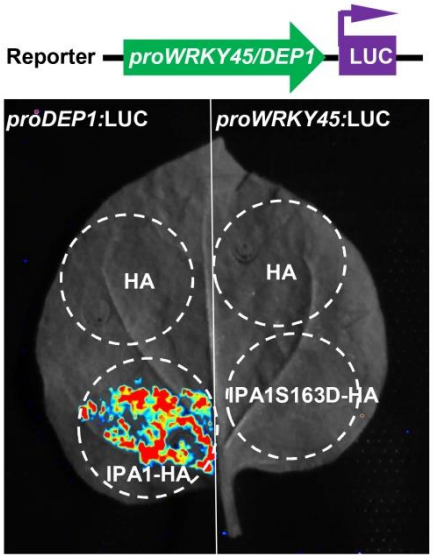
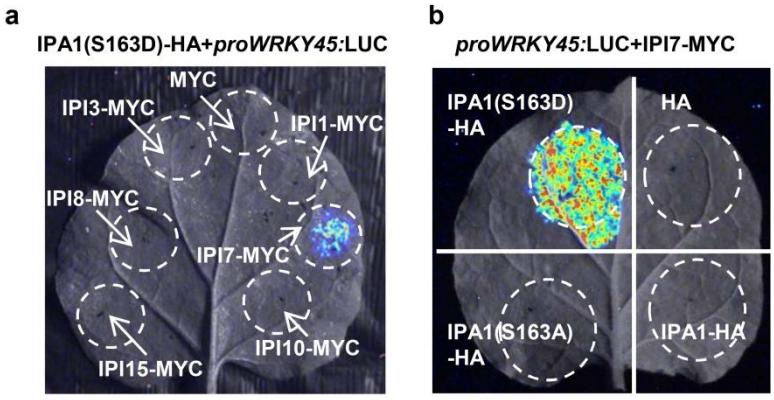
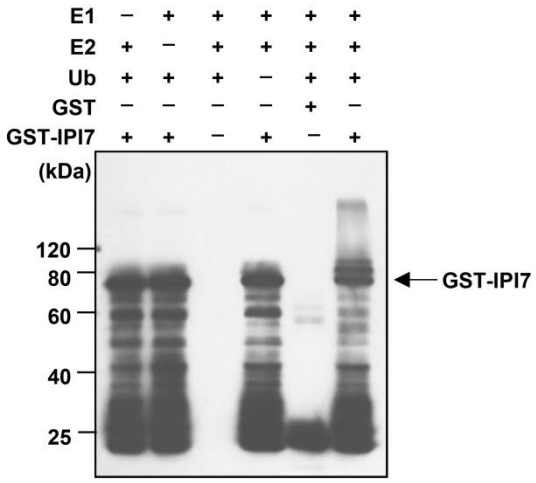


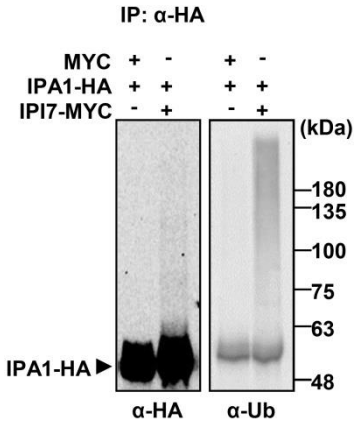
Supplementary Fig. 1. IPA1 alone activates *proDEP1:LUC* , but IPA1(S163D) alone is unable to activate *proWRKY45:LUC* expression. Transactivation of *proDEP1:*LUC by IPA1 and *proWRKY45:*LUC by IPA1(S163D) in tobacco leaves. *A. tumefaciens* carrying indicated plasmids were infiltrated into tobacco leaves. The luciferase (LUC) reporter is driven by *DEP1* (*proDEP1:*LUC) or *WRKY45* promoter (*proWRKY45:*LUC). IPA1 or IPA1(S163D) fused with an HA tag is driven by CaMV 35S promoter. D-luciferin was applied as the LUC substrate. Visible lights indicate that IPA1-HA activates expression of LUC driven by *DEP1*, which serves as a positive control.



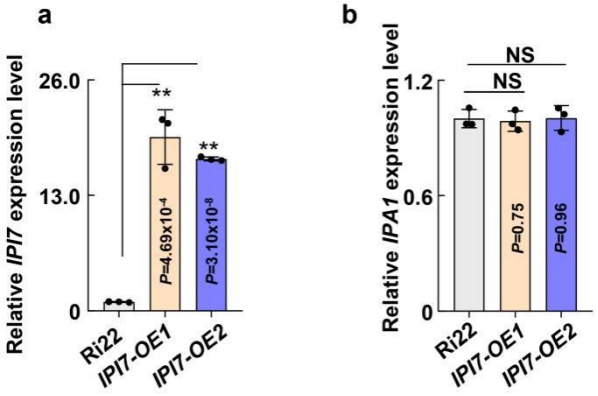
Supplementary Fig. 2. Determination of co-activators of IPA1(S163D) for transactivation of *WRKY45* promoter. a Each candidate co-activator was individually combined with IPA1(S163D) to test for their cooperative transactivation of *WRKY45* in an *A. tumefaciens*-mediated infiltration of tobacco leaves. **b** Transactivation assay of *proWRKY45:*LUC by IPA1(S163D) and IPA1(S163A) in the presence of IPI7-MYC. *A. tumefaciens* carrying indicated plasmids were infiltrated into tobacco leaves. The LUC reporter was driven by the *WRKY45* promoter (*proWRKY45:*LUC). D-luciferin was applied as the LUC substrate.



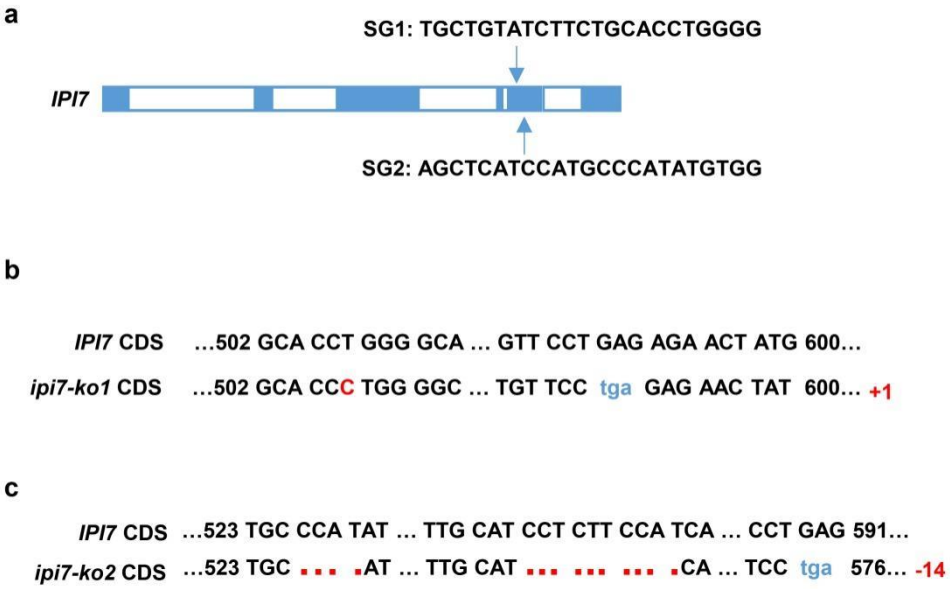
Supplementary Fig. 3. IPI7 is a functional E3 ubiquitin ligase. The E3 ubiquitin ligase activity of IPI7 was examined with purified GST-IPI7 protein in an *in vitro* assay. Immunoblotting was performed with a GST monoclonal antibody. Bands above the GST-IPI7 band represent ubiquitinated forms of GST-IPI7. The presence (+) or absence (-) of components in the reaction mixture is indicated.



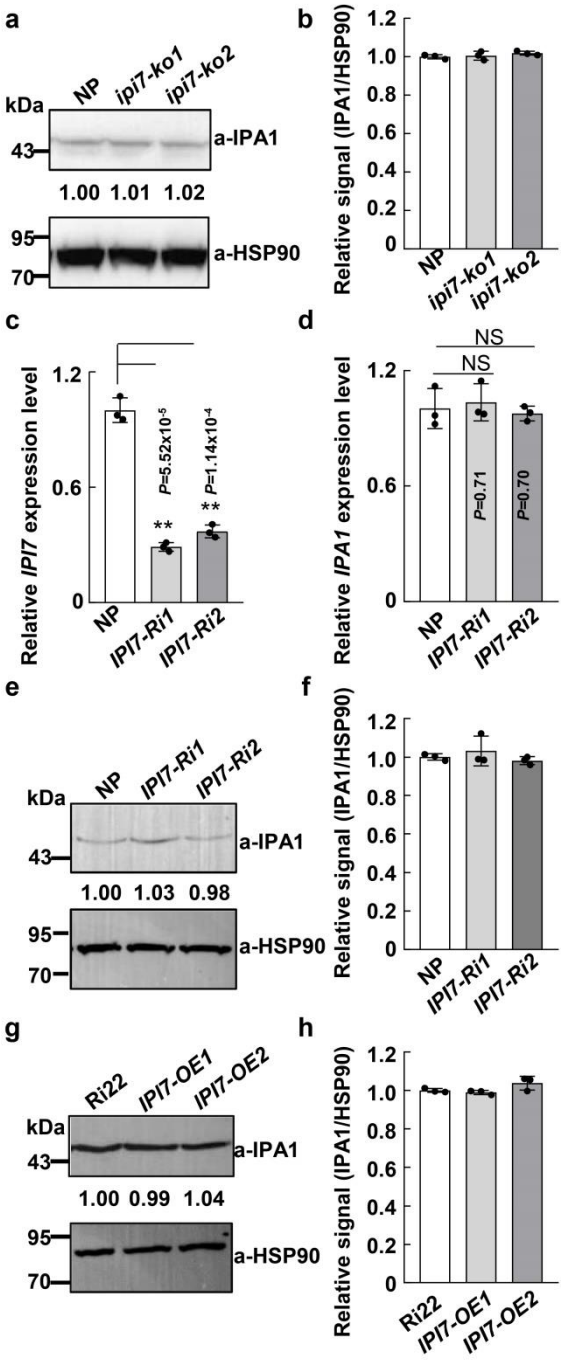
Supplementary Fig. 4. IPI7 promotes ubiquitination of IPA1. Enhanced ubiquitination of IPA1-HA with added IPI7-MYC in tobacco leaves. *A. tumefaciens* carrying indicated plasmids were infiltrated into tobacco leaves. Immunoprecipitation was performed with a HA antibody and immunoblotting was conducted with an HA antibody or a Ub antibody.



Supplementary Fig. 5. *IPI7* and *IPA1* RNA levels in *IPI7-OE* transgenic plants. a Transcript levels of *IPI7* in Ri22 and ubiquitin promoter-driven *IPI7*-overexpressing transgenic plants (*IPI7-OE-1* and *IPI7-OE-2*) were determined by RT-qPCR. **b** Transcript levels of *IPA1* in Ri22, *IPI7-OE-1* and *IPI7-OE-2* plants were determined by RT-qPCR. Rice *ubiquitin5* was used as the reference (Supplementary Data 1). Each value represents mean ± SD (n = 3 independent biological samples). Two-tailed *t*-test, ** indicates $P < 0.01$. NS indicates no significant differences between the compared pair.

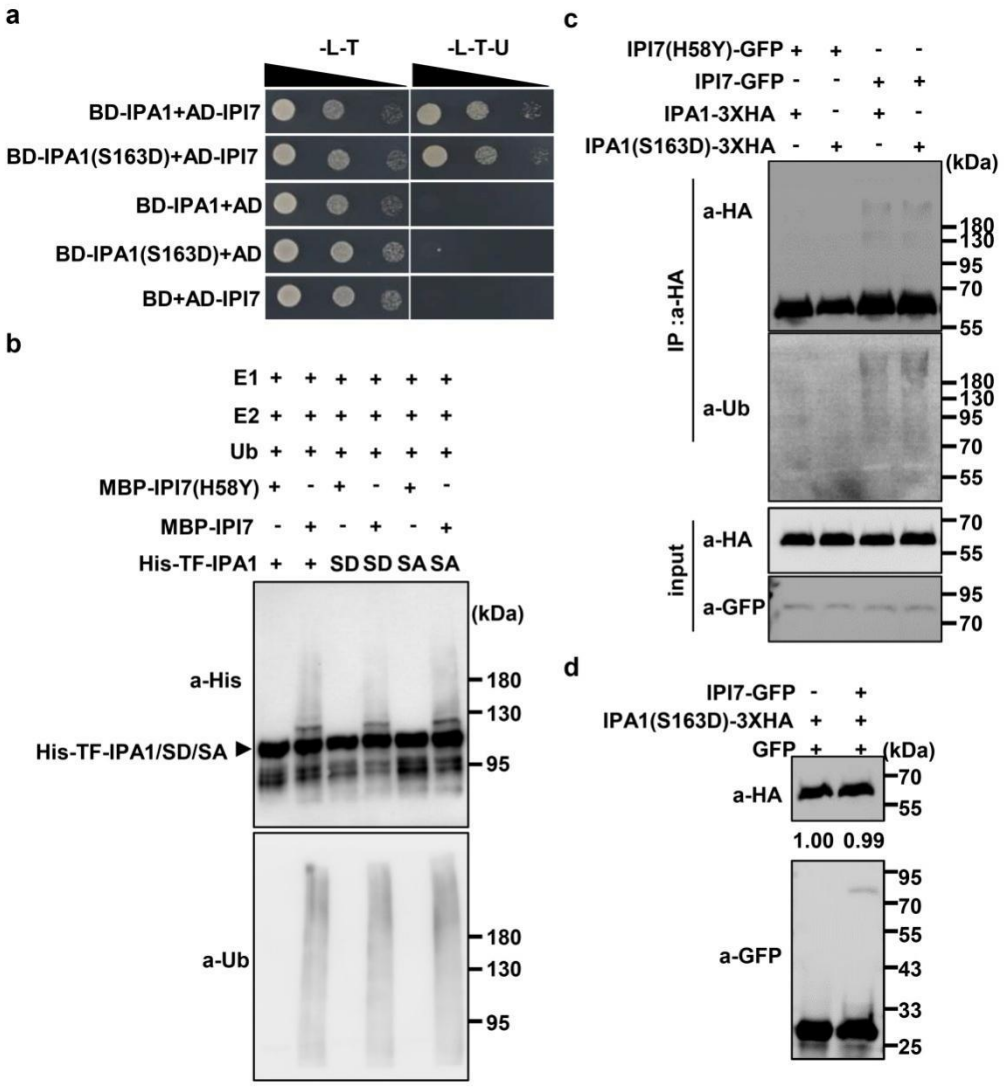


Supplementary Fig. 6. Generation of *IPI7* knockout plants by the CRISPR/Cas9 technology. a Schematic map of the genomic region of *IPI7* and the sgRNA target sites (SG1 and SG2). Arrows indicate the sgRNA target sites on the *IPI7* genomic sequence. Blue boxes indicate exons of *IPI7* and white boxes indicate introns. **b**, **c** Sequence alignment of the sgRNA target regions comparing wild-type with *ipi7-ko1* **(b)** and *ipi7-ko2* **(c)** mutants. The inserted or deleted bases are shown in red; the new stop codons introduced in *ipi7-ko* because of indels are marked in blue.



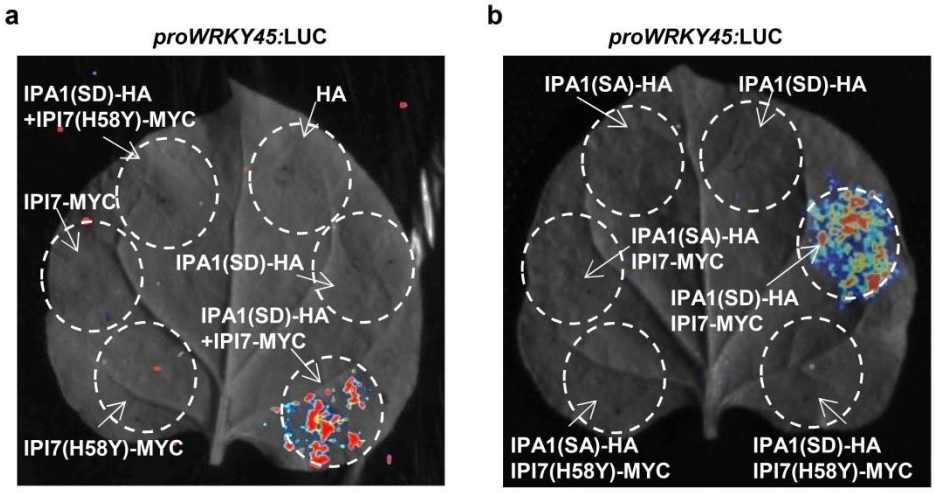
Supplementary Fig. 7. Stability of IPA1 protein is not affected by IPI7. a-b. The abundance of IPA1 protein in Nipponbare (NP) and *ipi7-ko* plants was compared (**a**). Statistical analysis of the protein bands in condition **a** was conducted using three independent assays (**b**). **c-d.** Transcript levels of *IPI7* (**c**) and *IPA1* (**d**) in NP and *IPI7* RNA interference (*IPI7-Ri*) plants were determined by RT-qPCR. Each value represents mean ± SD (n = 3 independent biological samples). Two-tailed *t*-test, ** indicates $P < 0.01$. NS indicates no significant differences between the compared pair. **e-f.** IPA1 protein levels in NP and *IPI7-Ri* plants. **g-h**. IPA1 protein levels in Ri22 and

IPI7-OE plants. Samples were collected from indicated plants. IPA1 protein was probed in immunoblots with an IPA1 antibody and quantitated by densitometry with normalization to Heat Shock Protein 90 (HSP90). Each value represents mean ± SD (n = 3 independent biological samples). Statistical analysis of protein bands was conducted using three independent assays.

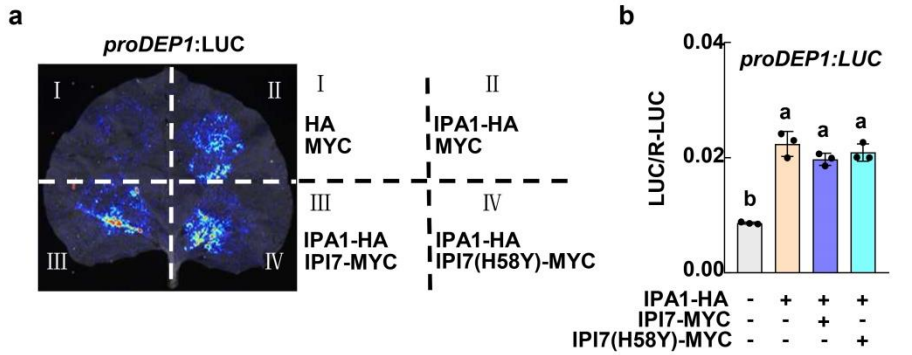


Supplementary Fig. 8. IPI7 interacts with and ubiquitinates IPA1(S163D). a Interaction between IPI7 and IPA1(S163D) in yeast cells. Wild type IPA1 and IPA1(S163D) proteins were individually fused with the GAL4 binding domain to generate BD-IPA1 and BD-IPA1(S163D), respectively, and IPI7 with the GAL4 activation domain to form AD-IPI7. Yeast clones growing on SD-L-T-U medium indicate protein interaction in cells. **b** *In vitro* ubiquitination of IPA1 (His-TF-IPA1), IPA1(S163D) [His-TF-IPA1-(S163D)] and IPA1(S163A) [His-TF-IPA1-(S163A)] by IPI7. MBP-IPI7(H58Y) was used as a negative control. Immunoblotting was performed with antibodies against His and Ub separately. **c.** *In vivo* ubiquitination of IPA1 and IPA1(S163D) by IPI7 in tobacco leaves. IPI7(H58Y)-GFP was used as a negative control. *A. tumefaciens* carrying indicated plasmids were infiltrated into tobacco leaves. Immunoprecipitation was performed with an HA antibody and immunoblotting was conducted with an HA, GFP or Ub antibody. **d.** Stability of

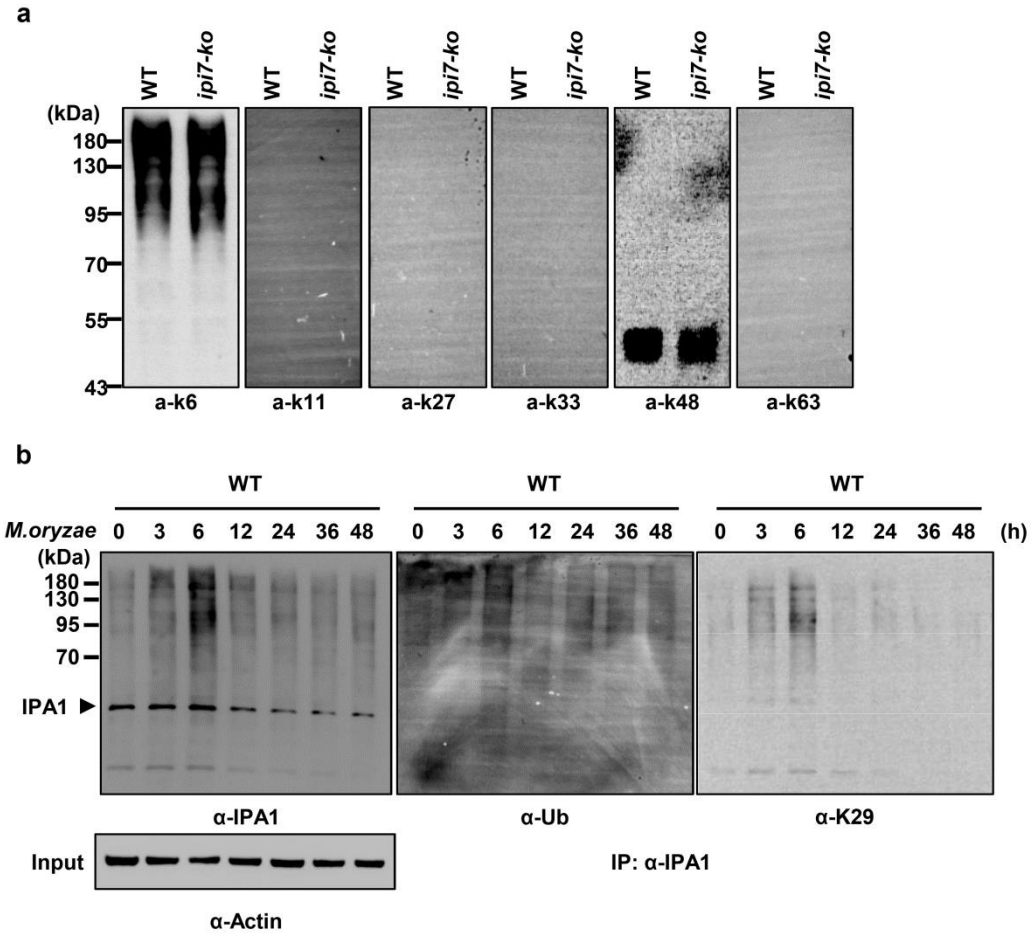
IPA1(S163D) protein in the presence or absence of IPI7 *in vivo*. *A. tumefaciens* carrying indicated constructs were co-infiltrated into tobacco leaves. GFP was used as an internal control for protein expression. The presence (+) or absence (-) of proteins expressed in leaves is indicated.



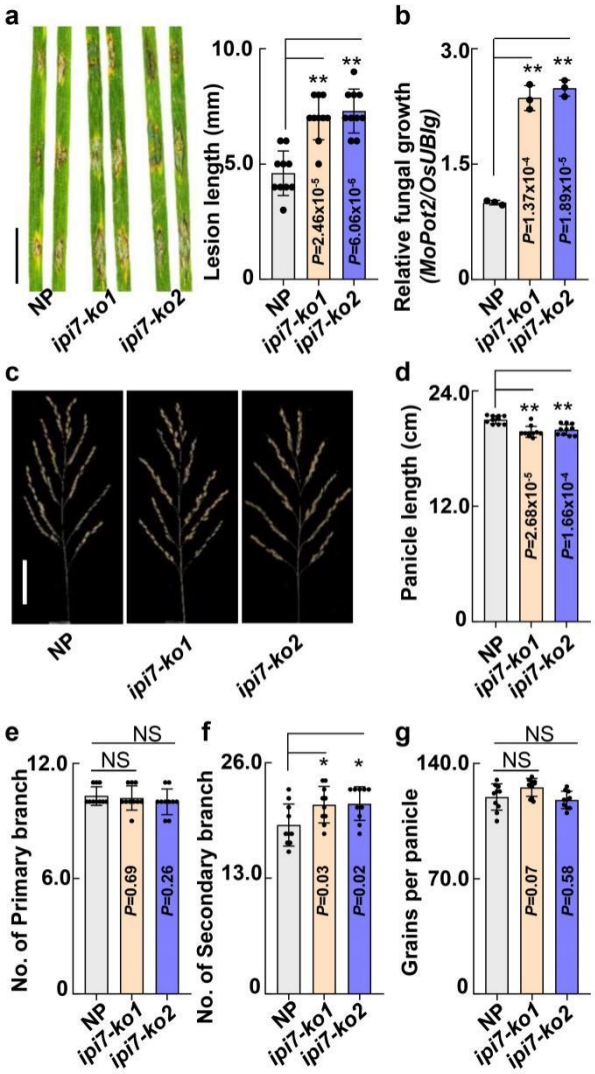
Supplementary Fig. 9. Effects of IPA1(S163D) and IPA1(S163A) on transactivation of *WRKY45* promoter. a The transactivation of *proWRKY45:*LUC was analyzed by co-expressing it with IPA1(SD)-HA and IPI7-MYC in tobacco leaves. **b** Co-expression of IPA1(SA)-HA and IPI7-MYC failed to activate the transactivation of *proWRKY45:*LUC. *A. tumefaciens* carrying indicated plasmids were infiltrated into tobacco leaves. The LUC reporter was driven by the *WRKY45* promoter. D-luciferin was applied as the LUC substrate. IPA1(SD) is IPA1(S163D); IPA1(SA) is IPA1(S163A). IPI7(H58Y) is a null mutant of IPI7.



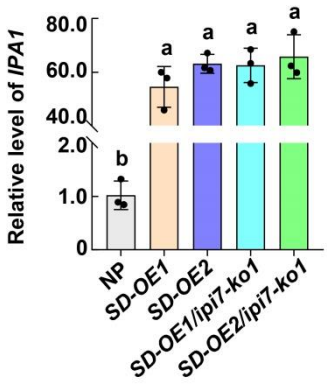
Supplementary Fig. 10. IPI7 does not affect transactivation of *DEP1* promoter by IPA1 in tobacco leaves. a Transactivation of *DEP1* by IPA1 with or without IPI7 in tobacco leaves. *A. tumefaciens* carrying indicated plasmids were infiltrated into tobacco leaves. The LUC reporter is driven by *DEP1* promoter (*proDEP1:*LUC). D-luciferin was applied as the LUC substrate. **b** Statistical analysis of (**a**). Each value represents mean ± SD (n = 3 independent biological samples). Renilla LUC was used as the internal reference. Different letters indicate significant differences determined by the Tukey-Kramer test, $P < 0.05$ (one-way ANOVA was conducted, followed by two-sided HSD test for multiple comparisons). The corresponding *P*-values can be found in the Source Data. Source data are provided as a Source Data file.



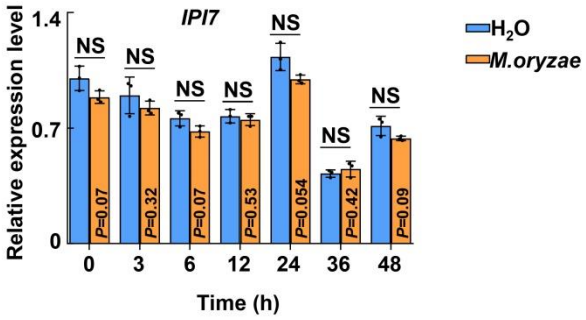
Supplementary Fig. 11. Detection of IPI7-mediated polyubiquitination of IPA1. a The *in vivo* ubiquitination assay was performed with wild type and *ipi7-ko* plants. Immunoprecipitation was performed with an IPA1 antibody; precipitated proteins were separated on a gel, blotted, and probed with an antibody against K6, K11, K27, K33, K48, or K63-polyubiquitin chain. **b.** Levels of K29-polyubiquitination of IPA1 upon *M. oryzae* infection. Leaves were collected at different time points after inoculation with *M. oryzae*. Immunoprecipitation was performed with an IPA1 antibody and immunoblotting was performed with an antibody against IPA1, Ub or K29. Proteins before immunoprecipitation (input) were probed with an antibody against actin for internal reference.



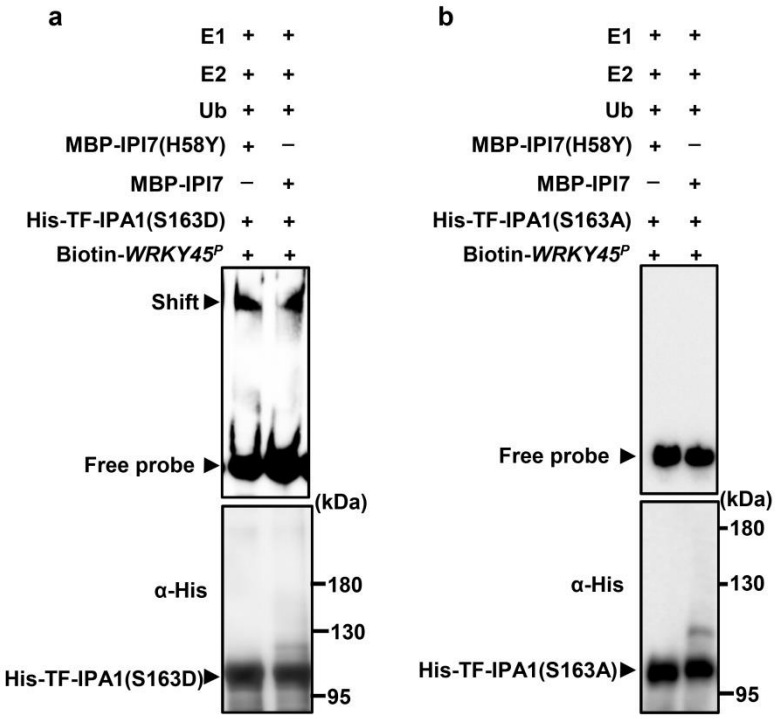
Supplementary Fig. 12. Knockout of *IPI7* impairs host resistance to blast disease without panicle morphology change. a, b Disease phenotypes of *ipi7-ko* plants after *M. oryzae* infection. NP and *ipi7-ko* plants were inoculated with *M. oryzae*. Lesion pictures and average lesion length **(a)**, and *M. oryzae* biomass in infected leaves determined by the *MoPot2* DNA content **(b)** are presented. Each value represents mean ± SD (n = 10 lengths of independent lesions are measured; n = 3 independent biological samples of *M. oryzae*). Two-tailed *t*-test, ** indicates $P < 0.01$. **c** Morphology of main panicles of NP and *ipi7-ko*. Bar = 5 cm. **d-g** Statistical analysis of main panicle length **(d)**, primary branch **(e)** and second branch numbers **(f)**, and grains per main panicle **(g)** of NP and *ipi7-ko*. Each value represents mean ± SD (n = 10 rice plants). Two-tailed *t*-test, ** indicates $P < 0.01$; * indicates $P < 0.05$. NS indicates no significant differences between compared pairs.



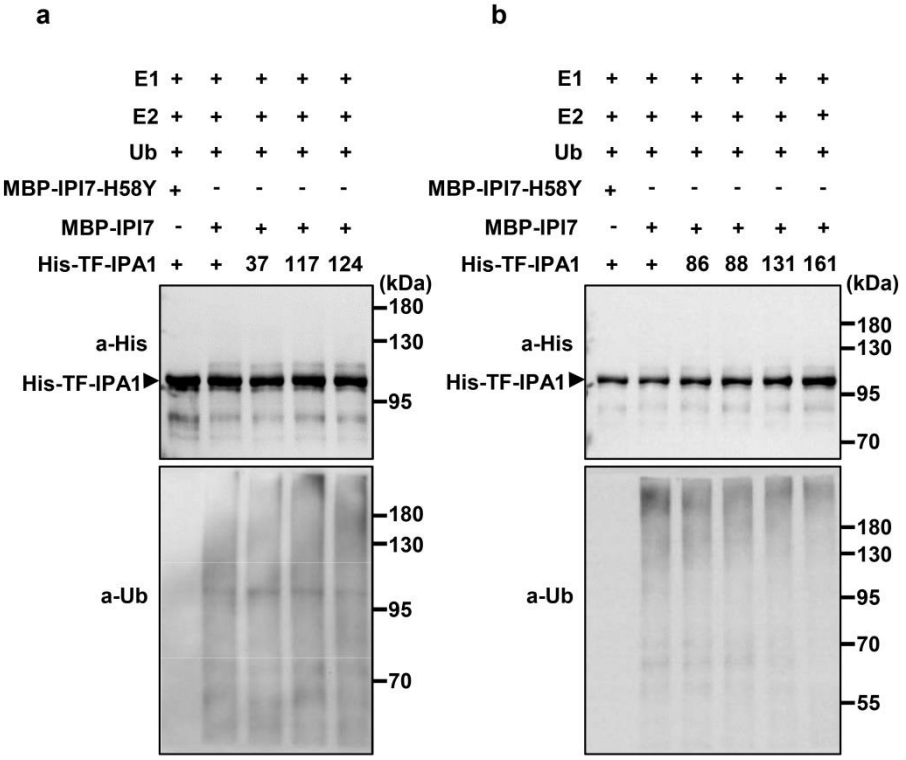
Supplementary Fig. 13. *IPA1* RNA level in transgenic plants. Confirmation of *IPA1(S163D)* overexpression in NP or *ipi7-ko1* background. RNA levels of *IPA1* were determined by RT-qPCR; rice *ubiquitin* was used as the reference. Each value represents mean ± SD (n = 3 independent biological samples). Different letters indicate significant differences determined by the Tukey-Kramer test, $P < 0.05$ (one-way ANOVA was conducted, followed by two-sided HSD test for multiple comparisons). The corresponding *P*-values can be found in the Source Data. Source data are provided as a Source Data file.



Supplementary Fig. 14 *IPI7* RNA levels upon *M. oryzae* infection or mock treatment. The transcript levels of *IPI7* were determined by RT-qPCR. Rice *ubiquitin* was used as the internal reference. Each value represents mean ± SD (n = 3 independent biological samples, Two-tailed *t*-test). NS indicates no significant differences between compared pairs.



Supplementary Fig. 15 IPI7-mediated ubiquitination does not affect IPA1(S163D) binding to the *WRKY45* promoter. DNA binding activities of IPA1(S163D) **(a)** and IPA1(S163A) **(b)** were assessed with or without IPI7-mediated ubiquitination. EMSA (top) and ubiquitination assays (bottom) were performed to detect the effects of IPI7 on the DNA binding activity of His-IPA1(S163D) to the *WRKY45* promoter (Biotin-*WRKY45*^{P}). The presence (+) or absence (-) of components in the reaction mixture is indicated.



Supplementary Fig. 16. IPI7 promotes ubiquitination of IPA1 lysine mutants *in vitro*. *In vitro* ubiquitination assays were performed for wildtype and mutant IPA1 proteins with or without MBP-IPI7. MBP-IPI7(H58Y) was used as a negative control. The number in the His-TF-IPA1 column represents the IPA1 protein carrying a mutation at the indicated number of amino acid (lysine), including (a) IPA1(K37R), IPA1(K117R), IPA1(K124R), (b) IPA1(K86R), IPA1(K88R), IPA1(K131R) and IPA1(K161R). Immunoblotting was performed with antibodies against His and Ub.