (D)

Sanger sequencing chromatograph of the CRISPR-cas9 target site in homozygous mutants of *crbzip72/crtrab1*.

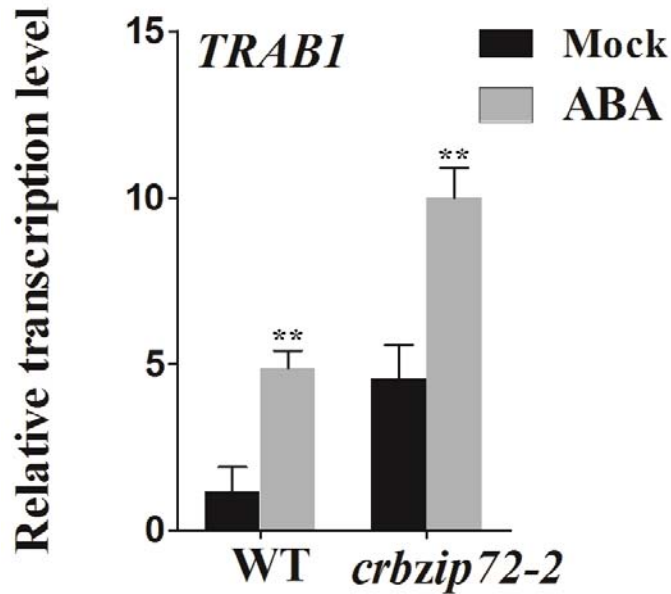


Fig. S7 qRT-PCR analysis for transcript accumulation of *TRAB1* in the seeds of *crbzip72* transgenic line. Seeds were grown on half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t*-test analysis: ** P<0.01.

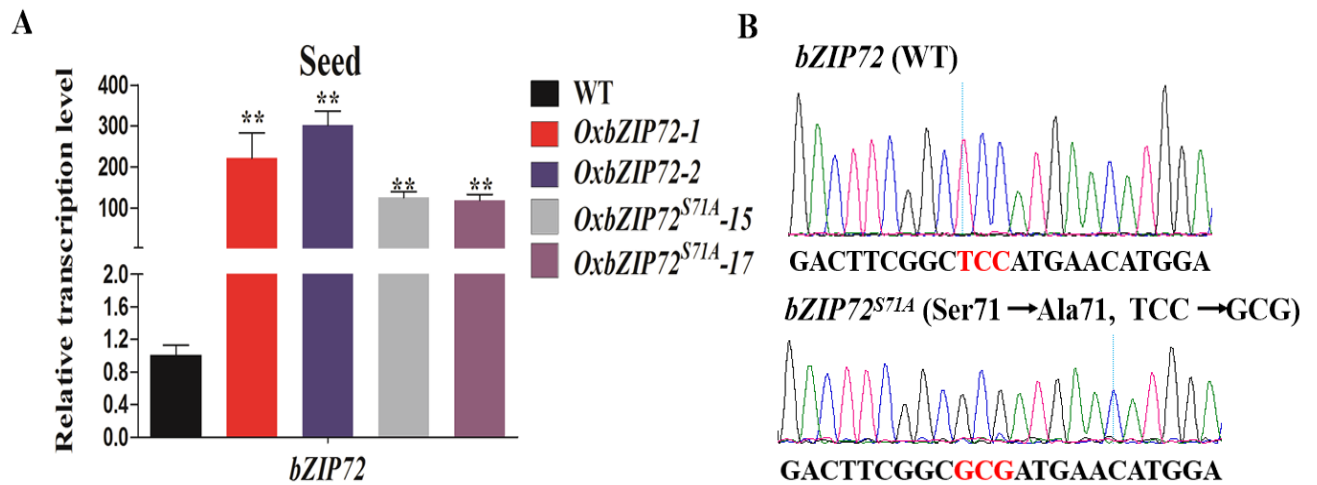


Fig. S8 Molecular characterization of *bZIP72* and *bZIP72^{S71A}* over-expressing transgenic lines. (A) qRT-PCR analysis for transcript accumulation of *bZIP72* in the seeds of *OxbZIP72* and *OxbZIP72^{S71A}* transgenic lines, respectively. Seeds were grown on half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t*-test analysis: ** P<0.01. (B) Sanger sequencing chromatograph of the mutated *bZIP72* cDNA in *OxbZIP72^{S71A}* transgenic lines. The letter in red represented the mutant sites.

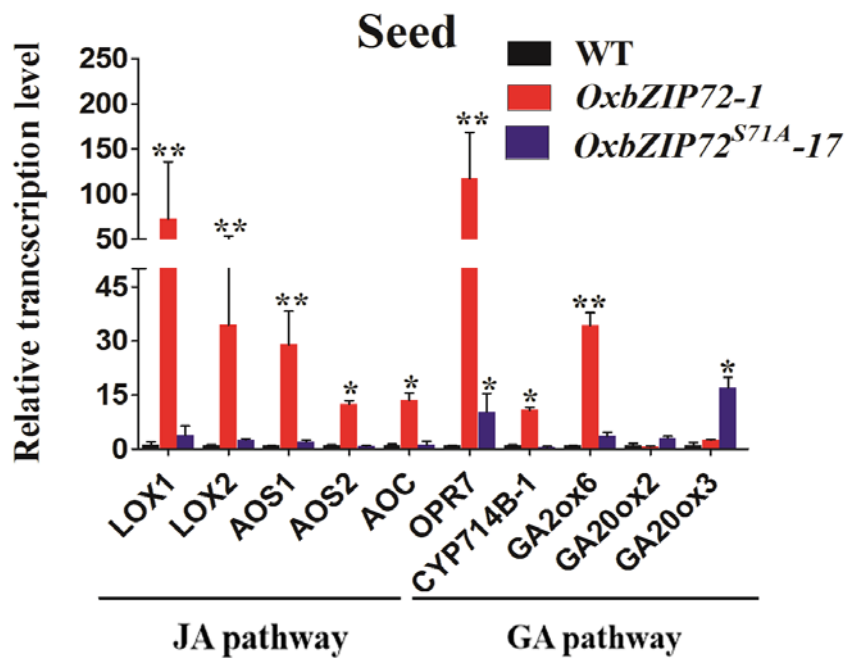


Fig. S9 qRT-PCR analysis for transcript accumulation of JA and GA pathway genes in the seeds of the WT, *OxbZIP72* and *OxbZIP72^{S71A}* transgenic lines. Seeds were grown on half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t*-test analysis: *P<0.05, ** P<0.01.

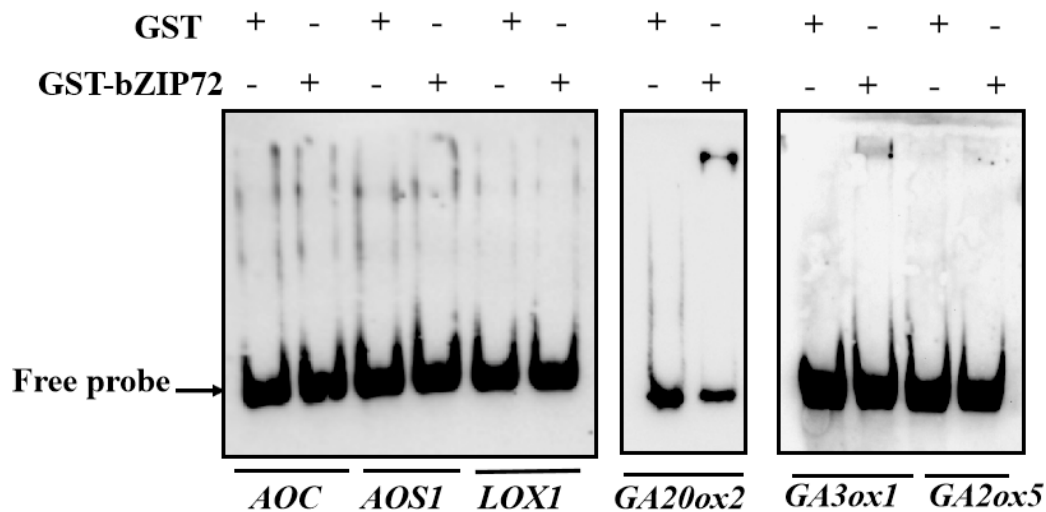


Fig. S10 EMSA of bZIP72 on JA pathway genes *AOC*, *AOS1* and *LOX1* promoter regions and GA pathway genes *GA3ox1*, *GA2ox5* and *GA20ox2* promoter regions.

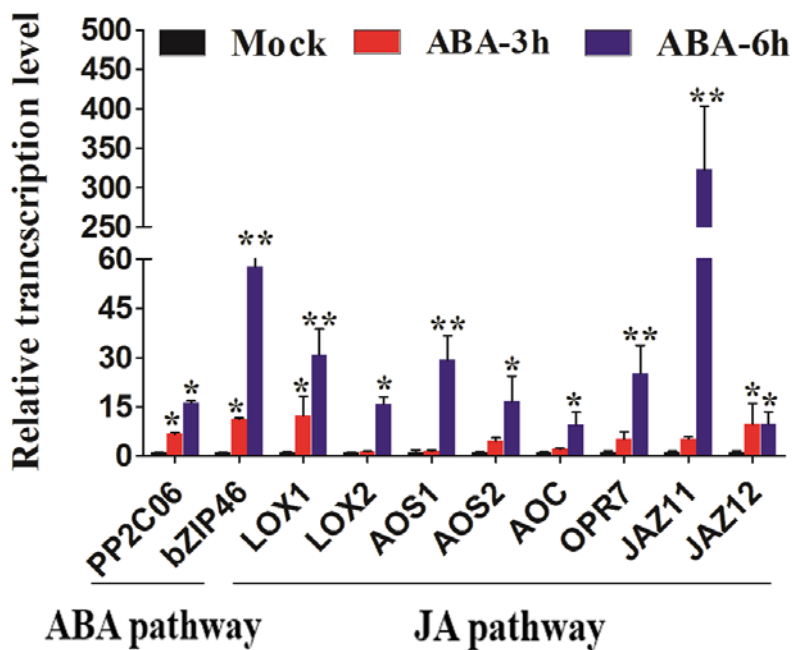


Fig. S11 qRT-PCR analysis for transcript accumulation of ABA and JA pathway genes in the seeds of the WT. Seeds were grown on half-strength MS medium treated with 0 or 10 μ M ABA for 3 or 6 hours before harvest for total RNA isolation, respectively. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of

differences between the mock-treated and ABA-treated seeds as determined by Student's *t*-test analysis: * $P < 0.05$, ** $P < 0.01$.

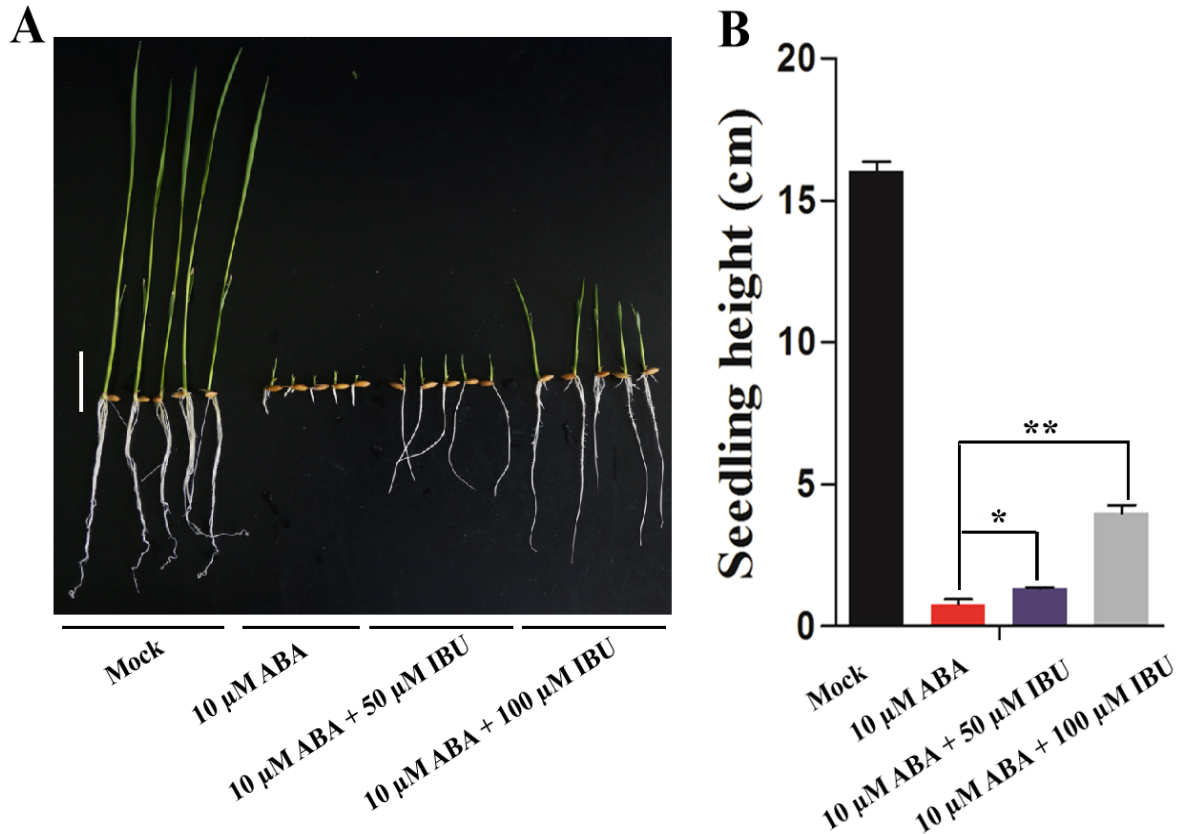


Fig. S12 IBU relieved the inhibition of ABA on post-germination growth. (A) Growth phenotypes of the WT after grown in nutrient solution containing 0, 10 μ M ABA, 10 μ M ABA + 50 μ M IBU or 10 μ M ABA + 100 μ M IBU for 7 days, respectively. Bar= 2.5 cm. (B) Seedling heights of the WT in accordance to (A). IBU: Ibuprofen. Error bars indicate SD with 50 biological replicates ($n=50$). Asterisks indicate the significance of differences between the ABA-treated seedlings and ABA+ IBU treated seedlings as determined by Student's *t*-test analysis: * $P < 0.05$, ** $P < 0.01$.

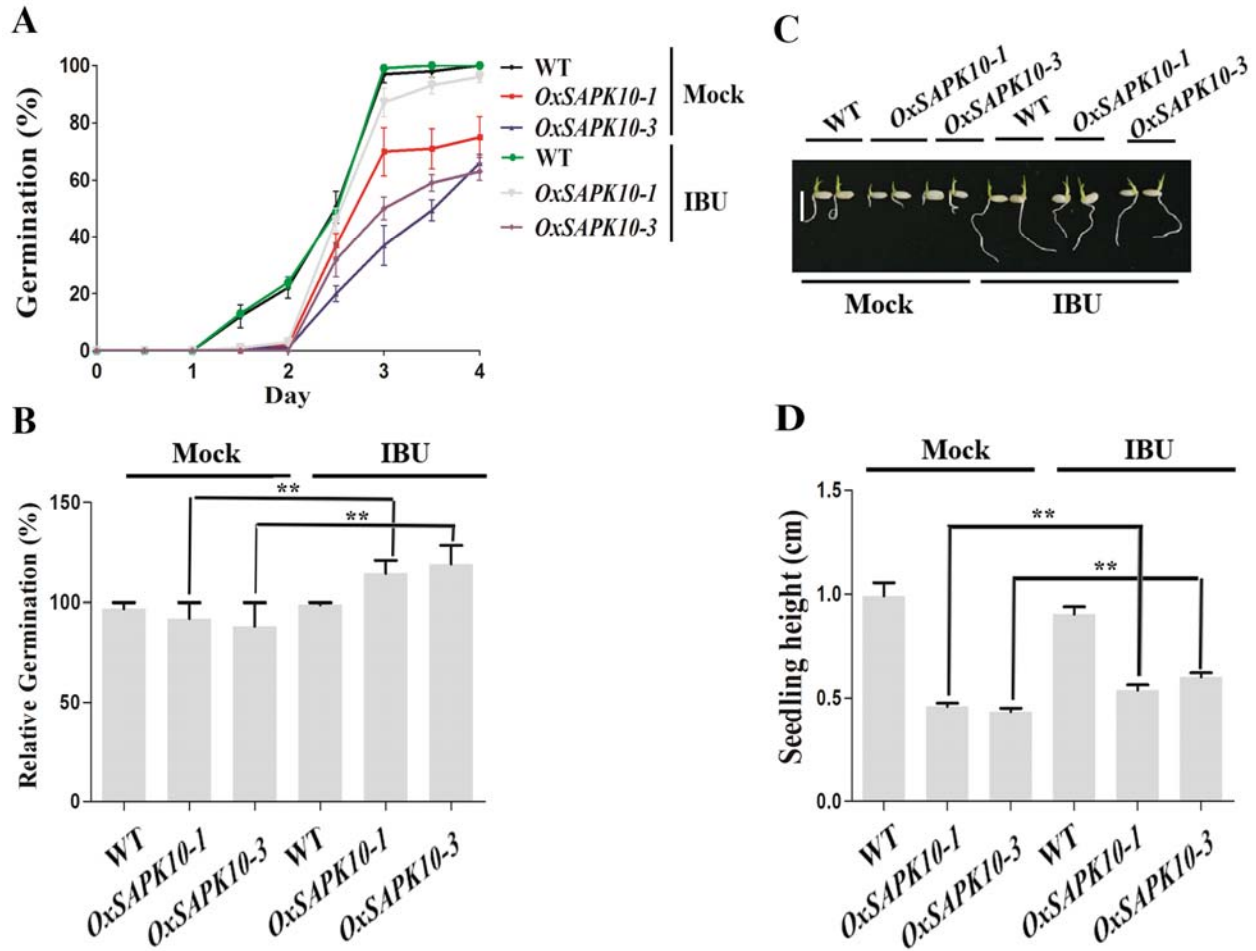


Fig. S13 SAPK10-mediated retarded seed germination was relieved by exogenous IBU applied. (A) Germination time courses of the WT and *OxSAPK10* grown on half-strength MS medium under mock or 100 μ M IBU treatment, respectively. (B) The relative germination of the WT and *OxSAPK10* seeds under 100 μ M IBU treatment was determined after 4 days and expressed as a percentage of those grown on Mock condition. (C) Germination phenotypes of the WT and *OxSAPK10* treated with 0 or 100 μ M IBU, respectively. Photographs were taken on day 4. Bar=1cm. (D) Seedling heights of the WT and *OxSAPK10* in accordance to (C). Error bars indicate SD with biological triplicates (n=3, each replicates containing 50 seeds) in (A) and 50 biological replicates (n=50) in (D). Asterisks indicate the significance of differences between the mock and IBU treatment in (B and D) (Student's *t*-test analysis; ** P<0.01).