

 $\overline{0.1}$

Supplemental Figure S1. Phylogeny of the human and tomato UBC domaincontaining proteins. Phylogenetic analysis of the human and tomato UBC domaincontaining proteins was performed using the same method and bootstrap trials as described for Figure 1. The phylogenetic tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Accession numbers of the tomato and human UBC domain-containing proteins are shown in the Supplemental Table I.

Supplemental Figure S2. Schematic representation of domain organization in the tomato UBC domain-containing proteins. The chromosomal loci and given gene names encoding the UBC domain-containing proteins are shown on the left side. The UBC fold and the extension of the UBC domain-containing proteins are represented as dark ellipse and light-gray line, respectively, both of which are drawn in scale to their length in the number of amino acids. The UBA domain of Sl-UBC27 is indicated as a dark-gray rectangle. The scale bar represents length of protein in amino acids.

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Supplemental Figure S3. The tomato ubiquitin E2 enzymes are classified into thirteen subgroups. Numbering of the groups was based on both the phylogenetic analysis of tomato ubiquitin E2s and the previously reported classification of Arabidopsis ubiquitin E2s (Zhao et al., 2013). Phylogenetic analysis of the forty tomato ubiquitin E2s using the amino acid sequence of core UBC domain was performed using the same method and bootstrap trials as described for Figure 1. The Roman numerals designate the different E2 groups.

Supplemental Figure S4. Purified tomato E2 proteins as shown by SDS-PAGE. Approximately 3 µg of thirty-five purified E2 proteins were separated by 10% SDS-PAGE and stained with Coomassie Brilliant Blue. The Roman numerals designate the group into which the E2s are classified. M represents the molecular weight markers. The numbers on the right denote the molecular mass of marker proteins in kD. The asterisks denote the band of corresponding purified E2 proteins. Except for UBC12, 14 and 36 that are 6xHis-tagged, all E2s are fused to GST.

 α -FLAG

UBC 4 5 6 16 - - 4 5 6 16 **DTT** $\ddot{}$ $\ddot{}$ ÷ ÷. L. \blacksquare $\mathsf{k}\mathsf{D}$ -80 -60 -50 -40 -30
 -25 Free Ub \rightarrow α -FLAG

Supplemental Figure S5. Examination of the ubiquitin-conjugating activity of tomato E2s by thioester formation assay. (**A**) Anti-FLAG Western blots were performed following thioester assay of different tomato ubiquitin E2s. The reactions of the assay were terminated by adding SDS sample loading buffer in the presence of 100 mM DTT (DTT +) or 4M Urea (DTT $-$). The formation of DTT-sensitive ubiquitin adducts by tomato E2s is shown as charged E2. The numbers on the right denote the molecular mass of marker proteins in kD. The experiment was repeated two times with similar results. (**B**) Tomato E2 mutants in which the cysteine residue at the active site mutated lost ubiquitin-conjugating activity. Thioester assay was performed using wild type tomato ubiquitin E2s and E2 mutants. The reactions of the assay were terminated by adding SDS sample loading buffer in the presence of 4M Urea (DTT–). The numbers on the right denote the molecular mass of marker proteins in kD. The experiment was repeated two times with similar results.

Supplemental Figure S6. AvrPtoB shows no specificity towards the tomato ubiquitin E2 enzymes Sl-UBC16, and 17. (**A**) AvrPtoB shows no specificity towards the tomato E2s Sl-UBC16 and 17 in *in vitro* ubiquitination assay. The E2s Sl-UBC16 and 17 demonstrated auto-ubiquitin-conjugation activity in the absence of an E3 ligase (lane 3 and 5). The presence of AvrPtoB enhanced their conjugation activity but did not alter the pattern of conjugates formed (lane 4 and 6). Sl-UBC28 was included as a control. The numbers on the top mark the lanes/reactions. (**B**) The enhancement of the conjugation activity of Sl-UBC16 by AvrPtoB is non-specific. The presence of a non-E3 ligase protein, GST-Fen also enhanced the auto-ubiquitin-conjugation activity of Sl-UBC16 (lane 5, as compared to lane 2), which is comparable to the effect of AvrPtoB (lane 1). In the absence of Sl-UBC16, no ubiquitin conjugation was observed (lane 3). The numbers on the right denote the molecular mass of marker proteins in kD.

Supplemental Figure S7. AvrPtoB interacts with tomato group III E2 members but not with E2s from other groups in BiFC assays. (**A**) Members of the group III E2s interact with AvrPtoB in *N. benthamiana* protoplasts. (**B**) Members of group VII, X, and XI tomato E2 did not interact with AvrPtoB in the BiFC assay. Tomato ubiquitin E1 enzyme UBA1 was used as positive control. Different construct pairs were transiently coexpressed in protoplasts isolated from *N. benthamiana* leaves. Cells were viewed with a confocal microscope under bright or laser light to detect cells and green fluorescence, respectively. The empty vector expressing N- and C-terminus of YFP (nYFP-EV and cYFP-EV) were used as negative control. EV, empty vector; FL, fluorescence; Chl., chlorophyll autofluorescence; Bright, bright field image. Scale bar = $20 \mu m$.

Supplemental Figure S8. Members of Group III E2 interacted with AvrPtoB in coimmunoprecipitation (Co-IP) assay. Group III members Ubc29 and 30 were randomly selected for the assay. AvrPtoB-HA and 10Myc-tagged E2s were transiently co-expressed in *N. benthamiana* leaves. The Co-IP was carried out with an anti-HA antibody (IP: anti-HA). The presence of corresponding proteins (Top panel) and the input (bottom panel) were detected by Western blot using anti-HA antibody for AvrPtoB-HA and anti-Myc antibody for 10Myc-tagged E2s.

UBC38 UBC39 UBC40 UBC16 UBC17

 $\overline{0.02}$

B

TRV TRV -*group III*

Supplemental Figure S9. Knocking down group III E2 genes in *N. benthamiana* **by Virus-Induced Gene Silencing (VIGS).** (**A**) The *N. benthamiana* group III E2 genes are highly homologous to their counterparts in tomato. The DNA sequences of *N. benthamiana* and tomato group III E2 genes' open reading frame (ORF) were used to generate the phylogenies. The same method and bootstrap trials as described for Figure 1 were employed for the phylogenetic analysis. The sequence IDs of the *N. benthamiana* ubiquitin E2 genes identified from the Sol Genomics Network database (SGN; http://solgenomics.net/) are shown in Supplemental Table I. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. (**B**) Phenotypes of the *N. benthamiana* plants in which the group III E2 genes are silenced. The non-silenced TRV empty-vector (TRV) was used as a control. Photographs were taken 4 weeks after the approximately 3-week old seedlings were infiltrated with TRV or TRV-*group III* E2 genes constructs. The upper panel shows the top view of the plants while the lower panel shows the side view. (**C**) The group III ubiquitin E2 genes are efficiently and specifically silenced in *N. benthamiana* using the TRV vector. The transcript level of group III E2 genes and closely related E2 genes Nb*-UBC16* and *17* (outside the group III) in non-silenced TRV control (TRV) and group III ubiquitin E2 genes-silenced (TRV-*group III*) *N. benthamiana* plants was examined as described (Mural et al., 2013). Nb*-EF1α* was used as an internal reference for the determination of the amount of cDNA template to be used. Experiments in (**B**) and (**C**) were repeated at least two times with similar results.

Supplemental Fig S10AAAGAGC
AACGAGC TCAAGGATCTCCAGAAAGATCCTCCTACCTCTTGCAG
TTAAGGATCTCCAGAAAGATCCTCCTACCTCTTGCAG CATTGGC
CATTGGC 100 **Nb-***UBC8* **Sl-***UBC8* CCAGTT
CCAGTA GC T GA A G A <mark>C</mark> A T G T T <mark>T</mark>
GC T GA A G A <mark>T</mark> A T G T T C 100 GCCTGACAGC
AGCTGACAGT 200 **Nb-***UBC8* **Sl-***UBC8* CCTTATGCTGG
CCCTAT<mark>T</mark>CTGG AGGT 200 **Nb-***UBC8* AGGA: 300 CAGCAATGG
TAGCAATGG AGCATTTGCCT
AGCATTTGCCT ATCTCC
ATCTCC **Sl-***UBC8* TTGAAGGAACACTGGAG 300 AGCTTTCAGGACAAAGGTT **Nb-***UBC8* AGGTATTGCTGTCAATCTGTT
AGGTACTGCTCTCTATCTGTT 400 \overline{G} **Sl-***UBC8* 400 ACAACTGCCCGGAGCTGGACTCAAAA<mark>GTA</mark>TGCCATGGG<mark>T</mark>TAG
ACAACTGCCCGGAGCTGGACTCAAAA<mark>ATT</mark>TGCCATGGGATAG 447 **Nb-***UBC8* **Sl-***UBC8* 447 **Nb-***UBC9* 100 A TGGCATCCAAGAGAATTC TGAAAGAGCTCAAGGATCTIGCAGAAAGATCCTCCCACCTCTTGTAGCGCTGGCCCAGTTGCTGAAGATATGTTTCACTGGC
A TGGCATCCAAGAGCATTCTGAAAGAGCTCAAGGATCTCCAGAAAGATCCTCCTACCTCTTGCAGTGCTGGCCCAGTTGCCGAAGATATGTTTCACTGCC **Sl-***UBC9* 100 200 **Nb-***UBC9* AAGCCACACTTATGGGTCCATCTGACAGTCC **Sl-***UBC9* **TTATGCTGG** GGAGTG **ATCCATTTAAGCCTCCAAAGGT** 200 **Nb-***UBC9* **AAACA AAGGAACA** GGAGCC 300 ATATCI
ATTTCC TTTCACCCAAACATCAACAGCAATGGCAGCATTTGCCTCGACATTCTGAAGGAACAATGGAGCCCGGCACTT **Sl-***UBC9* AGCTTTTAGGACAAAGGTT 300 AAGGTG<mark>TTGCTGTC</mark>CATCTG<mark>TTCTCT</mark>
AAGGTGCTGCTGTCAATCTGCTCTCT L<mark>i</mark>ctaacagaccc<mark>a</mark>aatccTgatgatcctttggtgcc
sctaacagacccTaatccCgatgatcctttggtgcc $G \Delta$ CΔ. **ACAAGAC** GATAAAAGCAAG 400 **Nb-***UBC9* **Sl-***UBC9* GAGATTGCTCATATGTACAAGACTGATAAAAGCAAGT 400 ACGAAGC **Nb-***UBC9* TGCTCGGAGCTGGAC CAAAA
CAAAA GGGT 447 A<mark>TATGCCATGGGTTAG</mark>
BTATGCAATGGGTTAG SI-UBC9 ACGAAGCAACTGCTCGGAGCTGGAC 447 100 **Nb-***UBC10* A TGGC TTCGAAACGAATATTGAAGGAGC TGAAGGA TCTCCAAAAGGA TCCTCC TACCTCCTGC TGC TGGCTGG ICCTGTTGGAGAGGACA TGTTTCACTGGC
A TGGC TTCGAAACGAATATTGAAGGAGC TGAAGGA TCTCCA<mark>G</mark>AAGGA TCCTC CTAC ITCATGCAGCGC TGGCC CTGTTGGAGAGGACA TGTTTCACT **Sl-***UBC10* 100 200 **Nb-***UBC10* AGGCTACAATAATGGGGCCCTCTGATAGCCCTTATGCTGGGGGTGTTTTTTTG **Sl-***UBC10* GTCACGATCCATTTTCCTCCGGATTATCCATTCAAGCCTCCTAAGGT 200 **Nb-***UBC10* GAAGGAGCAGTGGAGCC 300 **Sl-***UBC10* TGCTTTTAGGACAAAAGTTTTCCATCCAAATATCAA<mark>T</mark>AG<mark>C</mark>AATGGGAGTATATGCTTGGACATACTGAAGGAGCAGTGGAGCCCTGCATTAACTATTTCC 300 CAAT GACAAG AAGGTTTTGCTTTCAAT<mark>C</mark>TGCTCACTTTTGACGGA<mark>T</mark>CCAAACCCTGATGACCCC<mark>C</mark>T
AAGGTTTTGCTTTCAATTTGCTCACTTTTGACGGACCCAAACCCTGACGACCCCTT CTGAGATTGCTCACATGTACAAGAC 400 **Nb-***UBC10* STTCCTGAGATTGCTCACATGTACAAGACCGACAAGGCCAAAT
GTTCCTGAGATTGCTCACATGTACAAGAC<mark>T</mark>GACAAG<mark>T</mark>CCAAAT **Sl-***UBC10* 400 .
GAAGCAACCGCCAGGAGTTGGACCCAGAAGTACGCCATGGG<mark>CTAA</mark>
GAAGGAACCGCCAGGAGTTGGACCCAGAAGTATGCCATGGGTTAA 447 **Nb-***UBC10* A
A

CAAGGAACTAAGGGAGTTGCAAAGAGACCCTCCTACT ATGGCATCCAGGAGAATTCA
ATGGCATCAAGGAGAATTCA **TCATG** AGTGCAGGTCC CAGGATAT
CAGGATAT 100 **Nb-***UBC11* $TGGC$ ATTGGC
ATTGGC AAGGAACTAAGGGAGTTGCAAAGAGACCCTCCTACTTCATG **GTGGC Sl-***UBC11* 100 **Nb-***UBC11* AAGC
AAGC CAGCCCTTA<mark>TGCAGGTGGTGTTTT</mark>
TAGCCCTTTTGCAGGTGGTGTTTT **CETGA**
CETGA AAGGT
AAGGT 200 TACCCTTTCAAACC
TACCCTTTCAAACC GIGGCCAICCAIII
GTGGCCATCCATTT TACCATTAT AAATGAL **Sl-***UBC11* 200 **Nb-***UBC11* CATCCAAATATAAATAATAATGGAAATATTTGTTTGGACATTCTTAA<mark>G</mark>GATCAATGGAGTCCTGC
CATCCAAATATAAATAATAATGGAAATATTTGTTTGGACATTCTTAA<mark>A</mark>GATCAATGGAGTCCTGC 300 **Sl-***UBC11* GGCTTTCAAGACCAAAGT 300 GAGGTTTTGCT<mark>TTCCATATGTTCACTACTACAGATCCAAATCCAGAT</mark>GATCCATTGGT<mark>T</mark>CCAGAAAT
AAGGTTTTGCTATCGATATGTTCACTACTAACAGATCCAAATCCAGACGATCCATTGGTACCAGAAAT **AGAAAT** 400 **Nb-***UBC11* CTGATCGG
CTGATAGG 400 **Sl-***UBC11* **Nb-***UBC11* A TGAATCA <mark>A</mark> TGGC TCG TA A TTGGA CCCAAAA G<mark>IT</mark> TGC TA TGAA TTGA
A TGAATCA G TGGC TCG TA G TTGGA CCCAAAA A TA TGC TA TGAA TTGA 447 447 **Sl-***UBC11*

447

Sl-*UBC10*

CGAAG

ATGGCTTCAAAGAGGATTCAGAAGGAA<mark>C</mark>TGAAGGACTTGCAGAAAGACCCCCCTGCTTC
ATGGCTTCAAAGAGGATTCAGAAGGAA<mark>T</mark>TGAAGGACTTGCAGAAAGACCCCCCTGCTTC TTGCAGTGCAGG
CTGCAGTGCAGG 100 **Nb-***UBC12* AGGATATGTTCCAC<mark>TGGC</mark>
AGGATATGTTCCATTGGC **Sl-***UBC12* 100 **Nb-***UBC12* TATGGGTCCATCTGACAG<mark>C</mark>
AATGGGTCCATCTGACAGT 200 CCATTTTCTGGGGGTGTTTTCCTTGTGTC
CCGTTTTCTGGGGGTGTTTTCCTTGCATC ATTCAAGCCCCCAAAGGT
<mark>T</mark>TTCAAGCCCCCAAAGGT **Sl-***UBC12* 200 **Nb-***UBC12* SACATCTTAAAAGAACAATGGAGCCCTGC
GATATCCTAAAGGAACAATGGAGCCCTGC 300 C C A A A C A T C A A C A G T A A T G G T A G T A T T T G T C T <mark>G</mark>
C C A A A C A T C A A C A G T A A T G G T A G T A T T T G T C T T $CTCT$ CCITACIGIAICC
TCTTACTGTATCC **Sl-***UBC12* 300 400 **Nb-***UBC12* CATTITGCTCCTTGCTTACTGATCCCAATCCAGATGATCCCTTAGTGCCAGAGATTGCTCACATGTACAAGACTGATAGAGTGAAGAT
TATTTGCTCTTTGCTTACTGATCCCAATCCAGATGATCCTTTAGTGCCAGAGATTGCTCACATGTACAAGACGGATAGACCGAAGT AAGGTGCTGCTTTC 400 **Sl-***UBC12* ATGAGAGTACTGC<mark>T</mark>AGATCTTGGACCCAGAAATA<mark>TGCC</mark>ATGGGTTAG
ATGAGAGTACTGCCAGATCATGGACCCAGAAATACGCAATGGGTTGA 447 **Nb-***UBC12* **Sl-***UBC12* 447

A TGGCTTCGAA ACGGA TA TTGA AGGA GCTTA AGGA TCT
A TGGCTTC TAAGCGGA TA TTGA AGGA GCTTA AGGA OCT CCAGAAAGATCCTCCTACCTCTTG
TCAGAAAGATCCTCCTACTTCTTG **GAGAGGACATGT** 100 **Nb-***UBC28* AG
AG GCC
GCI GGCCCCGTCGGAGAGGACATGTTTCACTGGC 100 **Sl-***UBC28* 200 **Nb-***UBC28* AAGCIACAA IAA IGGGCCC I CCAGA IAGUCCC I ALAUCGGGGG I GI A I I I I I IAG I CA I I I I I I I I CC I CA I I A I C
AAGCTACAA TAATGGGCCCTCCAGATAG TCCCTATGG TGGGGG TG TA TTTITAGTCACTAT TCA I TTICCTCCTGATTA TCCATTTAAACCTCCTAAGGT **Sl-***UBC28* 200 **Nb-***UBC28* **ACCACAAAACT TAATAGCAATGG** CCACAT CAACCACCAATCCACCCO **TCCAAAT** 300 AGTATATGCTTGGACATATTGAAGGAGCAATGGAGCCCTGCCTTGACTATTTCC **Sl-***UBC28* TGCTTTTAGGACAAAAGTTTTCCATCCAAATATTAATAGCAATGG 300 GACAGGGCAAAAT **Nb-***UBC28* CATATGTACAAGAC **GATGA
GATGA** $\frac{1}{100}$ **GAGATT** 400 CTTGGTGCCTGAGATTGCTCATATGTACAAGAC
TTTGGTGCCTGAGATTGCTCATATGTACAAGAC **Sl-***UBC28* AAGGTTTTGCTTTCA TTGACGGATCCAAA **GACAGGGCAAAAT** 400 **Nb-***UBC28* SCCCGGAGTTGGACCCAGAAGTATG<mark>C</mark>
SCCCGGAGTTGGACCCAGAAGTATGC C<mark>ATGGGTT</mark>
TATGGGTT 447 **Sl-***UBC28* 447

Nb-*UBC40* **Sl-***UBC40*

Nb-*UBC40* **Sl-***UBC40*

 $\frac{447}{447}$

Supplemental Figure S10. Homologs of the group III E2 genes from *N. benthamiana* **share high nucleotide sequence identity to their counterpart of tomato.** The group III E2 genes from *N. benthamiana* were searched using the BLAST algorithm (Camacho et al., 2009) against the Sol Genomics Network (SGN, http://solgenomics.net) database with the counterparts from tomato as the queries. The Clustal X algorithm (Larkin et al., 2007) was used for the sequence alignment. The sequences underlined red were the fragments used for building the TRV*-group III* VIGS construct. The DNA fragment from Nb*-UBC9* was designed for silencing Nb*-UBC8*, *9* and *38*; the DNA fragment from Nb*-UBC28* was designed for silencing Nb*-UBC10*, *28* and *31*; the fragment from Nb*-UBC11* for silencing Nb*-UBC11* and *29*; and the fragment from Nb*-UBC39* for silencing Nb*-UBC39* and *40*. The blue-underlined were sequences that are putatively targeted in VIGS by the redunderlined sequence of the corresponding gene from the same clade in the phylogenetic tree (Figure S6A).

Supplemental Figure S11. Silencing group III E2 genes diminished AvrPtoBpromoted degradation of Fen in *N. benthamiana* **protoplasts.** AvrPtoB-HA and Fen-HA were transiently co-expressed in protoplasts isolated from leaves of group III E2 genes-silenced (TRV-*group III*) and non-silenced TRV control (TRV) *N. benthamiana* plants. Protoplasts were harvested and lysed at 21 h after the protoplasts were transfected with DNA carrying corresponding genes to isolate total proteins. Western blot was performed using anti-HA antibody for detecting Fen-HA and AvrPtoB-HA. Staining of ribulose 1, 5-bisphosphate carboxylase–oxygenase (Rubisco) subunits by Coomassie blue demonstrated equal loading. Marker minus (-) denotes the corresponding gene was not transfected into the protoplasts. The experiment was repeated two times with similar results.

Supplemental Figure S12. Silencing group III E2 genes does not influence multiple ETI elicitors-triggered programmed cell death (PCD). *Agrobacterium*-mediated transient expression of Fen, AvrPto/Pto, Rx2/CP, RBP1/Gpa2, RPS2/AvrRpt2, AvrPtoB1- 387/Pto, AvrPtoB1-387, BAX, avrB and Inf1 were performed in group III ubiquitin E2 genes-silenced (TRV-*group III*) and non-silenced TRV control (TRV) *N. benthamiana* plants as described (Mural et al., 2013). *Agrobacterium*-mediated transient expression of empty vector (EV) was performed as the control. At least three spots of infiltration were performed for each elicitor or EV on four different plants with typical result being shown. Photographs were taken on day four after infiltration. The experiment was repeated at least two times with similar results.

Mock DC3000Δ*AvrPto***Δ***AvrPtoB***+***AvrPtoB*

Supplemental Figure S13. Effect of AvrPtoB on the expression of group III E2 genes. Real time PCR analysis of the transcript level of group III E2 genes in *Pst-* or mocktreated tomato plants. Tomato RG-pto11 plants were inoculated with *Pst* strain DC3000Δ*avrPtoΔavrPtoB* expressing AvrPtoB or mock (10 mM MgCl₂). Samples were collected at 0 and 6 h post inoculation. The Y-axis depicts the relative expression of the gene being tested. The experiment was performed using three technical repeats in each of the three biological replicates. Asterisks denote significant difference (P<0.05) in the expression of the E2 gene on the plants inoculated with *Pst* strain DC3000*ΔavrPtoΔavrPtoB* expressing AvrPtoB compared with mock inoculation.

A 120 Relative transcript level (%) **Relative transcript level (%) 100 80** ■ TRV **60 TRV-***group III* **TRV-***NbUBC12/10/28/31* **40 TRV-***NbUBC10/28/31* **20 TRV-***NbUBC12* **0** ¹² ¹⁰ ²⁸ ³¹ ⁸ ⁹ ¹¹ ²⁹ ³⁰ ³⁸ ³⁹ ⁴⁰ ¹⁶ *UBC8 UBC9 UBC12 UBC10 UBC28 UBC31 UBC11 UBC29 UBC30 UBC38 UBC39 UBC40 UBC16*

B

- **1. TRV**
- **2. TRV-***group III*
- **3. TRV-***NbUBC12*
- **4. TRV-***NbUBC10/28/31*
- **5. TRV-***NbUBC12/10/28/31*

Supplemental Figure S14. Specific silencing of E2 genes Nb*-UBC12* **alone, Nb***-UBC10***,** *28* **and** *31* **together, or Nb***-UBC10***,** *12, 28* **and** *31* **together in** *N. benthamiana* **by Virus-Induced Gene Silencing.** (**A**) Nb*-UBC10*, *12*, *28* and *31* genes were specifically and efficiently silenced in *N. benthamiana* by TRV-based VIGS. The transcript level of group III E2 genes and a closely-related E2 gene outside the group III, Nb*-UBC16* in various VIGS-treated *N. benthamiana* plants was examined as described (Mural et al., 2013). Nb*-EF1α* was used as an internal reference for the determination of the amount of cDNA template to be used. The experiment was repeated three times with similar results. (**B**) Phenotypes of the *N. benthamiana* plants in which Nb-*UBC12* alone, three E2 genes Nb*-UBC10*, *28* and *31*, or Nb*-UBC10, 12*, *28* and *31* were specifically silenced. The non-silenced TRV-infected plant and *group III*-silenced plant were included as control. Photographs were taken 4 weeks after the \sim 3-week-old seedlings were infiltrated with TRV vector-based VIGS constructs. The upper panel shows the side view of the plants while the lower panel shows the top view.

B

Supplemental Figure S15. Specific silencing of E2 genes Nb*-UBC11, 29, 39 and 40* **together in** *N. benthamiana* **by Virus-Induced Gene Silencing.** (**A**) Nb*-UBC11*, *29*, *39* and *40* genes were efficiently silenced in *N. benthamiana* by TRV-based VIGS. The Nb-*UBC8*, *9* and *38* were also very slightly knocked down in the plants. The transcript level of group III E2 genes in VIGS-treated *N. benthamiana* plants was examined as described (Mural et al., 2013). Nb*-EF1α* was used as an internal reference for the determination of the amount of cDNA template to be used. The experiment was repeated three times with similar results. (**B**) Phenotypes of the *N. benthamiana* plants in which Nb*-UBC11*, *29, 39* and *40* were silenced. The non-silenced TRV-infected plant and *group III*-silenced plant were included as control. Photographs were taken 4 weeks after the \sim 3-week-old seedlings were infiltrated with TRV vector-based VIGS constructs. The upper panel shows the side view of the plants while the lower panel shows the top view.

D

Supplemental Figure S16. UBC11, 29, 39 and 40 of group III play a more important role in PTI. (A) Silencing the E2 genes *UBC11*, *29, 39* and *40* resulted in reduced ROS production induced by flg22 in a chemiluminescence assay. The diminishment of ROS on the *UBC11*/*29*/*39*/*40*-silenced plants was to a less extent than that on the group IIIknocked down plants. **(B)** Knocking down the E2 genes *UBC11*, *29, 39* and *40* downregulates the induction of PTI reporter gene *Wrky28* by flg22. The expression of *N. benthamiana* PTI marker gene *Wrky28* was performed as described in Figure 6. The experiment was performed with three technical repeats in each of the three biological replicates. Error bars indicate standard deviation. Asterisks mark significant reduction of the expression of *Wrky28*in group III E2 genes-silenced and *UBC11*/*29*/*39*/*40*-silenced plants compared to non-silenced TRV control plants $(P < 0.05)$. **(C)** Bacterial populations of the *Pst* strain DC3000*ΔhopQ1-1* on leaves of various VIGS-treated plants. Experiments were performed as described in Figure 8D and repeated three times with similar results. Asterisks indicate significantly increased bacterial growth on *group III*silenced plants compared to the non-silenced control plants based on the one-way ANOVA ($P < 0.01$). **(D)** No effect on the degradation of Fen caused by AvrPtoB was observed on *N. benthamiana* plants in which the expression of Nb*-UBC11*, *29*, *39* and *40* was knocked down. The experiment was performed as shown in Figure 5B and was repeated two times with similar results.

Supplemental Table I. List of UBC domain-containing proteins from tomato, Arabidopsis, *N. benthamiana*, and human.

Supplemental Table II. List of primers used in this study.

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Supplemental Table I List of UBC domain-containing proteins from tomato, Arabidopsis, *N. benthamiana* **, and human**

Arabidopsis UBCs

Tomato UBCs

N. benthamina **ubiquitin E2s**

Human ubiquitin E2s

Supplemental Table II List of primers used in this study

Name Sequence(5'-3') purpose Sl-UBC8-EcoRI-F ACGGATCCATGGCATCCAAGCGGATTC Sl-*UBC8* in the pGEX-4T-1 vector SI-UBC8-XhoI-R CGCTCGAGCTATCCCATGGCAAATTTTTG SI-UBC8 in the pGEX-4T-1 vector Sl-UBC31-EcoRI-F TAGAATTCATGGCTTCGAAACGGATATT Sl-*UBC31* in the pGEX-4T-1 vector Sl-UBC31-XhoI-R TTCTCGAGTTAACCCATTGCATATTTCTGG Sl-*UBC31* in the pGEX-4T-1 vector Sl-UBC39-EcoRI-F AGGAATTCATGGCGTCGAAGCGCATAT Sl-*UBC39* in the pGEX-4T-1 vector Sl-UBC39-XhoI-R TGCTCGAGTTATCCCATCGCATATTTTTGA Sl-*UBC39* in the pGEX-4T-1 vector Sl-UBC40-EcoRI-F TTGAATTCATGGCGTCGAAGAGGATATT Sl-*UBC40* in the pGEX-4T-1 vector Sl-UBC40-XhoI-R AACTCGAGTCATCCCATTGCATATTTCTGA Sl-*UBC40* in the pGEX-4T-1 vector Sl-UBC3-EcoRI-F AGGAATTCATGTCGACACCGGCGAAG Sl-*UBC3* in the pGEX-4T-1 vector Sl-UBC3-XhoI-R CGCTCGAGTCAGTCTGCTGTCCAGCTT Sl-*UBC3* in the pGEX-4T-1 vector SI-UBC5-EcoRI-F TAGGATCCATGTCTTCTCCAAGCAAACG SI-UBC5 in the pGEX-4T-1 vector SI-UBC5-XhoI-R TGCTCGAGTCATGGATCAACAGGGCCT SI-UBC5 in the pGEX-4T-1 vector Sl-UBC6-EcoRI-F CAGAATTCATGTCTTCCCCTAGCAAACG Sl-*UBC6* in the pGEX-4T-1 vector Sl-UBC6-XhoI-R TGCTCGAGTTAGGGATCTGCTTTTCCAG Sl-*UBC6* in the pGEX-4T-1 vector Sl-UBC16-BamHI-F GAGGATCCATGACTAGTGCTTCTGCTTC Sl-*UBC16* in the pGEX-4T-1 vector Sl-UBC16-XhoI-R CACTCGAGCTACACTTTATCGTCATGGAA Sl-*UBC16* in the pGEX-4T-1 vector Sl-UBC17-BamHI-F AAGGATCCATGTCGGCCTCCTCTGCC Sl-*UBC17* in the pGEX-4T-1 vector Sl-UBC17-XhoI-R TGCTCGAGTCACACCTTATCATCATGGAA Sl-*UBC17* in the pGEX-4T-1 vector Sl-UBC20-BamHI-F GTGGATCCATGGCGACAATGAACAGTGG Sl-*UBC20* in the pGEX-4T-1 vector Sl-UBC20-XhoI-R CGCTCGAGCTACACACTAGGCTTGTATAG Sl-*UBC20* in the pGEX-4T-1 vector Sl-UBC27-BamHI-F GAGGATCCATGGTGGACTTGGCTAGGG Sl-*UBC27* in the pGEX-4T-1 vector Sl-UBC27-XhoI-R CGCTCGAGTTAGCTGGACAACAGCTTTTC Sl-*UBC27* in the pGEX-4T-1 vector Sl-UBC32-BamHI-F GTGGATCCATGGCGGAAGACAAGTATAAT Sl-*UBC32* in the pGEX-4T-1 vector Sl-UBC32-XhoI-R CGCTCGAGTTACGATTCATCCATAAAGACA Sl-*UBC32* in the pGEX-4T-1 vector Sl-UBC1-EcoRI-F CGGAATTCATGTCGACTCCAGCT Sl-*UBC1* in the pGEX-4T-1 vector SI-UBC1-XhoI-R AACTCGAGTCAGCAGTCAGCAGTCCA SI-UBC1 in the pGEX-4T-1 vector Sl-UBC2-EcoRI-F CGGAATTCATGTCAACTCCTTCA Sl-*UBC2* in the pGEX-4T-1 vector Sl-UBC2-XhoI-R AACTCGAGTCAGTCTGCAGTCCA Sl-*UBC2* in the pGEX-4T-1 vector Sl-UBC15-EcoRI-F CGGAATTCATGTCTTCTCCAAGC Sl-*UBC15* in the pGEX-4T-1 vector Sl-UBC15-XhoI-R AACTCGAGTCAAGGATCAGCATG Sl-*UBC15* in the pGEX-4T-1 vector Sl-UBC21-EcoRI-F CGGAATTCATGCAGGCTTCAAGG Sl-*UBC21* in the pGEX-4T-1 vector Sl-UBC21-XhoI-R AACTCGAGTTAGCCCTTCTTGGG Sl-*UBC21* in the pGEX-4T-1 vector Sl-UBC22-EcoRI-F CGGAATTCATGGCAACTAATGAA Sl-*UBC22* in the pGEX-4T-1 vector SI-UBC22-XhoI-R CGCTCGAGTTATAATCTCTTCAA Sl-UBC22 in the pGEX-4T-1 vector SI-UBC41-EcoRI-F CGGAATTCATGTCGACGCCGGCT Sl-UBC41 in the pGEX-4T-1 vector Sl-UBC41-XhoI-R AACTCGAGTCAGTCCGCCGTCCA Sl-*UBC41* in the pGEX-4T-1 vector Sl-UBC13-ORF-F CGGAATTCATGGCTAACAGC Sl-*UBC13* in the pGEX-4T-1 vector Sl-UBC13-ORF-R CCGCTCGAGTCATGCACCACTAG Sl-*UBC13* in the pGEX-4T-1 vector Sl-UBC13-2-ORF-F CGGAATTCATGGCTAACAGC Sl-*UBC13-2* in the pGEX-4T-1 vector Sl-UBC13-2-ORF-R CCGCTCGAGTCATGCACCACTAG Sl-*UBC13-2* in the pGEX-4T-1 vector

SI-UBC14-GW-F CACCATGGCTTCACAAGCTAGTC SI-UBC14 ORF gateway cloning in pDEST17 vector Sl-UBC14-GW-R TTCTCGAGCTACATTTCTTGTGACCGTC Sl-*UBC14* ORF gateway cloning in pDEST17 vector SI-UBC36-F-GW CACCATGGCTTCTTCACAAGCCG SI-UBC36 ORF gateway cloning in pDEST17 vector SI-UBC36-R CGCTCGAGTCACAACATCTCTTGTGATTTC SI-UBC36 ORF gateway cloning in pDEST17 vector Sl-UBC12-F-GW CACCATGGCTTCAAAGAGGATTCAG Sl-*UBC12* ORF gateway cloning in pDEST17 vector SI-UBC12-R CGCTCGAGTCAACCCATTGCGTATTTCT SI-*UBC12* ORF gateway cloning in pDEST17 vector SIUbc7-GW-F CACCATGGCTTCAACTTCTCCTTC SI-UBC7ORF gateway cloning in pDEST15 vector SIUbc7-GW-R TTACATCATTTCTTGAGACCG SI-UBC7 ORF gateway cloning in pDEST15 vector Sl-UBC10-ORF-F CACCATGGCTTCGAAACGAATATTGAA Sl-*UBC10* ORF gateway cloning in pDEST15 vector SI-UBC10-ORF-R TTAACCCATGGCATACTTCTG SI-UBC10 ORF gateway cloning in pDEST15 vector SI-UBC35-ORF-F CACCATGGCTTCAGCTTCTCCTTC SI-UBC35 ORF gateway cloning in pDEST15 vector SI-UBC35-ORF-R TTACGTCATTTCTTGGGACCG SI-UBC35 ORF gateway cloning in pDEST15 vector SI-UBC26-ORF-F CACCATGGATGAGGCAAACAAGAAC SI-UBC26 ORF gateway cloning in pDEST15 vector Sl-UBC26-ORF-R TTAACTAACCCTTGGCTGTTTGA Sl-*UBC26* ORF gateway cloning in pDEST15 vector Sl-UBC25-ORF-F CACCATGGAGACTCATAAACAAGTAG Sl-*UBC25* ORF gateway cloning in pDEST15 vector SI-UBC25-ORF-R GCTACTCAGTCCCATTCTGT Sl-UBC25 ORF gateway cloning in pDEST15 vector Sl-UBC28-ORF-F CACCATGGCTTCTAAGCGGATATTG Sl-*UBC28* ORF gateway cloning in pDEST15 vector SI-UBC28-ORF-R TTAACCCATAGCATACTTCTG SI-UBC28 ORF gateway cloning in pDEST15 vector Sl-UBC33-ORF-F CACCATGGCAGAAAAAGCATGTGTAA Sl-*UBC33* ORF gateway cloning in pDEST15 vector SI-UBC33-ORF-R TCAAAGCTGAAGCAGAGGCA Sl-*UBC33* ORF gateway cloning in pDEST15 vector Sl-UBC4-GW-F CACCATGTCTTCCCCAAGCAAAAG Sl-*UBC4* ORF gateway cloning in pDEST15 vector

SI-UBC4-GW-R TTATGGATCAACAGGTCCTG SI-UBC4 ORF gateway cloning in pDEST15 vector SI-UBC7-GW-F CACCATGGCTTCAACTTCTCCTTC SI-UBC7 ORF gateway cloning in pDEST15 vector SI-UBC7-GW-R TTACATCATTTCTTGAGACCG SI-UBC7 ORF gateway cloning in pDEST15 vector SI-UBC9-GW-F CACCATGGCATCCAAGAGGATTCT Sl-UBC9 ORF gateway cloning in pDEST15 vector SI-UBC9-GW-R CTAACCCATTGCATACTTTTG SI-UBC9 ORF gateway cloning in pDEST15 vector Sl-UBC11-GW-F CACCATGGCATCAAGGAGAATTCAA Sl-*UBC11* ORF gateway cloning in pDEST15 vector Sl-UBC11-GW-R TCAATTCATAGCATATTTTTGGG Sl-*UBC11* ORF gateway cloning in pDEST15 vector SI-UBC23-GW-1F CACCATGGATGAGTCTGAGTCTAC SI-UBC23 ORF gateway cloning in pDEST15 vector SI-UBC23-GW-1R TCCTGCCAAGCAACATCAAC SI-UBC23 ORF gateway cloning in pDEST15 vector SI-UBC23-GW-2F AGATCCGCAAAGTTGTGGTT Sl-UBC23 ORF gateway cloning in pDEST15 vector SI-UBC23-GW-2R GCAGCAGGTAGCCTTGGA Sl-UBC23 ORF gateway cloning in pDEST15 vector SI-UBC23-GW-3F GATTCATGGTCCCAAAGGTC SI-UBC23 ORF gateway cloning in pDEST15 vector SI-UBC23-GW23-3R CTACAATTGGTGGAGATGTTG SI-UBC23 ORF gateway cloning in pDEST15 vector Sl-UBC24-GW-1F CACCATGGATACATCTCTAAGTGAC Sl-*UBC24* ORF gateway cloning in pDEST15 vector Sl-UBC24-GW-1R GCCAACAATAATACCGATACA Sl-*UBC24* ORF gateway cloning in pDEST15 vector SI-UBC24-GW-2F CTGTTTAGGTGATGCGGTTT SL-UBC24 ORF gateway cloning in pDEST15 vector SI-UBC24-GW-2R TTAATCGGACAACTGACTGC SI-UBC24 ORF gateway cloning in pDEST15 vector Sl-UBC29-GW-F CACCATGGCATCCAGGAGAATTCA Sl-*UBC29* ORF gateway cloning in pDEST15 vector SI-UBC29-GW-R TCAGTTCATGGCATACTTTTG SI-UBC29 ORF gateway cloning in pDEST15 vector SI-UBC30-GW-F CACCATGGCTTCCAAGCGGATCT Sl-*UBC30* ORF gateway cloning in pDEST15 vector SI-UBC30-GW-R TTAGCCCATGGCATACTTCT Sl-UBC30 ORF gateway cloning in pDEST15 vector SI-UBC34-GW-F CACCATGGCAGAAAAGGCATGTGT SI-UBC34 ORF gateway cloning in pDEST15 vector SI-UBC34-GW-R TCAAAGCTGAAGTAGCGGCA Sl-UBC34 ORF gateway cloning in pDEST15 vector SI-UBC37-GW-1F CACCATGGCTCAAGAGGCGCGG SI-UBC37 ORF gateway cloning in pDEST15 vector Sl-UBC37-GW-1R TGAAATCGGTTGACTGAAGC Sl-*UBC37* ORF gateway cloning in pDEST15 vector SI-UBC37-GW-2F CAGGCTATAAGCAATTCAGG SI-UBC37 ORF gateway cloning in pDEST15 vector Sl-UBC37-GW-2R TCAAGATATTGGATAAAAAGATTG Sl-*UBC37* ORF gateway cloning in pDEST15 vector SI-UBC38new-GW-F CACCATGGCGTCCAAGCGGATTCT Sl-*UBC38* ORF gateway cloning in pDEST15 vector SI-UBC38-GW-R CTAACCCATGGCGTACTTTT Sl-UBC38 ORF gateway cloning in pDEST15 vector Nb-UBC9-GW-F CACCATGGCATCCAAGAGGATTCT Group III VIGS fragment Nb*-UBC9* cloning Nb-UBC9-VIGS-R TGTAGCCTGCCAGTGAAACAT Group III VIGS fragment Nb*-UBC9* cloning Nb-UBC28-VIGS-F GAAGATATGTTTCACTGGCA Group III VIGS fragment Nb*-UBC28* cloning Nb-UBC28-VIGS-R TATTTATATTTGGATGGAAAACTTTTG Group III VIGS fragment Nb*-UBC28* cloning Nb-UBC11-VIGS-F CAAAAGTATTTCCATCCAAATATA Group III VIGS fragment Nb-*UBC11* cloning Nb-UBC11-VIGS-R TCGTCTGGATTTGGATCTGT Group III VIGS fragment Nb-*UBC11* cloning Nb-UBC39-VIGS-F ACAGATCCAAACCCAGACGA Group III VIGS fragment Nb-*UBC39* cloning Nb-UBC39-R TGCTCGAGTTATCCCATCGCATATTTTTGA Group III VIGS fragment Nb-*UBC39* cloning Nb-UBC12-VIGS-F ATGGGATAACTCGAGCATCTATCCATTTCC Group III VIGS fragment Nb-*UBC12* cloning Nb-UBC12-VIGS-R TGCTGTTTATGTTTGGGTGG Group III VIGS fragment Nb-*UBC12* cloning Nb-UBC30-VIGS-F GGTTTTCCACCCAAACATAAACAGCA Group III VIGS fragment Nb-*UBC30* cloning Nb-UBC30-VIGS-R GATTAGGGTCCGTCAGAAGTG Group III VIGS fragment Nb-*UBC30* cloning Nb-UBC12VIGS-G-F CACCAGTATTTGTCTGGACATCTTAAAAG VIGS fragment cloning forsilencing Nb-*UBC12* alone or the four E2 genes Nb-*UBC12* , *10* , *28* and *31.* Nb-UBC12VIGS-G-R AAGCAAAACCTTGGTGAGCAGCACCTTGGATACAGT VIGS fragment cloning forsilencing Nb-*UBC12* alone or the four E2 genes Nb-*UBC12* , *10* , *28* and *31.* Nb-UBC10VIGS-G-F CACCAAGGTTTTGCTTTCAATCTGC VIGS fragment cloning for silencing the three E2 genes Nb-*UBC10, 28* and *31* or the four E2 genes Nb-*UBC12* , *10* , *28* and *31.* Nb-UBC10VIGS-G-R TTATTGTAGCTTGGTGGCAATCTCAGGAACAAGGGG VIGS fragment cloning for silencing the three E2 genes Nb-*UBC10, 28* and *31* or the four E2 genes Nb-*UBC12* , *10* , *28* and *31.* Nb-UBC28VIGS-G-F CACCAAGCTACAATAATGGGCCC VIGS fragment cloning for silencing the three E2 genes Nb-*UBC10, 28* and *31* or the four E2 genes Nb-*UBC12* , *10* , *28* and *31.* Nb-UBC28VIGS-G-R AATGGATAGTGACTAAAAATAC VIGS fragment cloning for silencing the three E2 genes Nb-*UBC10, 28* and *31* or the four E2 genes Nb-*UBC12* , *10* , *28* and *31.* Nb-UBC11VIGS-F CACCGACAGCCCTTATGCAGGTGG VIGS fragment cloning for silencing the three E2 genes Nb-*UBC11, 29, 39 and 40.* Nb-UBC11VIGS-R GAATTCTCCTCAGGAGGAAAATGGATGGCC VIGS fragment cloning for silencing the three E2 genes Nb-*UBC11, 29, 39 and 40.* Nb-UBC29VIGS-F TTTCCTCCTGAGGAGAATTCTCAAGGAGC VIGS fragment cloning for silencing the three E2 genes Nb-*UBC11, 29, 39 and 40.* Nb-UBC29VIGS-R CTACTGGACCTGCACTGCATGAAG VIGS fragment cloning for silencing the three E2 genes Nb-*UBC11, 29, 39 and 40.*

Nb-UBC16-RT-R TGGTATGGGAGTTGGAGTCA RT-PCR for Nb-*UBC16* Nb-UBC17-RT-F TGGGTGATTGAAGTGATTGGG RT-PCR for Nb-*UBC17* Nb-UBC17-RT-R TCAAGGGTCAGAAGGACACC RT-PCR for Nb-*UBC17* Sl-EF1a-RT-F TCCAAAGATGGTCAGACCCGTGAA Real-time PCR for reference gene Sl-*EF1a* Sl-EF1a-RT-R ATACCTAGCCTTGGAGTACTTGGG Real-time PCR for reference gene Sl-*EF1a* SI-UBC8-RT-F CATTTTGAAGGAACAGTGGAGC Real-time PCR for SI-UBC8 SI-UBC8-RT-R CTCCGGGCAGTTGTCTCATAC Real-time PCR for SI-*UBC8* Sl-UBC9-RT-F TTCCAAGGTGCTGCTGTCAATC Real-time PCR for Sl-*UBC9* Sl-UBC9-RT-R ATGGGTCTGAGCAGCAACTAAC Real-time PCR for Sl-*UBC9* Sl-UBC10-RT-F TGCTGGGGGTGTTTTTTTGGTC Real-time PCR for Sl-*UBC10* SI-UBC10-RT-R AAGGGGTCGTCAGGGTTTGGGT Real-time PCR for SI-*UBC10* SI-UBC11-RT-F AGGTCCTGTGGCTCAGGATATA Real-time PCR for SI-UBC11 SI-UBC11-RT-R CTTAGGGGGTTTGAAAGGGTAG Real-time PCR for SI-UBC11 Sl-UBC12-RT-F TCTTACTGTATCCAAGGTGCTGCT Real-time PCR for Sl-*UBC12* Sl-UBC12-RT-R GTGTTCAACCCATTGCGTATTTCT Real-time PCR for Sl-*UBC12* Sl-UBC28-RT-F TACAAGACAGACAGGGCAAAATA Real-time PCR for Sl-*UBC28* Sl-UBC28-RT-R GGGAAGGTAGAGGACAGAGAGAC Real-time PCR for Sl-*UBC28* Sl-UBC29-RT-F CATGCAGTGCAGGTCCAGTAGC Real-time PCR for Sl-*UBC29* SI-UBC29-RT-R CTTGGGAGGTTTGAAAGGGTAA Real-time PCR for SI-*UBC29* SI-UBC30-RT-F AGTCCTTATTCCGGTGGAGTTT Real-time PCR for SI-UBC30 Sl-UBC30-RT-R CTTCCGTTGCTGTTTATGTTTG Real-time PCR for Sl-*UBC30* Sl-UBC31-RT-F AGGTTTTGCTTTCAATTTGCTC Real-time PCR for Sl-*UBC31* Sl-UBC31-RT-R GGCAGTTGATTCGTATTTGGC Real-time PCR for Sl-*UBC31* SI-UBC38-RT-F TCCTACTTCTTGCAGTGCTGGT Real-time PCR for SI-*UBC38* SI-UBC38-RT-R CATTGCTGTTGATGTTCGGGTG Real-time PCR for SI-*UBC38* Sl-UBC39-RT-F TTTCCCCCCTGATTATCCTTTC Real-time PCR for Sl-*UBC39* SI-UBC39-RT-R GTGGTCTCGTATTTGGCCCTGT Real-time PCR for SI-UBC39 Sl-UBC40-RT-F CCCTTATGCTGGAGGTGTATT Real-time PCR for Sl-*UBC40* SI-UBC40-RT-R ATCATCTGGGTTTGGGTCTGT Real-time PCR for SI-UBC40 Nb-EF1a-F TACTGGTGGTTTTGAAGCTG Real-time PCR for reference gene Nb-*EF1a* Nb-EF1a-R ATACCTAGCCTTGGAGTACTTGGG Real-time PCR for reference gene Nb-*EF1a* Nb-Wrky28-F GCATTCATGACAAAGAGTGAGGTT Real-time PCR for Nb-*Wrky28* Nb-Wrky28-R GACATTTTTGACTTGTGCACCTAT Real-time PCR for Nb-*Wrky28* Nb-Pti5-F CCTCCAAGTTTGAGCTCAGATAGT Real-time PCR for Nb-Pti5 Nb-Pti5-R CCAAGAAATTCTCCATGTACTCTGTC Real-time PCR for Nb-Pti5 Nb-Acre31-F GAGAAACTGGGATTGCCTGAAGGA Real-time PCR for Nb-Acre31 Nb-Acre31-R AACTTGGCCATCGTGATCTTGGTC Real-time PCR for Nb-*Acre31* Nb-Gras2-F TCATGAGGCGTTACTCGGAGCATT Real-time PCR for Nb-Gras2 Nb-Gras2-R TACCTAGCACCAAGCAGATGCAGA Real-time PCR for Nb-*Gras2* AvrPtoB307-smaI-R CCCGGGTACATGTCTTTCAAGGGCCGTG *AvrPtoB* ORF cloning for constrcucting pSPYNE173- *AvrPtoB1-307* Sl-UBC8-XhoI-F CACCCTCGAGATGGCATCCAAGCGGATTCTC Sl-*UBC8*ORF cloning for constrcucting pSPYCE(M)-*SlUBC8* Sl-UBC8-SmaI-R CCCGGGTCCCATGGCAAATTTTTGAGTC Sl-*UBC8* ORF cloning for constrcucting pSPYCE(M)-*SlUBC8* Sl-UBC9-XhoI-F CACCCTCGAGATGGCATCCAAGAGGATTCTG Sl-*UBC9* ORF cloning for constrcucting pSPYCE(M)-*SlUBC9* SI-UBC9-SmaI-R CCCGGGACCCATTGCATACTTTTGGG SI-*UBC9* ORF cloning for constrcucting pSPYCE(M)-*SlUBC9* Sl-UBC11-XhoI-F CACCCTCGAGATGGCATCAAGGAGAATTCAAA Sl-*UBC11* ORF cloning for constrcucting pSPYCE(M)- *SlUBC11* SI-UBC11-SmaI-R CCCGGGATTCATAGCATATTTTTGGGTC SI-UBC11 ORF cloning for constrcucting pSPYCE(M)-*SlUBC11* Sl-UBC28-XhoI-F CACCCTCGAGATGGCTTCTAAGCGGATATTG Sl-*UBC28* ORF cloning for constrcucting pSPYCE(M)- *SlUBC28* Sl-UBC28-SmaI-R CCCGGGACCCATAGCATACTTCTGGG Sl-*UBC28* ORF cloning for constrcucting pSPYCE(M)- *SlUBC28* Sl-UBC29-XhoI-F CACCCTCGAGATGGCATCCAGGAGAATTCAAAAG Sl-*UBC29* ORF cloning for constrcucting pSPYCE(M)- *SlUBC29* SI-UBC29-SmaI-R CCCGGGGTTCATGGCATACTTTTGAG SI-UBC29 ORF cloning for constrcucting pSPYCE(M)-*SlUBC29* SI-UBC30-XhoI-F ggtaccctcgagATGGCTTCCAAGCGGATC SI-*UBC30* ORF cloning for constrcucting pSPYCE(M)-*SlUBC30* SI-UBC30-SmaI-R GGGctgcagGCCCATGGCATACTTCTGGG SI-UBC30 ORF cloning for constrcucting pSPYCE(M)-*SlUBC30* Sl-UBC31-XhoI-F CACCCTCGAGATGGCTTCGAAACGGATATTG Sl-*UBC31* ORF cloning for constrcucting pSPYCE(M)- *SlUBC31*

