Supplementary Information:

Article title: Ubiquitylation of PHYTOSULFOKINE RECEPTOR 1 modulates defense response in tomato

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The following supporting data are available for this article:

Supplemental Figure S1 Phylogenetic analysis of partial PUBs from tomato and Arabidopsis

Supplemental Figure S2 Effect of PSK application on PSKR1-PUB14 interaction

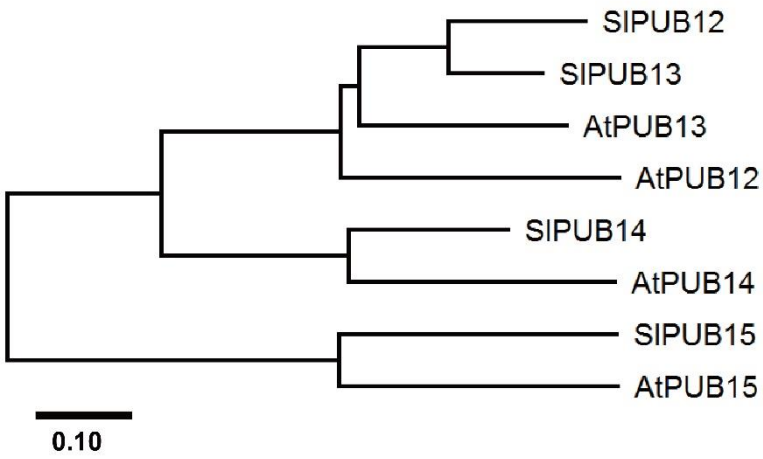
Supplemental Figure S3 Effects of *B. cinerea* inoculation on the transcript expression of *PUBs* in tomato plants

Supplemental Figure S4 Gene silence efficiency of VIGS tomato plants

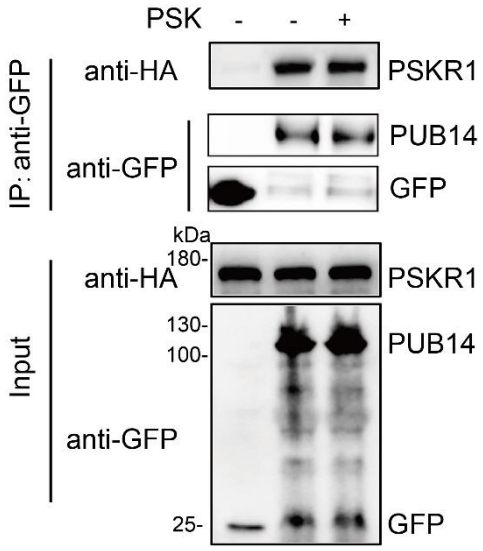
Supplemental Figure S5 PUB12 and PUB13 have the E3 ligase enzyme activity

Supplemental Figure S6 Schematic diagram showing Lys-748 and Lys-905 ubiquitination sites of PSKR1 by mass spectrum

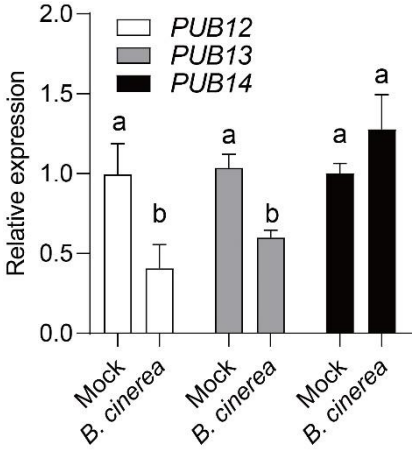
Supplemental Figure S1 Primers used in this study



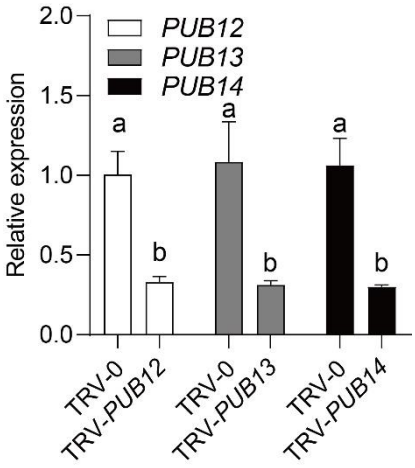
Supplemental Figure S1. Phylogenetic analysis of partial PUBs from tomato and Arabidopsis. The amino acid sequences of PUB12/13/14/15 were used for generating the phylogenetic tree by the neighbor-joining method using MEGAX program with 1000 bootstrap trails.



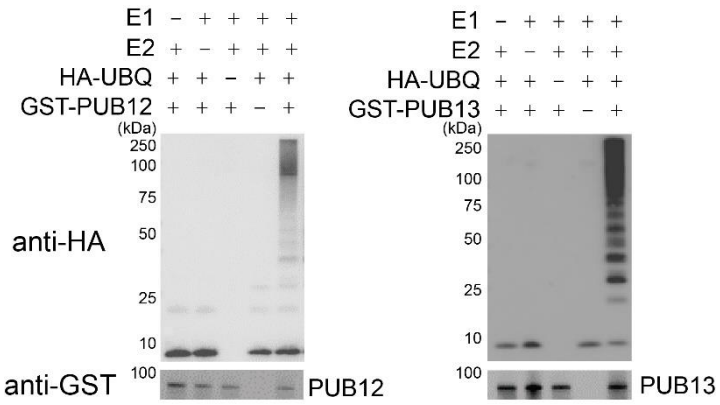
Supplemental Figure S2. Effect of PSK application on PSKR1-PUB14 interaction. 5-week-old fully expanded *N. benthamiana* leaves were co-transfected with PSKR1-HA and SlPUB14-GFP, or GFP control. After 48 h of inoculation, half of the leaves were infiltrated with 10 μM PSK, and the other parts were infiltrated with dH_2O control for 1 h before the sample collection for CoIP. The associations were detected by anti-HA and anti-GFP immunoblots. The protein levels before IP were detected by anti-HA immunoblot and anti-GFP antibody, respectively.



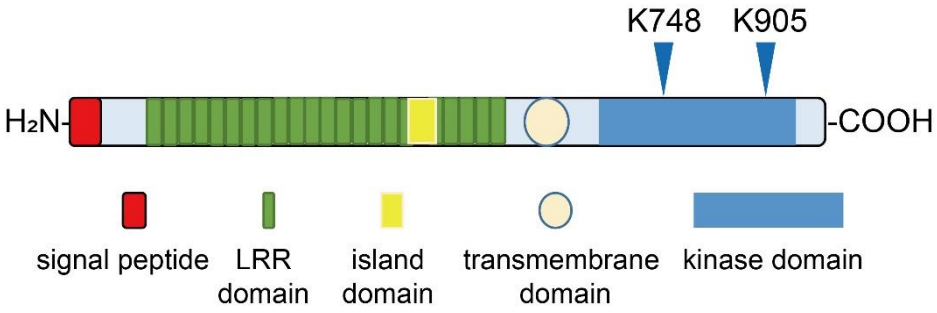
Supplemental Figure S3. Effects of *B. cinerea* inoculation on the transcript expression of *PUBs* in tomato plants. Five-week-old tomato plants were inoculated with *B. cinerea*, and the leaf samples were collected at 1 day post inoculation for RT-qPCR analysis. The transcript abundance of each gene under mock treatment was defined as 1. Data are presented as the means of three biological replicates (± SD, n=3), and different letters indicate significant differences ($P < 0.05$) according to Tukey's test.



Supplemental Figure S4. Gene silence efficiency of VIGS tomato plants. Approximately 4 weeks after the infiltration of Agrobacterium suspension carrying VIGS vectors, each plant was sampled from the uppermost 1 to 2 fully expanded leaf for gene silencing efficiency analysis. The transcript abundance of each gene in TRV-0 plants was defined as 1. Data are presented as the means of three biological replicates (± SD, n=3), and different letters indicate significant differences ($P < 0.05$) according to Tukey's test.



Supplemental Figure S5. PUB12 and PUB13 have the E3 ligase enzyme activity. Ubiquitinated forms of GST-fusion proteins were evaluated in the presence of E1, E2, and ubiquitin. The reactions were analyzed by immunoblots using anti-HA antibody and anti-GST antibody.



Supplemental Figure S6. Schematic diagram showing Lys-748 and Lys-905 ubiquitination sites of PSKR1 by MS.

Supplemental Table S1. Primers used in this study

Gene	Accession No.	Primer pairs	Assay
PUB12	Solyc11g066040	F:5'- ACAGTGGTGTGAAGCGAATG -3' R:5'- GGCTGCCAGATTTGAGCTTT -3'	qPCR
		F:5'- CGgaattcTGATGTCCTAAGGAGGTT -3' R:5'- CGggatccAATAGGGCAACGAAAA -3'	pTRV2
		F:5'- GgaattcATGGGAGAAGAAGATAAAGGAGAA -3' R:5'- ACGCgtcgacACTCTCAATGGCATTAGTT -3'	pGEX-4T-1
		F:5'- ggggacaagtttgtacaaaaaagcaggctttATGGGAGAAGAAGATAAAGG -3' R:5'-ggggaccactttgtacaagaaagctgggtcACTCTCAATGGCATTAGT -3'	pDONR-Zeo
		F:5'- CCCttaattaaCATGGGAGAAGAAGATAAAGG -3' R:5'- GGGactagtACTCTCAATGGCATTAGTTG -3'	p2YC
PUB13	Solyc06g076040	F:5'- TATGTGTTGCGGAGCCTCAT -3' R:5'- TGCATGCGGATGCTGATTTA -3'	qPCR
		F:5'- CGgaattcGAGCCAAAGGAAGGGTT -3' R:5'- CGggatccCCGCTACAAGTTGAAGAC -3'	pTRV2
		F:5'- GgaattcATGGAAGAAGGAAGAGGAG -3' R:5'- CCGctcgagTCAGCATTCCAGGACATTTG -3'	pGEX-4T-1
		F:5'- ggggacaagtttgtacaaaaaagcaggctttATGGAAGAAGGAAGAGGA -3' R:5'- ggggaccactttgtacaagaaagctgggtcGCATTCCAGGACATTTGTCGA -3'	pDONR-Zeo
		F:5'- CCCttaattaaCATGGAAGAAGGAAGAGGAG -3' R:5'- GGGactagtGCATTCCAGGACATTTGTCG -3'	p2YC
		F:5'- AggcgcgccATGGAAGAAGGAAGAGGAG -3' R:5'- GGggtaccGCATTCCAGGACATTTGTCG -3'	pAC004

PUB14	Solyc11g008390	F:5’- TGATGGATGAAGCACTTGCG -3’ R:5’- TCGGTTACGAGGAGAACCTG -3’	qPCR
		F:5’- GgaattcATGGGTCATCAGAAAGA -3’ R:5’- CGggatccATTGTAAGGAATGTGGC -3’	pTRV2
		F:5’- GgaattcATGGGTCATCAGAAAGAGGA -3’ R:5’- CctcgagTGATTCAACTGGATCGACTC -3’	pGEX-4T-1
		F:5’- ggggacaagtttgtacaaaaaagcaggctttATGGGTCATCAGAAAGAGG -3’ R:5’- ggggaccactttgtacaagaaagctgggtcTGATTCAACTGGATCGAC -3’	pDONR-Zeo
		F:5’- atttacgaacgatagttaattaaCATGGGTCATCAGAAAGAGGAATTAA -3’ R:5’- actgccacctcctccactagtTGATTCAACTGGATCGACTCGTT -3’	p2YC
PUB15	Solyc04g082440	F:5’- atttacgaacgatagttaattaaCATGGTATCAGCTGGGGAAATAGAA -3’ R:5’- actgccacctcctccactagtATATGGGATCTGCTCAGTTTTGG -3’	p2YC
		F:5’- ccgcgtggatccccggaattcATGGTATCAGCTGGGGAAATAGAA -3’ R:5’- gtcacgatgcggccgctcgagATATGGGATCTGCTCAGTTTTGG -3’	pGEX-4T-1
PSKR1	Solyc01g008140	F:5’- cagcaaatgggtcgcggatccCGGGCAAGCAGTCGAAAA -3’ R:5’- tgcggccgcaagcttgtcgacTCAAATAACATTTCCTTTGCGTGCTG -3’	pET-28a
		F:5’- TGCtctagaATGCGGGCAAGCAGTCGAAAAGT -3’ R:5’- AActgcagCACATGAACATCAGGTGGT -3’	pMAL-c2x
		F:5’- ggggacaagtttgtacaaaaaagcaggctttATGGGTGTGTTGCAAGTTTG -3’ R:5’- ggggaccactttgtacaagaaagctgggtcCTAAAACACATGAACATCAGGTGG -3’	pDONR-Zeo
		F:5’- TTggcgcgccATGGTGATTTGGGAGTTTCT -3’ R:5’- CGGggtaccCCTCTCCTTTACACTTGCGATTG -3’	pAC004
		F:5’- CCCttaattaaCATGGGTGTGTTGCAAGTTTG -3’ R:5’- GGGactagtAAACACATGAACATCAGGTG -3’	p2YN
		F:5’- AGGGTCTCTATTGaacaaagcaccagtggtct -3’; R:5’- AGGGTCTCTAAACTGGGTTAGTTCCTGATTATCtgcaccagccgggaatcg -3’	pHEE401 (for CRISPR）

		F:5’- GGAAACATTCCGGATGTGTT -3’ R:5’- AGGCTAGAATCAGTAGGCAA -3’	*pskr1* mutant identification
ACTIN	Solyc03g078400	F: 5’-TGGTCGGAATGGGACAGAAG -3’ R: 5’-CTCAGTCAGGAGAACAGGGT -3’	qPCR
BcACTIN	XM_001553318.1	F: 5’-GGTAACATTGTTATGTCTGG -3’ R: 5’-CTTGACCTTCATCGACG -3’	qPCR
UBA(E1)	Solyc09g018450	F:5’- ACGCgtcgacAAATGCTTCCTGTGAAGAGGTC -3’ R:5’- CCGctcgagCTACCTGAAATAAATAGAGAC -3’	pET-28a
UBC8(E2)	Solyc12g056100	F:5’- CGggatccATGGCATCCAAGCGGATTCTCA -3’ R:5’- ACGCgtcgacCTATCCCATGGCAAATTTTTG -3’	pET-28a
UBQ	Solyc01g096290	F:5’- accatggggcgcgccggtaccATGCAGATCTTCGTGAAAACTCTTAC -3’ R:5’- gtccttatagtccatggatccCTTGATCTTCTTCTTTGGCCTCA -3’	pAC007
MRN1	Solyc12g006530	F:5’- GGCGGTTTAGCAGCATGGGA -3’ R:5’- TGCATTCGACGTACTCGTGCT -3’	qPCR
RLKR	Solyc02g079990	F:5’- AGGACCTAGGTGCAACTTCCGT -3’ R:5’- TCCGACCTGGTGGTGGAGAA -3’	qPCR
SAG12	Solyc02g076910	F:5’- ACCGGCCAACAACGAGAAGG -3’ R:5’- CCCATCGATTGCCACCGACA -3’	qPCR
PAD3	Solyc09g092600	F:5’- TCGTGGGCTATTGCAAGGGA -3’ R:5’- GATCCACCGTCGCAACACCA -3’	qPCR