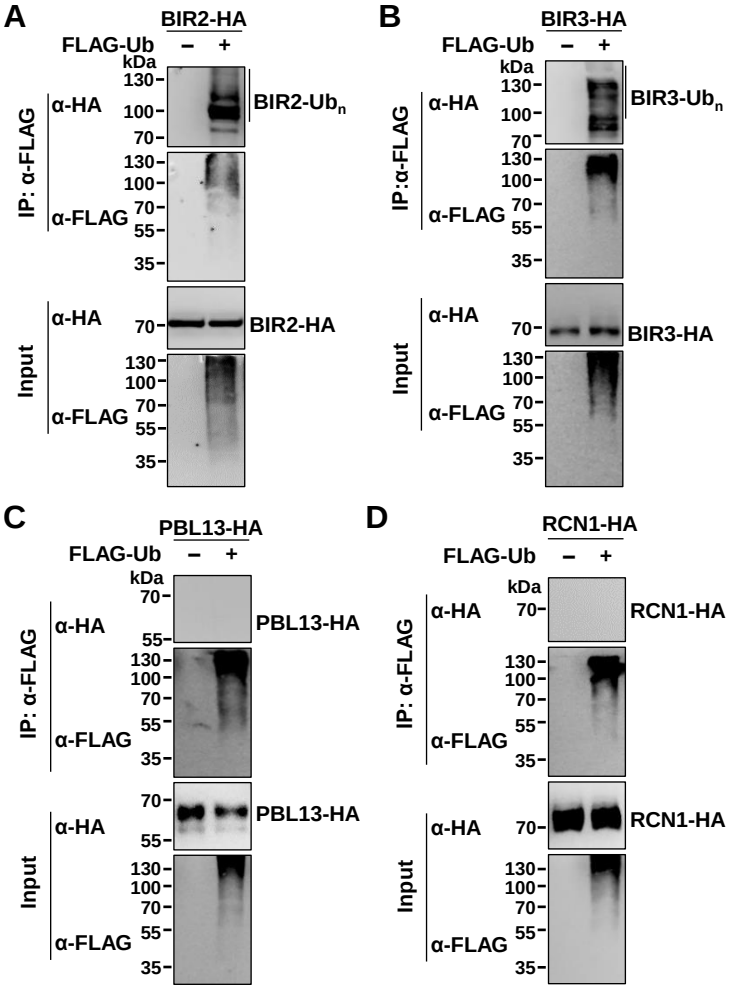
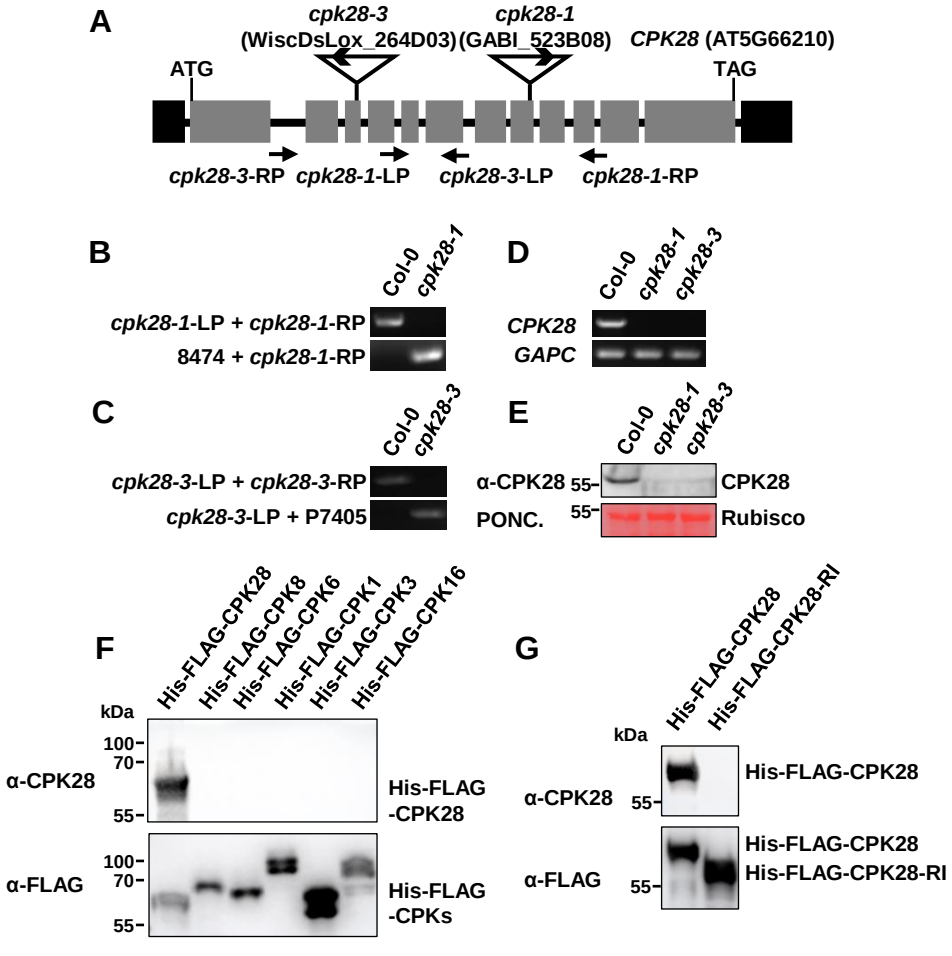
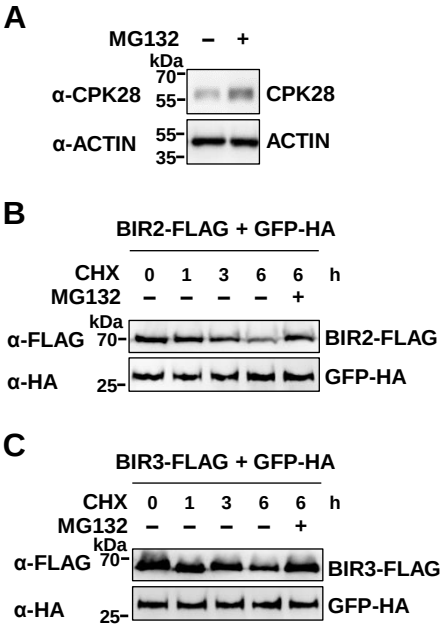


Supplemental Figure S1. BIR2 and BIR3 undergo ubiquitination. (Supports Figure 1)
(A-D) *In vivo* ubiquitination assays for BIR2, BIR3, PBL13-HA, and RCN-HA. FLAG-Ub was co-expressed with BIR2-HA, BIR3-HA, PBL13-HA, or RCN-HA in Arabidopsis protoplasts. Total ubiquitinated proteins were immunoprecipitated with anti-FLAG antibodies, and ubiquitinated BIR2-HA, BIR3-HA, PBL13-HA, or RCN-HA proteins were detected by immunoblotting with anti-HA antibodies.



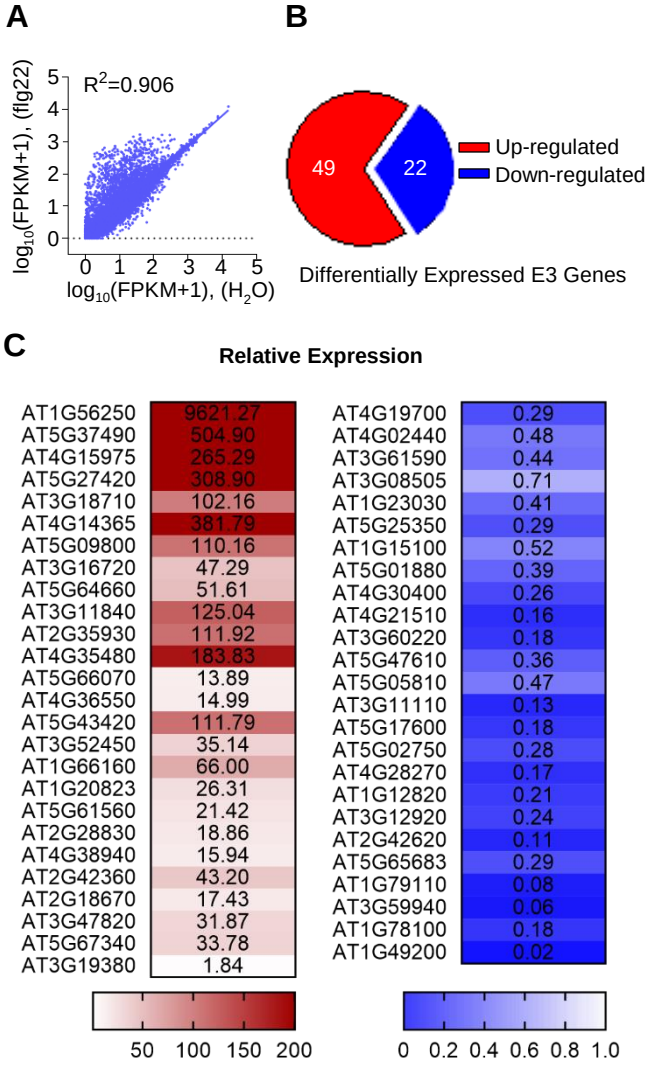
Supplemental Figure S2. Identification of *cpk28* mutants and verification of the specificity of anti-CPK28 antibodies. (Supports Figure 1)

(A) Schematic diagram of *CPK28* gene structure. Black lines, gray boxes, and black boxes indicate introns, exons, and untranslated regions (UTRs), respectively. The approximate positions of the primers used for genotyping are indicated. (B and C) Identification of the homozygous T-DNA insertion mutants of *CPK28*. T-DNA-specific primers and gene-specific primers were used for genotyping *cpk28-1* and *cpk28-3*. (D) Measurement of *CPK28* transcript levels in *cpk28-1* and *cpk28-3*. *CPK28* transcript levels were measured by RT-PCR; *GAPC* was used as an internal control. (E) Verification of the specificity of anti-CPK28 antibodies. Total proteins were extracted from 7-d-old Col-0, *cpk28-1*, and *cpk28-3* seedlings. CPK28 proteins were detected by immunoblotting with anti-CPK28 antibodies. Ponceau (PONC.) staining was used as the loading control. (F) Anti-CPK28 antibodies specifically recognize recombinant CPK28 proteins. Recombinant His-FLAG-CPK28, His-FLAG-CPK8, His-FLAG-CPK6, His-FLAG-CPK1, His-FLAG-CPK3, and His-FLAG-CPK16 proteins expressed in *E.coli* were purified. Equal amounts of CPKs were subjected to SDS-PAGE followed by immunoblotting with anti-CPK28 or anti-FLAG antibodies. (G) Anti-CPK28 antibodies do not recognize recombinant CPK28-RI proteins. The recombinant His-FLAG-CPK28 and His-FLAG-CPK28-RI proteins were subjected to immunoblotting with anti-CPK28 or anti-FLAG antibodies.



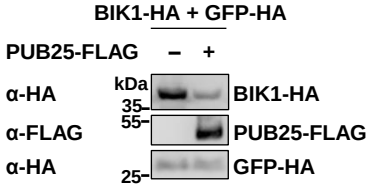
Supplemental Figure S3. Various negative immune regulators undergo proteasomal degradation. (Supports Figure 1)

(A) CPK28 protein accumulation increases after MG132 treatment. Seven-day-old Col-0 seedlings (10 seedlings per sample) were treated with 50 μM MG132 for 6 h. CPK28 proteins were detected by immunoblotting with anti-CPK28 antibodies. Actin detected with anti-ACTIN antibodies was used as the loading control. (B and C) BIR2 and BIR3 undergo proteasomal degradation. BIR2-FLAG or BIR3-FLAG was expressed in protoplasts. GFP-HA was used as an internal transfection control. The protoplasts were treated with 50 μM CHX for the indicated times in the presence or absence of 20 μM MG132. BIR2/3-FLAG and GFP-HA were detected by immunoblotting with anti-FLAG and anti-HA antibodies, respectively.



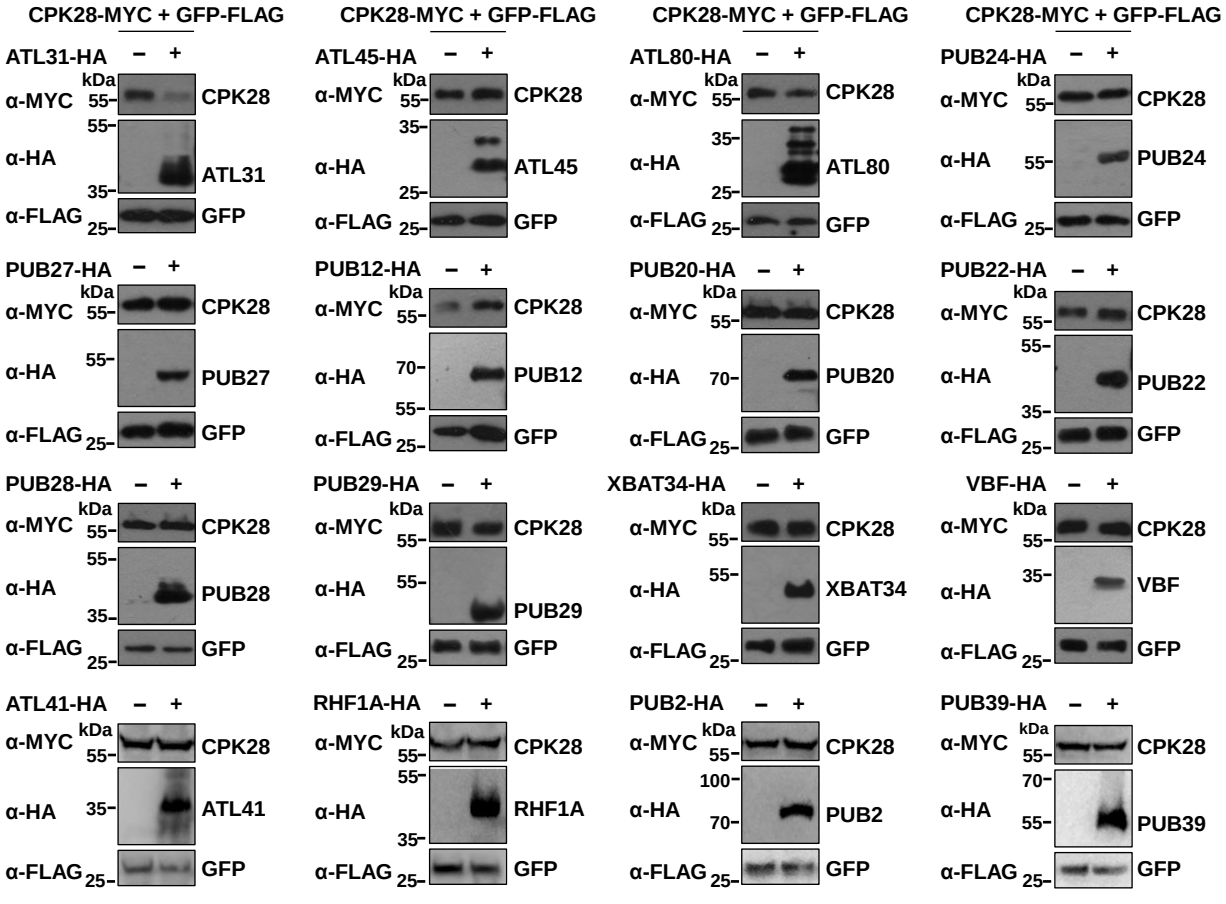
Supplemental Figure S4. Screening of differentially expressed ubiquitin ligase genes upon flg22 treatment. (Supports Figure 2)

(A) Scatter plots of whole-genome transcript fragments per kilobase of million mapped reads (FPKM) in 7-day-old Arabidopsis seedlings treated with water and 4 μM flg22 for 30 min. The correlation coefficient (R^2) of the expression profiles of all transcripts between water- and flg22-treated seedlings is almost linear (0.906), indicating that flg22 treatment does not affect gene transcription on the whole. The *x* and *y* axes show gene expression levels in water- and flg22-treated Arabidopsis seedlings, respectively. (B) The number of up- and downregulated ubiquitin ligase genes after flg22 treatment. Differentially expressed genes (DEGs, fold change ≥ 2 or ≤ 0.5) were determined by RNA-seq analysis, FDR < 0.05. (C) RT-qPCR analysis of the differentially expressed ubiquitin ligase genes. The expression of the differentially expressed ubiquitin ligase genes determined by RNA-seq analysis was confirmed by RT-qPCR. Relative transcript levels were normalized to those of *GAPC*. The gene expression level in seedlings with water treatment was set to 1.0. Red indicates upregulated genes; blue indicates downregulated genes.



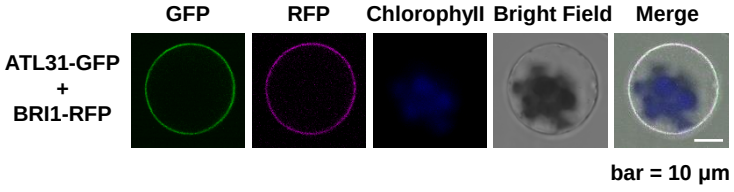
Supplemental Figure S5. Validation of the feasibility of the protoplast cell-based screening system. (Supports Figure 2)

The feasibility of the screening system was validated by co-expressing *PUB25-FLAG* and *BIK1-HA* in Arabidopsis protoplasts. GFP-HA was used as an internal protoplast transfection control. The accumulation of BIK1-HA was examined by immunoblotting with anti-HA antibodies.



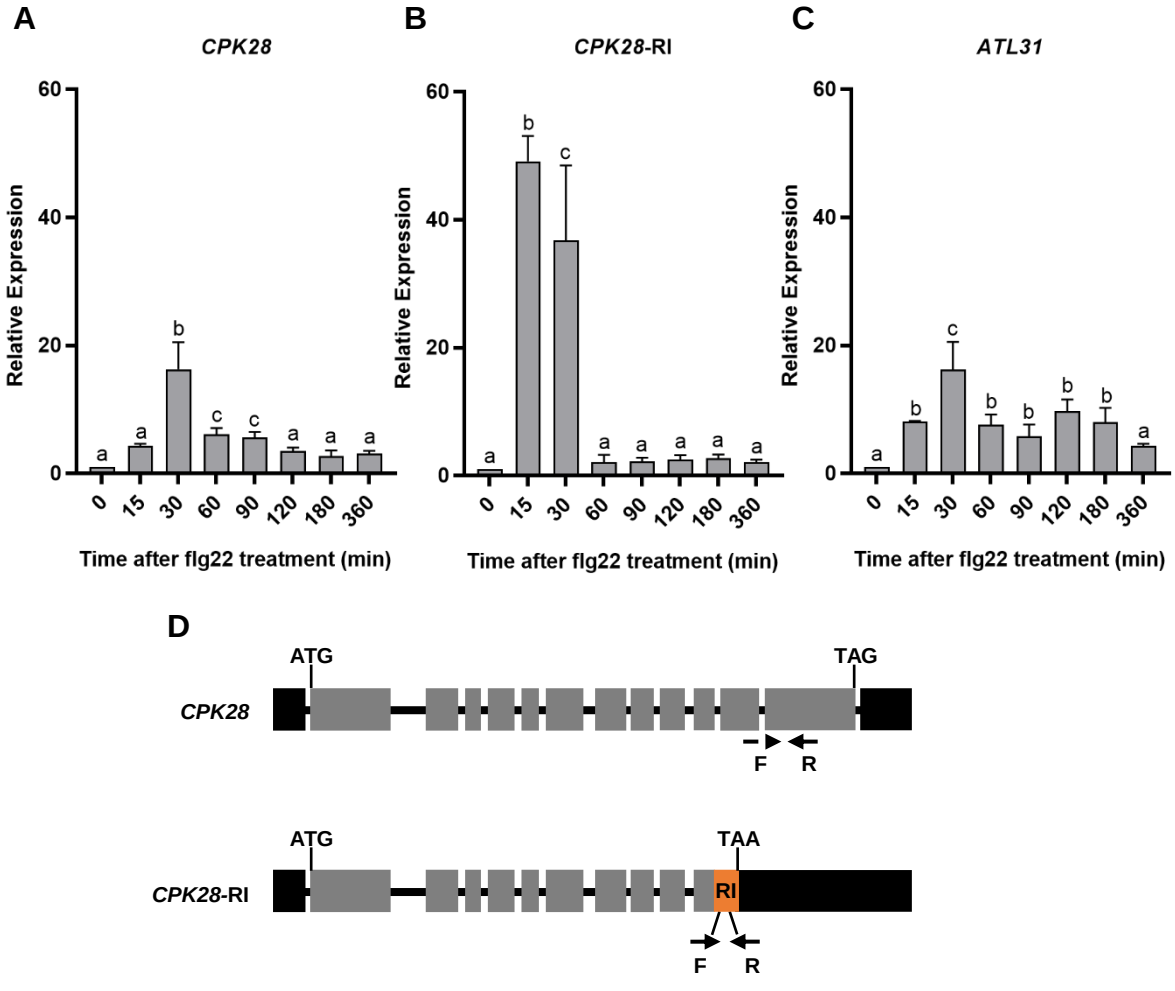
Supplemental Figure S6. Screening of ubiquitin ligases that can potentially mediate CPK28 degradation. (Supports Figure 2)

CPK28-MYC was co-expressed with a ubiquitin ligase gene in Arabidopsis protoplasts. GFP-FLAG was used as an internal transfection control and was detected with anti-FLAG antibodies. The accumulation of CPK28-MYC was analyzed with anti-MYC antibodies.



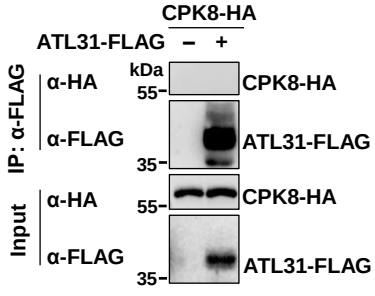
Supplemental Figure S7. ATL31 is localized to the plasma membrane in Arabidopsis protoplasts. (Supports Figure 2)

ATL31-GFP was co-expressed together with BRI1-RFP (a known integral plasma membrane protein) in Arabidopsis protoplasts. Autofluorescence of chlorophyll and green and red fluorescent proteins were visualized via confocal microscopy. A total of 10 protoplasts were observed. Scale bar = 10 μm.

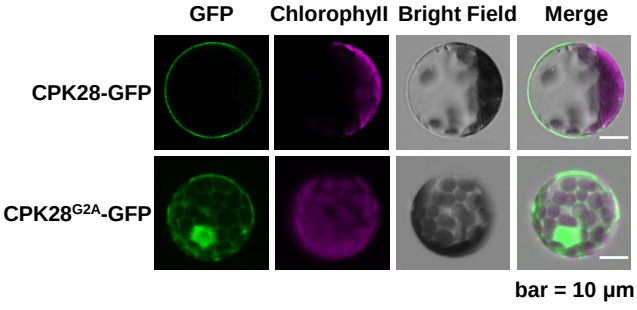


Supplemental Figure S8. Measurement of transcript levels of *ATL31*, *CPK28*, and the *CPK28*-RI splice variant upon flg22 treatment. (Supports Figure 2)

(A-C) Seven-day-old Arabidopsis seedlings were treated with 4 μM flg22 for the indicated times. The transcript levels of *CPK28* (A), the *CPK28*-RI splice variant (B), and *ATL31* (C) were analyzed by RT-qPCR and were then normalized to that of *GAPC*; the values represent the expression fold change versus the 0 min samples. Values represent the means ± SD of three independent replicates using independent seedling samples grown and treated with flg22 under the same conditions. Lowercase letters indicate significant differences with $P < 0.05$ (one-way ANOVA with Tukey's multiple comparisons test, Supplemental Table S4). (D) The approximate positions of the primers used for qPCR. Black lines, gray boxes, and black boxes indicate introns, exons, and untranslated regions (UTRs), respectively. RI: retained intron. Arrows indicate primers used for qPCR (not drawn to scale). F: forward primer, R: reverse primer. The primers used for measuring *CPK28*-RI transcript levels were described previously (Dressano et al., 2020).

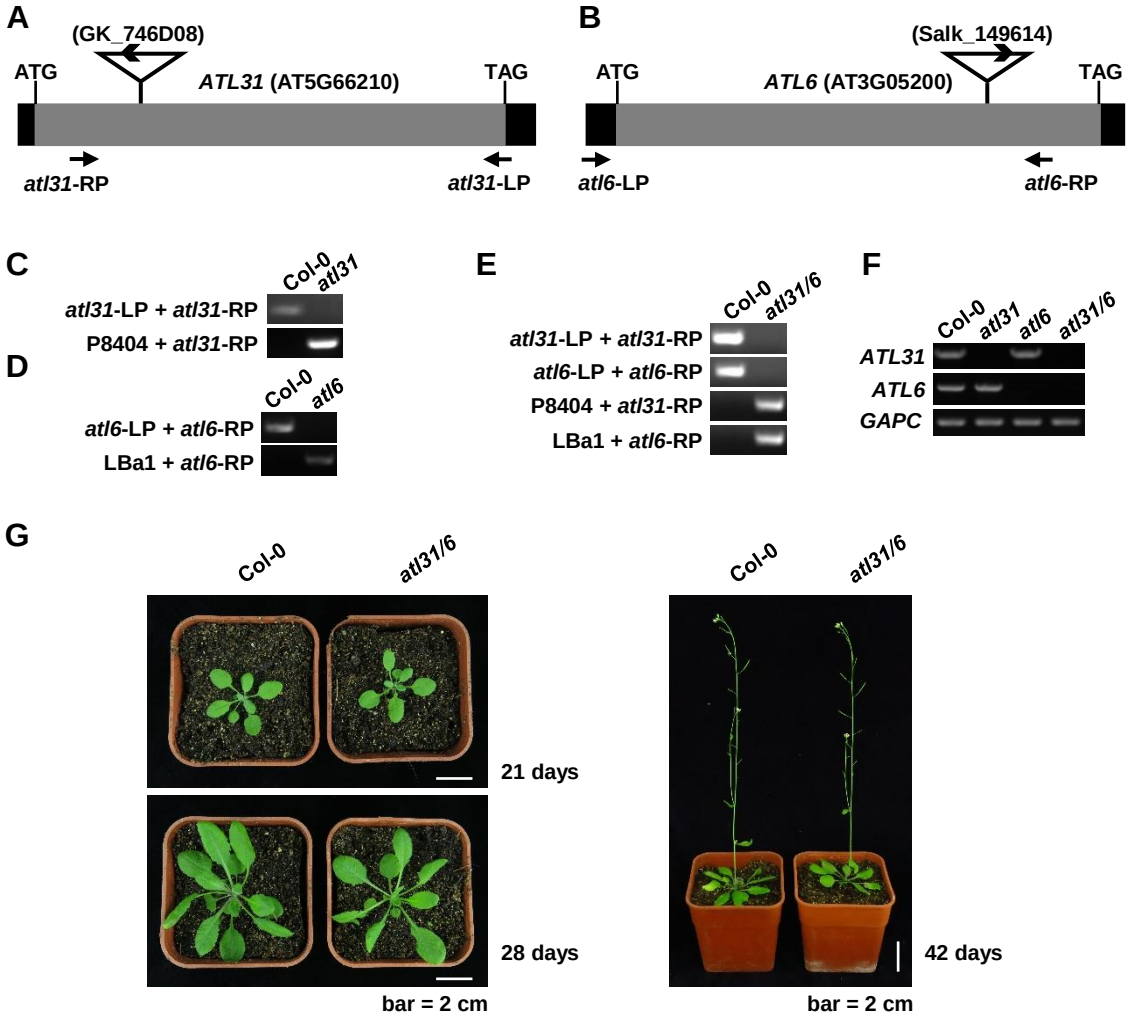


Supplemental Figure S9. ATL31 does not associate with CPK8. (Supports Figure 2)
ATL31-FLAG was co-expressed with CPK8-HA in Arabidopsis protoplasts. IPs were performed using anti-FLAG antibodies. The immunoprecipitated ATL31-FLAG proteins were immunoblotted with anti-FLAG antibodies, and the associated proteins were detected with anti-HA antibodies (top two panels). The expression of ATL31-FLAG and CPK8-HA is shown in the bottom two panels.



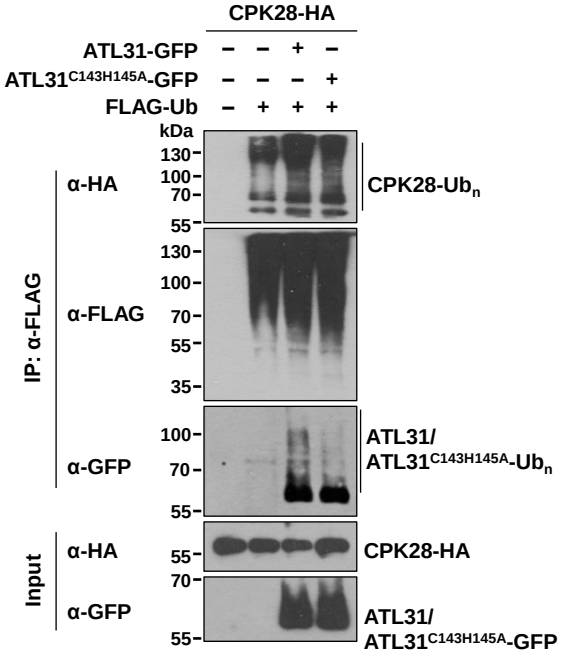
Supplemental Figure S10. The CPK28^{G2A} variant loses its plasma membrane localization in protoplasts. (Supports Figure 3)

CPK28-GFP or CPK28^{G2A}-GFP was expressed in Arabidopsis protoplasts. Green fluorescent protein and chlorophyll autofluorescence were visualized by confocal microscopy. Ten protoplasts were observed for each transfection. Scale bar = 10 µm.

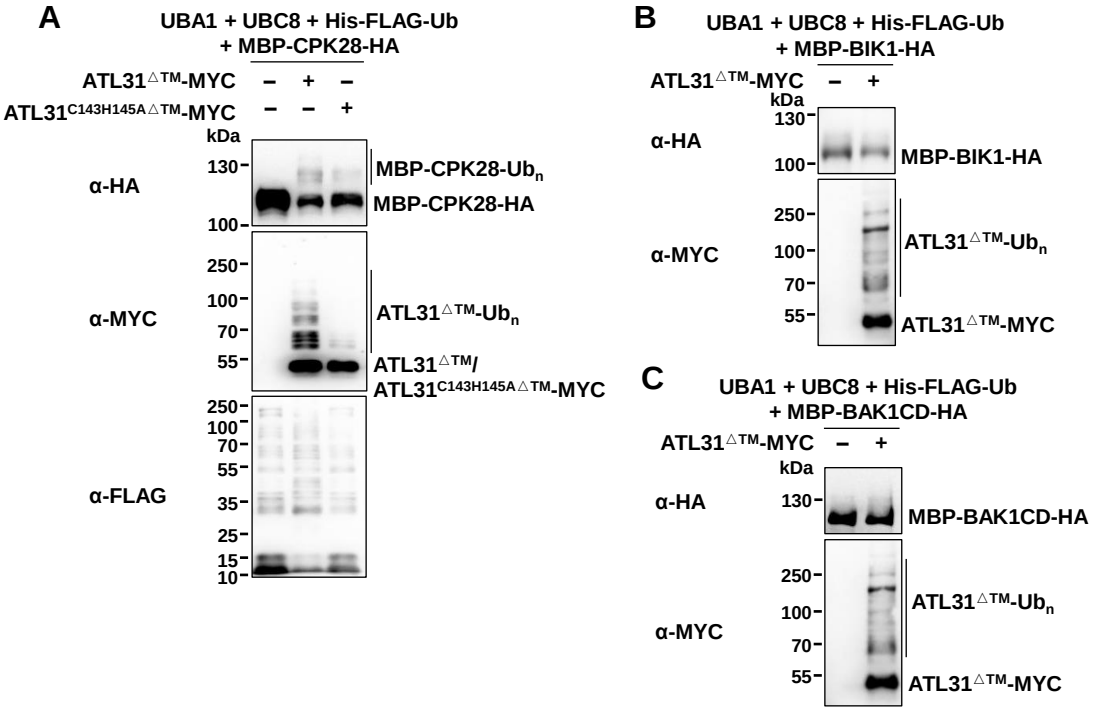


Supplemental Figure S11. Identification of the homozygous T-DNA insertion mutants *atl31* and *atl6* and generation of the *atl31 atl6* double mutant. (Supports Figure 4)

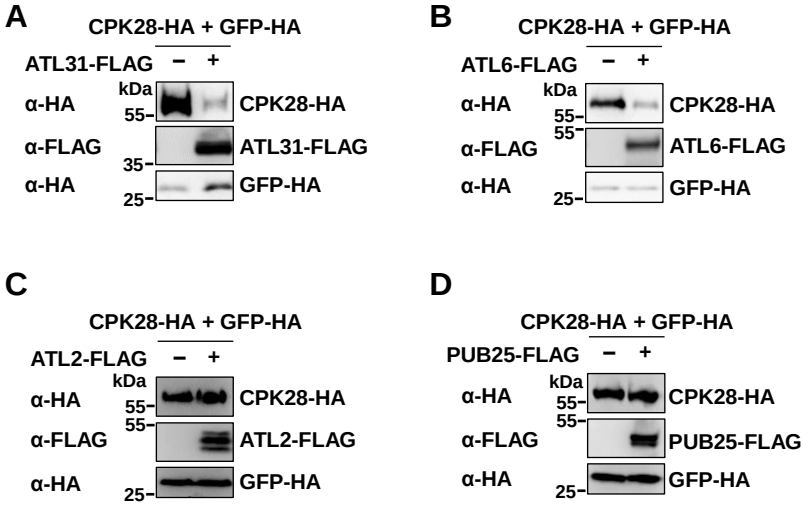
(A and B) Schematic diagrams of the *ATL31* and *ATL6* gene structures. Gray boxes indicate exons, and black boxes represent UTRs. The approximate positions of primers used for genotyping are indicated. (C and D) Identification of the homozygous T-DNA insertion mutants *atl31* and *atl6*. T-DNA-specific primers and gene-specific primers were used for genotyping. (E) Identification of the homozygous *atl31 atl6* double mutant, which was generated by crossing *atl31* and *atl6*. (F) Measurement of *ATL31/6* transcript levels in different mutants by RT-PCR; *GAPC* was used as an internal control. (G) The growth phenotypes of *atl31 atl6* and Col-0 plants at 21, 28, and 42 d post germination under a 12 h light/12 h dark cycle. Scale bar = 2 cm.



Supplemental Figure S12. ATL31 promotes the ubiquitination of CPK28. (Supports Figure 4)
FLAG-Ub, CPK28-HA, and ATL31-GFP or ATL31^{C143H145A}-GFP were co-expressed in Arabidopsis protoplasts. IPs were carried out with anti-FLAG antibodies. Ubiquitinated CPK28-HA proteins and the autoubiquitination of ATL31-GFP or ATL31^{C143H145A}-GFP were detected by immunoblotting with anti-HA and anti-GFP antibodies, respectively.

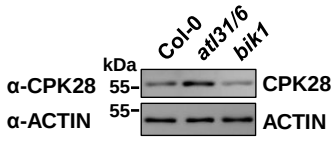


Supplemental Figure S13. The specificity of CPK28 ubiquitination by ATL31. (Supports Figure 4)
(A) The ubiquitination of CPK28 by ATL31$^{C143H145A\Delta TM}$ was weaker than that by ATL31$^{\Delta TM}$. ATL$^{31C143H145A\Delta TM}$-MYC or $^{ATL31\Delta TM}$-MYC, AtUBA1 (E1), AtUBC8 (E2), His-FLAG-Ub and MBP-CPK28-HA were co-expressed in *E. coli*. The bacterial lysates were subjected to immunoblotting analysis with anti-HA antibodies to detect CPK28 ubiquitination, with anti-MYC antibodies to detect ATL31$^{C143H145A\Delta TM}$-MYC autoubiquitination, or with anti-FLAG antibodies to detect ubiquitin conjugates and free ubiquitin chains. (B and C) ATL31 does not ubiquitinate BIK1 or BAK1CD. ATL31$^{\Delta TM}$, AtUBA1, AtUBC8, His-FLAG-Ub and MBP-BIK1/BAK1CD-HA were co-expressed in *E. coli*. The bacterial lysates were subjected to immunoblotting analysis. BAK1CD, the cytosolic domain of BAK1.



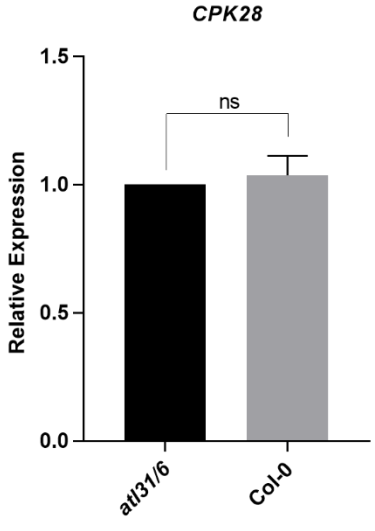
Supplemental Figure S14. Overexpressing *ATL31/6* reduces CPK28 protein accumulation in Arabidopsis protoplasts. (Supports Figure 5)

(A-C) Overexpressing *ATL31/6* but not *ATL2* reduces CPK28 protein accumulation. *CPK28-HA* was co-expressed with *ATLs-FLAG* in Arabidopsis protoplasts. *GFP-HA* was used as an internal transfection control. CPK28-HA proteins were detected by immunoblotting with anti-HA antibodies. (D) PUB25 does not affect CPK28 accumulation. *PUB25-FLAG* was co-expressed with *CPK28-HA* in protoplasts. CPK28-HA/PUB25-FLAG/GFP-HA proteins were detected by immunoblotting with the indicated antibodies.

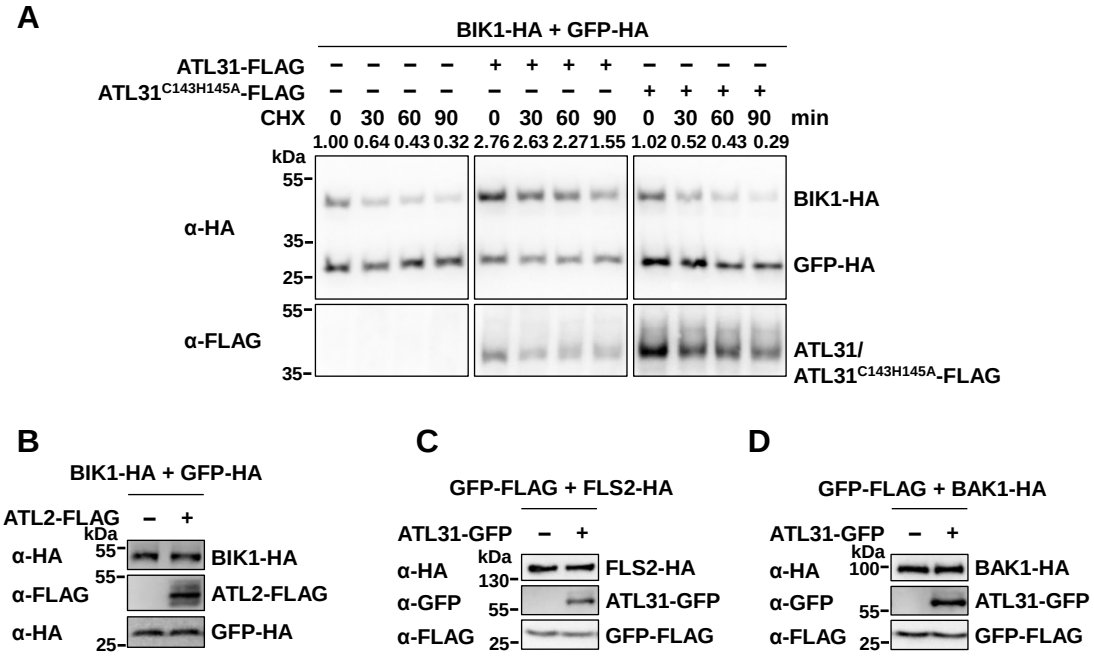


Supplemental Figure S15. **CPK28 protein accumulation in Col-0, *atl31 atl6*, and *bik1* plants. (Supports Figure 6)**

Total proteins were isolated from 7-day-old Col-0, *atl31 atl6*, and *bik1* seedlings. CPK28 proteins were detected by immunoblotting with anti-CPK28 antibodies. ACTIN was used as a loading control.

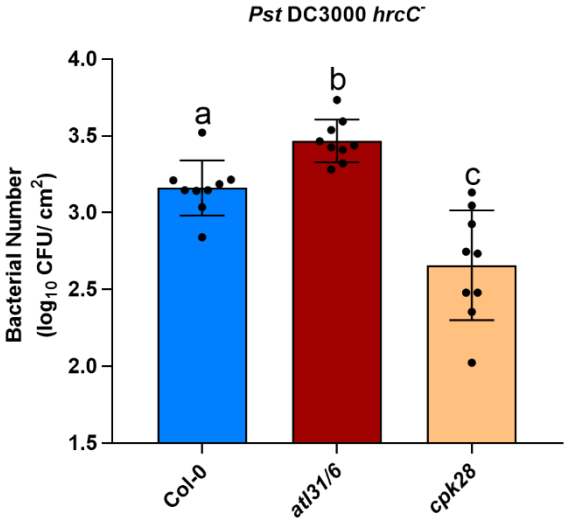


Supplemental Figure S16. *CPK28* transcript levels in *atl31 atl6* and Col-0. (Supports Figure 6)
Total RNA was extracted from 7-d-old *atl31 atl6* and Col-0 seedlings. *CPK28* mRNA levels were measured by RT-qPCR. The expression levels of *CPK28* were normalized to those of *GAPC*. Value is mean ± SD of three independent replicates using independent seedling samples grown under the same conditions. Statistical significance compared with Col-0 was determined by Student's *t* tests; ns, not significant (Supplemental Table S3).



Supplemental Figure S17. ATL31 specifically promotes BIK1 protein accumulation in protoplasts. (Supports Figure 7)

(A) ATL31-FLAG promotes BIK1-HA accumulation. BIK1-HA was co-expressed with ATL31-FLAG or ATL31^{C143H145A}-FLAG in protoplasts. GFP-HA was used as an internal transfection control. The protoplasts were treated with 50 μM CHX for the indicated times before harvesting. BIK1-HA proteins were detected by immunoblotting with anti-HA antibodies. The relative protein levels of BIK1-HA were normalized to those of GFP-HA. The relative protein level of BIK1-HA at the beginning of CHX treatment (in the absence of ATL31-FLAG or ATL31^{C143H145A}-FLAG and CHX 0) was set to 1. The density of BIK1-HA and GFP-HA protein bands was quantified using EvolutionCapt v18.10 software. (B) ATL2 does not affect BIK1 accumulation. BIK1-HA was co-expressed with ATL2-FLAG in protoplasts. GFP-HA was used as an internal transfection control. BIK1-HA proteins were detected by immunoblotting with anti-HA antibodies. (C and D) The accumulation of FLS2-HA and BAK1-HA is not affected by overexpressing *ATL31-GFP* in protoplasts. FLS2-HA or BAK1-HA was co-expressed with or without ATL31-GFP in Arabidopsis protoplasts. GFP-FLAG was used as an internal transfection control. FLS2-HA and BAK1-HA proteins were detected with anti-HA antibodies.



Supplemental Figure S18. Growth of *Pst* DC3000 *hrcC*⁻ in *atl31 atl6*, *cpk28-1*, and Col-0 plants. (Supports Figure 8)

Four-week-old *atl31 atl6*, *cpk28-1*, and Col-0 plants were infiltrated with *Pst* DC3000 *hrcC*⁻. The bacterial titer (represented as CFU/cm^2) was determined 3 d post inoculation. Individual data points are shown with means ± SD (n = 9 plants from three biological replicates using independent plant samples grown and inoculated under the same conditions). Different letters indicate significant differences with $P < 0.05$ (one-way ANOVA with Tukey's multiple comparisons test, Supplemental Table S4).

Supplemental Table S1. The differentially expressed ubiquitin ligase genes upon flg22 treatment.

Gene_id	log2FoldChange (flg22+/flg22-)	Gene name	Gene description
AT1G56250	8.8904	*VBF*	F-box protein VBF [Source:UniProtKB/Swiss-Prot;Acc:Q9C7K0]
AT5G37490	7.4595	*PUB21*	U-box domain-containing protein 21 [Source:UniProtKB/Swiss-Prot;Acc:Q5PNY6]
AT4G15975	7.2726	*ATL17*	RING/U-box superfamily protein [Source:TAIR;Acc:AT4G15975]
AT5G27420	6.955	*ATL31*	E3 ubiquitin-protein ligase ATL31 [Source:UniProtKB/Swiss-Prot;Acc:Q8LGA5]
AT3G18710	6.1499	*PUB29*	U-box domain-containing protein 29 [Source:UniProtKB/Swiss-Prot;Acc:Q9LSA6]
AT4G14365	5.9729	*XBAT34*	Putative E3 ubiquitin-protein ligase XBAT34 [Source:UniProtKB/Swiss-Prot;Acc:Q9FPH0]
AT5G09800	5.4157	*PUB28*	U-box domain-containing protein 28 [Source:UniProtKB/Swiss-Prot;Acc:Q9LXE3]
AT3G16720	4.9232	*ATL2*	RING-H2 finger protein ATL2 [Source:UniProtKB/Swiss-Prot;Acc:Q8L9T5]
AT5G64660	4.8441	*PUB27*	U-box domain-containing protein 27 [Source:UniProtKB/Swiss-Prot;Acc:Q9FLF4]
AT3G11840	4.8005	*PUB24*	plant U-box 24 [Source:TAIR;Acc:AT3G11840]
AT2G35930	4.7634	*PUB23*	E3 ubiquitin-protein ligase PUB23 [Source:UniProtKB/Swiss-Prot;Acc:Q84TG3]
AT4G35480	4.744	*ATL45*	RING-H2 finger protein ATL45 [Source:UniProtKB/Swiss-Prot;Acc:Q9ZT49]
AT5G66070	4.1965	*ATL27*	NEP1-interacting protein-like 1 [Source:UniProtKB/Swiss-Prot;Acc:Q9FKX5]
AT4G36550	4.1297	*PUB5*	U-box domain-containing protein 5 [Source:UniProtKB/Swiss-Prot;Acc:O23225]
AT5G43420	4.0169	*ATL16*	RING-H2 finger protein ATL16 [Source:UniProtKB/Swiss-Prot;Acc:Q9LSW9]
AT3G52450	3.8814	*PUB22*	E3 ubiquitin-protein ligase PUB22 [Source:UniProtKB/Swiss-Prot;Acc:Q9SVC6]
AT1G66160	3.8703	*PUB20*	U-box domain-containing protein 20 [Source:UniProtKB/Swiss-Prot;Acc:Q9C8D1]
AT1G20823	3.6774	*ATL80*	RING-H2 finger protein ATL80 [Source:UniProtKB/Swiss-Prot;Acc:Q9LM69]
AT5G61560	3.6737	*PUB51*	U-box domain-containing protein 51 [Source:UniProtKB/Swiss-Prot;Acc:Q9FKG5]
AT2G28830	3.1279	*PUB12*	PLANT U-BOX 12 [Source:TAIR;Acc:AT2G28830]
AT4G38940	2.9313	-	F-box/kelch-repeat protein At4g38940 [Source:UniProtKB/Swiss-Prot;Acc:Q9SVJ9]
AT2G42360	2.91	*ATL41*	E3 ubiquitin-protein ligase ATL41 [Source:UniProtKB/Swiss-Prot;Acc:Q9SLC3]
AT2G18670	2.7057	*ATL56*	RING-H2 finger protein ATL56 [Source:UniProtKB/Swiss-Prot;Acc:Q9ZV51]
AT3G47820	2.6812	*PUB39*	U-box domain-containing protein 39 [Source:UniProtKB/Swiss-Prot;Acc:Q9STT1]
AT5G67340	2.6322	*PUB2*	U-box domain-containing protein 2 [Source:UniProtKB/Swiss-Prot;Acc:Q5XEZ8]
AT3G05200	2.5888	*ATL6*	E3 ubiquitin-protein ligase ATL6 [Source:UniProtKB/Swiss-Prot;Acc:Q8RXX9]
AT1G29340	2.5534	*PUB17*	U-box domain-containing protein 17 [Source:UniProtKB/Swiss-Prot;Acc:Q9C7R6]
AT5G65920	2.4963	*PUB31*	U-box domain-containing protein 31 [Source:UniProtKB/Swiss-Prot;Acc:Q9FHN9]
AT4G14220	2.4171	*RHF1A*	E3 ubiquitin-protein ligase RHF1A [Source:UniProtKB/Swiss-Prot;Acc:Q4TU14]
AT4G30370	2.2359	*ATL14*	RING-H2 finger protein ATL14 [Source:UniProtKB/Swiss-Prot;Acc:Q9M0C3]
AT1G60190	2.2039	*PUB19*	U-box domain-containing protein 19 [Source:UniProtKB/Swiss-Prot;Acc:O80742]
AT2G35000	1.9332	*ATL9*	E3 ubiquitin-protein ligase ATL9 [Source:UniProtKB/Swiss-Prot;Acc:O64763]
AT5G67250	1.7937	*SKIP2*	F-box protein SKIP2 [Source:UniProtKB/Swiss-Prot;Acc:Q9FE83]
AT5G10380	1.7074	*ATL55*	E3 ubiquitin-protein ligase RING1 [Source:UniProtKB/Swiss-Prot;Acc:Q9LX93]
AT5G01830	1.5629	*PUB16*	U-box domain-containing protein 16 [Source:UniProtKB/Swiss-Prot;Acc:Q9LZW3]
AT3G19380	1.5596	*PUB25*	U-box domain-containing protein 25 [Source:UniProtKB/Swiss-Prot;Acc:Q9LT79]
AT1G67530	1.524	*PUB7*	U-box domain-containing protein 7 [Source:UniProtKB/Swiss-Prot;Acc:Q9CAG5]
AT4G09100	1.5181	*ATL39*	RING-H2 finger protein ATL39 [Source:UniProtKB/Swiss-Prot;Acc:Q9M0R7]
AT5G62560	1.5054	*PUB41*	U-box domain-containing protein 41 [Source:UniProtKB/Swiss-Prot;Acc:Q0WUF6]
AT2G46160	1.4834	*ATL67*	RING-H2 finger protein ATL67 [Source:UniProtKB/Swiss-Prot;Acc:O82353]

AT5G65200	1.4398	*PUB38*	U-box domain-containing protein 38 [Source:UniProtKB/Swiss-Prot;Acc:Q9FJP6]
AT2G42350	1.3867	*ATL40*	RING-H2 finger protein ATL40 [Source:UniProtKB/Swiss-Prot;Acc:Q9SLC4]
AT1G63090	1.3251	*PP2A11*	F-box protein PP2-A11 [Source:UniProtKB/Swiss-Prot;Acc:Q9CAN4]
AT3G49810	1.3167	*PUB30*	U-box domain-containing protein 30 [Source:UniProtKB/Swiss-Prot;Acc:Q058P4]
AT1G03770	1.2752	*RING1B*	Putative E3 ubiquitin-protein ligase RING1b [Source:UniProtKB/Swiss-Prot;Acc:Q0WX00]
AT1G10560	1.1997	*PUB18*	U-box domain-containing protein 18 [Source:UniProtKB/Swiss-Prot;Acc:Q9XIJ5]
AT4G03510	1.1163	*RMA1*	E3 ubiquitin-protein ligase RMA1 [Source:UniProtKB/Swiss-Prot;Acc:O64425]
AT1G22510	1.0918	-	RING/U-box protein with domain of unknown function (DUF 1232) [Source:TAIR;Acc:AT1G22510]
AT1G02860	1.0143	*BAH1*	E3 ubiquitin-protein ligase BAH1 [Source:UniProtKB/Swiss-Prot;Acc:Q9SRX9]
AT3G61590	-1.0213	*HWS*	F-box/kelch-repeat protein At3g61590 [Source:UniProtKB/Swiss-Prot;Acc:Q9M310]
AT3G08505	-1.09	*MKRN*	E3 ubiquitin-protein ligase makorin [Source:UniProtKB/Swiss-Prot;Acc:Q6IDS6]
AT1G23030	-1.1001	*PUB11*	U-box domain-containing protein 11 [Source:UniProtKB/Swiss-Prot;Acc:Q8GUG9]
AT5G25350	-1.1297	*EBF2*	EIN3-binding F-box protein 2 [Source:UniProtKB/Swiss-Prot;Acc:Q708Y0]
AT1G15100	-1.2141	*RHA2A*	E3 ubiquitin-protein ligase RHA2A [Source:UniProtKB/Swiss-Prot;Acc:Q9ZT50]
AT5G01880	-1.3185	*ATL74*	RING-H2 finger protein ATL74 [Source:UniProtKB/Swiss-Prot;Acc:Q9LZV8]
AT4G30400	-1.3623	*ATL13*	RING-H2 finger protein ATL13 [Source:UniProtKB/Swiss-Prot;Acc:Q940Q4]
AT4G21510	-1.3792	*SKIP27*	F-box protein SKIP27 [Source:UniProtKB/Swiss-Prot;Acc:O65416]
AT3G60220	-1.393	*ATL4*	E3 ubiquitin-protein ligase ATL4 [Source:UniProtKB/Swiss-Prot;Acc:Q9LY41]
AT5G47610	-1.4042	*ATL79*	RING-H2 finger protein ATL79 [Source:UniProtKB/Swiss-Prot;Acc:Q9FGJ6]
AT5G05810	-1.4827	*ATL43*	RING/U-box superfamily protein [Source:TAIR;Acc:AT5G05810]
AT3G11110	-1.5826	*ATL66*	RING-H2 finger protein ATL66 [Source:UniProtKB/Swiss-Prot;Acc:Q9SRM0]
AT5G17600	-1.6737	*ATL52*	RING-H2 finger protein ATL52 [Source:UniProtKB/Swiss-Prot;Acc:Q9LF64]
AT5G02750	-1.6958	*SGR9*	E3 ubiquitin-protein ligase SGR9, amyloplastic [Source:UniProtKB/Swiss-Prot;Acc:Q8GXF8]
AT4G28270	-1.7752	*RMA2*	E3 ubiquitin-protein ligase RMA2 [Source:UniProtKB/Swiss-Prot;Acc:P93030]
AT1G12820	-1.8288	*AFB3*	Protein AUXIN SIGNALING F-BOX 3 [Source:UniProtKB/Swiss-Prot;Acc:Q9LPW7]
AT3G12920	-1.9985	*BRG3*	Probable BOI-related E3 ubiquitin-protein ligase 3 [Source:UniProtKB/Swiss-Prot;Acc:Q9LDD1]
AT2G42620	-2.0627	*MAX2*	F-box protein MAX2 [Source:UniProtKB/Swiss-Prot;Acc:Q9SIM9]
AT1G79110	-2.3222	*BRG2*	Probable BOI-related E3 ubiquitin-protein ligase 2 [Source:UniProtKB/Swiss-Prot;Acc:F4IDI6]
AT3G59940	-2.5916	*SKIP20*	F-box/kelch-repeat protein SKIP20 [Source:UniProtKB/Swiss-Prot;Acc:Q9M1Y1]
AT1G78100	-2.8165	-	F-box protein At1g78100 [Source:UniProtKB/Swiss-Prot;Acc:Q9C9S2]
AT1G49200	-3.3542	*ATL75*	RING-H2 finger protein ATL75 [Source:UniProtKB/Swiss-Prot;Acc:Q94BY6]

Supplemental Table S2. Primers used for genotyping, cloning, and RT-qPCR.

Genotyping Primers	
Name	Sequence (5'→3')
atl31-LP	ACATCACCGAACACTAAACCG
atl31-RP	CTACTATTATCCGTGTCGGCG
atl6-LP	ATTGGAGGAACCTTCCTGTTG
atl6-RP	TCCTCGAACTCCCTCCTCTAG
cpk28-1-LP	ATCTTCTGCTTTTGCTCCTCC
cpk28-1-RP	GATGCAATTGCAATGGAAAAC
cpk28-3-LP	CAGTTCTATCCCAAAAAGGCC
cpk28-3-RP	TCCAGCCCTTACTAGGGTTTC
Molecular Cloning and Site-Directed Mutation Primers	
Name	Sequence (5'→3')
CPK28-F	GC**GGATCC**ATGGGTGTCTGTTTCTCCGCCA
*CPK28*_G2A-F	GC**GGATCC**ATGG**c**TGTCTGTTTCTCCGC
CPK28-R	AA**AGGCCT**TCGAAGATTCCTGTGACCTGCA
CPK28-RI-R	AA**AGGCCT**TGAAACTACGTCTCCCTCTGAT
CPK8-F	GC**GGATCC**ATGGGAAATTGTTGTGCGAGCC
CPK8-R	AA**AGGCCT**ATTTTCGCCTTCTAATTGCAAT
CPK6-F	GC**GGATCC**ATGGGCAATTCATGTCGTGGTT
CPK6-R	AA**AGGCCT**CACATCTCTCATGCTGATGTTT
CPK1-F	GC**GGATCC**ATGGGTAATACTTGTGTTGGAC
CPK1-R	AA**AGGCCT**GAGTTTAAGAGCAATGCTAAAG
CPK3-F	GC**GGATCC**ATGGGCCACAGACACAGCAAGT
CPK3-R	AA**AGGCCT**CATTCTGCGTCGGTTTGGCACC
CPK16-F	GC**GGATCC**ATGGGTCTCTGTTTCTCCTCCG
CPK16-R	AA**AGGCCT**GACCTTGCGAGAAATAAGATAA
BIK1-F	GC**GGATCC**ATGGGTTCTTGCTTCAGT
BIK1-R	AA**AGGCCT**CACAAGGTGCCTGCCAAA
ATL31-F	GC**GGATCC**ATGGATCCCATAAAACACATC
*ATL31*_△TM-F	GC**GGATCC**ATGCGTCACTGTACTGGC
A*TL31*-R	AA**AGGCCT**AACCGGTAGCCTAAGGGAACC
*ATL31*_C143H145A-F	CTGTTGCCTAAA**gct**GAT**gcc**GTGTTTCATCCT
*ATL31*_C143H145A-R	AGGATGAAACAC**ggc**ATC**agc**TTTAGGCAACAG
ATL6-F	GC**GGATCC**ATGAGAAGCTCCGATCATAT
ATL6-R	AA**AGGCCT**AACCGGTAATCTCACCGAAC
BIR2-F	GC**GGATCC**ATGAAAGAGATCGGCTCAAA
BIR2-R	AA**AGGCCT**CACTTTCTCGTTCTCTTGCG
BIR3-F	GC**GGATCC**ATGAAGAAGATCTTCATCAC
BIR3-R	AA**AGGCCT**AGCTTCTTGTTTGTTGAAGA
RCN1-F	GC**GGATCC**ATGGCTATGGTAGATGAACC
RCN1-R	AA**AGGCCT**GGATTGTGCTGCTGTGGAAC
PBL13-F	GC**GGATCC**ATGGTTTTGTGTTTCCAAGA
PBL13-R	AA**AGGCCT**GTACCGTTCCCCTCCGGCCT
FLS2-F	GC**GGATCC**ATGAAGTTACTCTCAAAGAC
FLS2-R	AA**AGGCCT**AACTTCTCGATCCTCGTTAC
BAK1-F	GC**GGATCC**ATGGAACGAAGATTAATGAT
BAK1-R	AA**AGGCCT**TCTTGGACCCGAGGGGTATT
PCRK1-F	GC**GGATCC**ATGAAGTGTTTCTTGTTCTC
PCRK1-R	AA**AGGCCT**ACAAGCTCTTATTGTCTTTG
BRI1-F	GC**GGATCC**ATGAAGACTTTTTCAAGCTT
BRI1-R	AA**AGGCCT**TAATTTTCCTTCAGGAACTT
ATL2-F	GC**GGATCC**ATGAACTCCAACGACCAGGATC
ATL2-R	AA**AGGCCT**CCTACTCTCTTCTCCTCCCCGT
ATL15-F	GC**GGATCC**ATGAACCCCAAAGGTAGAAC
ATL15-R	AA**AGGCCT**GACAGGGCTCGCATCGCCGG
ATL41-F	GC**GGATCC**ATGAGCTCCAACGACAGAGA
ATL41-R	AA**AGGCCT**ATGTCTCTCAAGATCTGCCA
ATL45-F	GC**GGATCC**ATGGCTCGCTGCGCTTGG
ATL45-R	AA**AGGCCT**AGGAAGAAAAGCAGGAAT
ATL80-F	GC**GGATCC**ATGGCTCGCCTTCTCTTTCG

ATL80-R	AA**AGGCCT**TGGCAAGAAGGAGTTGGGG
PUB2-F	GC**GGATCC**ATGATGGTACATATGGAGGT
PUB2-R	AA**AGGCCT**GCCTCTCCTCTGATTGCTTT
PUB12-F	GC**GGATCC**ATGCTAAGGATTTGCTTTCTT
PUB12-R	AA**AGGCCT**GATTAGGGAGATTTGATCTTC
PUB20-F	GC**GGATCC**ATGGGACTTTCATTGAGAGTG
PUB20-R	AA**AGGCCT**AAATGGTTTCTTAACATGTTT
PUB22-F	GC**GGATCC**ATGGATCAAGAGATAGAGATT
PUB22-R	AA**AGGCCT**AGCAGGATACGAATCATACAA
PUB24-F	GC**GGATCC**ATGGATCAAGAAGAGGAAGA
PUB24-R	AA**AGGCCT**GATCTTTGGCCCTTTGGGTG
PUB25-F	GC**GGATCC**ATGCCTAGGAATATAGAACCA
PUB25-R	AA**AGGCCT**AAAAGGGACCACTTGGCTGC
PUB27-F	GC**GGATCC**ATGAGGAAAGATGATCTCTGC
PUB27-R	AA**AGGCCT**AAACGGCATGATATGTGTCGT
PUB28-F	GC**GGATCC**ATGAGGAGCGATGATCTTTAC
PUB28-R	AA**AGGCCT**AAACGGCATAATATGTGTCGT
PUB29-F	GC**GGATCC**ATGGGGAGAGATGAAACAGAGACG
PUB29-R	AA**AGGCCT**AAAAGGCATAATATGAGTAGTCTTG
PUB39-F	GC**GGATCC**ATGGGCGACACCGGTAGACA
PUB39-R	AA**AGGCCT**AAACTGAGAGGAATAAGCAA
XBAT34-F	GC**GGATCC**ATGGGGCAACAACAATCACAG
XBAT34-R	AA**AGGCCT**AACATGATATAGCTTAATGAC
VBF-F	GC**GGATCC**ATGATGATGTTACCAGAAGCATGC
VBF-R	AA**AGGCCT**TGTTTTAGGCCTCACTTCAATACCA
RHF1A-F	GC**GGATCC**ATGTCTAATTTCACTTATAC
RHF1A-R	AA**AGGCCT**GCACGTTTCTCCTCCCTGCA

Quantitative Real Time PCR Primers

Name	Sequence (5'→3')	
ATL31-F	TTGTTTCTACTATTATCCGTGTCG	
ATL31-R	ACGCTCCTTTACCAATCTTCTG	
ATL6-F	CCAACAAATCAACCGTATAACT	
ATL6-R	AACTGAAACGTCGAGACCAC	
CPK28-F	GGAACTTCGAATGCACACGGGG	
CPK28-R	GCAGGGCTTGGTGCTCTCTGTG	
CPK28-RI-F	GCATCTCTGGGGGTATCAGA	
CPK28-RI-R	CACAACGGCTCATTATGAAA	(Dressano et al., 2020)
GAPC-F	TTGGTGACAACAGGTCAAGCA	
GAPC-R	AAACTTGTCGCTCAATGCAAT	(Czechowski et al., 2005)

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Dressano, K., Weckwerth, P.R., Poretsky, E., Takahashi, Y., Villarreal, C., Shen, Z., Schroeder, J.I., Briggs, S.P., and Huffaker, A. (2020). Dynamic regulation of Pep-induced immunity through post-translational control of defence transcript splicing. Nat Plants. **6:** 1008-1019.

Supplemental Table S3. Student's *t* test tables.

Figure Number	P-Value	T	df	One- or two-tailed P value?
Figure 8C Col-0 flg22+ vs. flg22-	<0.0001	10.71	286	Two-tailed
Figure 8C *atl31atl6* flg22+ vs. flg22-	0.1338	1.504	286	Two-tailed
Figure 8C *bik1* flg22+ vs. flg22-	0.3763	0.8861	286	Two-tailed
Figure 8D Col-0 ABA+ vs. ABA-	<0.0001	19.67	198	Two-tailed
Figure 8D *atl31atl6* ABA+ vs. ABA-	<0.0001	13.52	286	Two-tailed
Figure 8D *bik1* ABA+ vs. ABA-	<0.0001	15.31	286	Two-tailed
Figure 8E	<0.0001	5.027	22	Two-tailed
Supplemental Figure 16	0.4393	0.8579	4	Two-tailed

Supplemental Table S4. ANOVA tables.

Sum Sq = Sum of squares; df = degrees of freedom; Mean Sq = Mean Squares

Figure Number		df	Sum Sq	Mean Sq	F-Value	P-Value
Supplemental Figure 8A	Treatment	7	1213	173.3	64.22	P<0.0001
	Residual	16	43.18	2.699		
	Total	23	1256			
Supplemental Figure 8B	Treatment	7	3477	496.7	100.6	P<0.0001
	Residual	16	79	4.938		
	Total	23	3556			
Supplemental Figure 8C	Treatment	7	11870	1696	51.48	P<0.0001
	Residual	16	527.1	32.94		
	Total	23	12397			
Supplemental Figure 18	Treatment	2	3.006	1.503	25.21	P<0.0001
	Residual	24	1.431	0.05963		
	Total	26	4.437			