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Figure 1

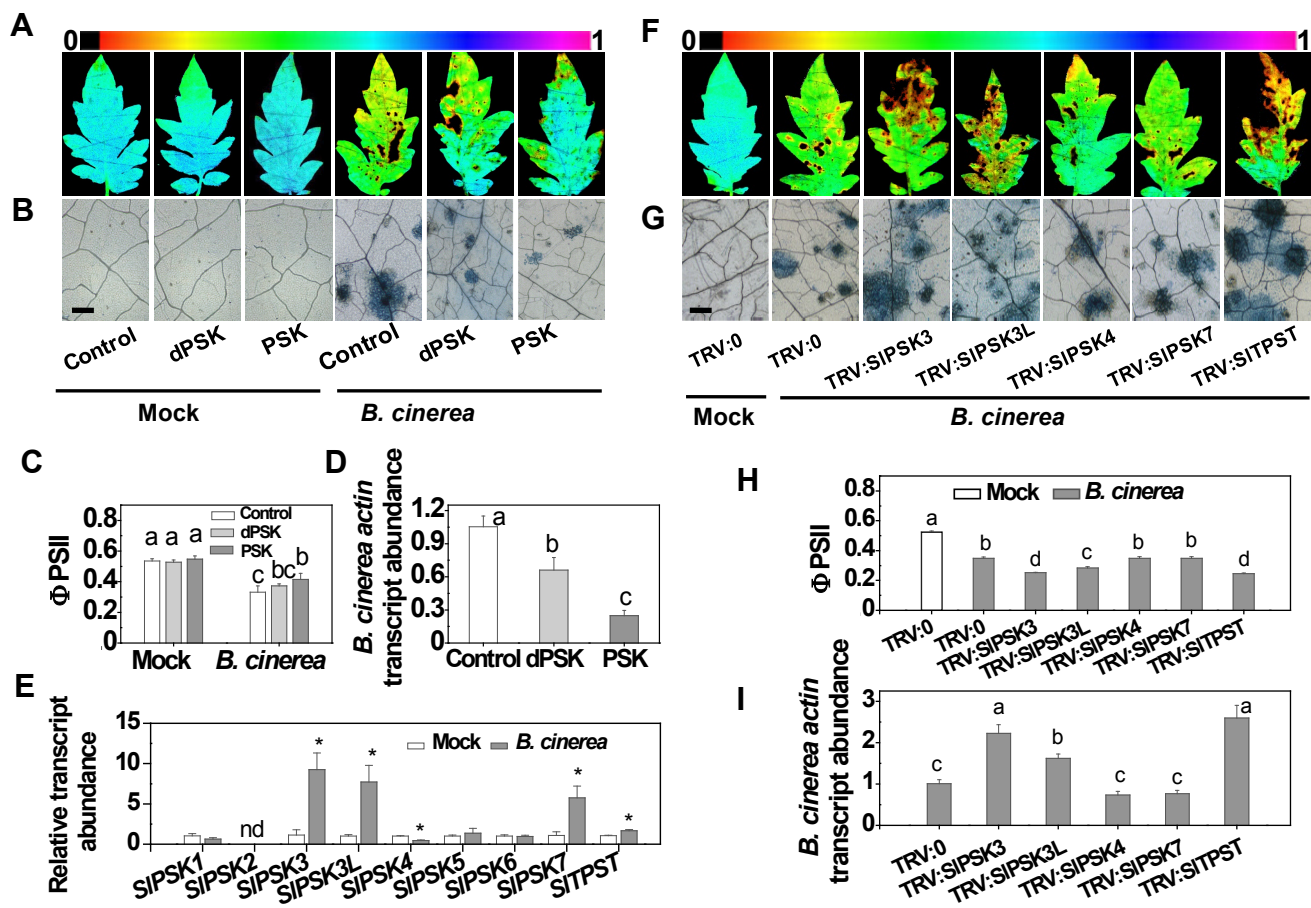


Figure 1. PSK signaling confers tomato plants immunity against *B. cinerea*.

(A-D) Tomato defense to *B. cinerea* is promoted by exogenous PSK. Four-week-old tomato plants were treated with 10 μM PSK, 10 μM dPSK, or water as control 12 h before *B. cinerea* inoculation. (A) Representative chlorophyll fluorescence imaging of the photochemical quantum yield of photosystem II (ΦPSII) at 2 days post *B. cinerea* inoculation (dpi). (B) Representative images of trypan blue staining for cell death in leaves at 2 dpi. Scale bar = 250 μm. (C) The quantification data of ΦPSII at 2 dpi. (D) Relative *B. cinerea actin* transcript abundance in infected leaves at 1 dpi.

(E) Effects of *B. cinerea* inoculation on the transcript abundance of PSK precursor genes and tyrosine sulfation processing gene *S1TPST* in leaves at 0.5 dpi. The transcript abundance of each gene under mock-inoculated condition were defined as 1. nd, not detected. The asterisk indicates a significant effect of *B. cinerea* inoculation with tomato plants.

(F-I) Effects of PSK-biosynthesis related genes silencing on tomato innate immunity against *B. cinerea*. (F) Representative chlorophyll fluorescence imaging of ΦPSII at 2 dpi. (G) Trypan blue staining for cell death in leaves at 2 dpi. Scale bar = 250 μm. (H) The quantification data of ΦPSII at 2 dpi. (I) Relative *B. cinerea actin* transcript abundance in infected leaves at 1 dpi.

The results in (C, D, E, H, and I) are presented as the mean values ± SD; *n* = 3. Different letters indicate significant differences between treatments (*P* < 0.05, Tukey's test). The above experiments were repeated three times with similar results.

Figure 2

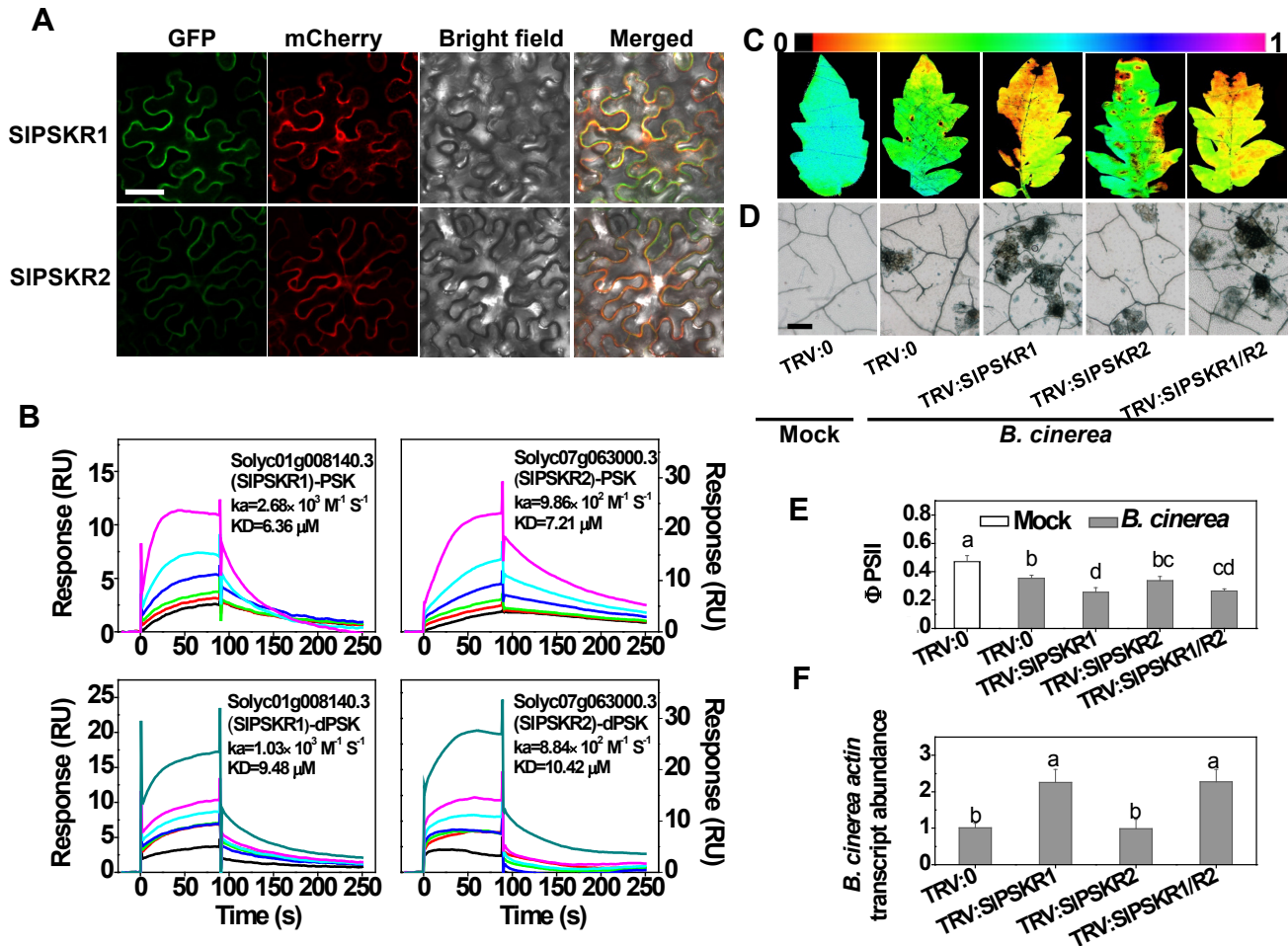


Figure 2. Characterization of tomato PSK receptors.

(A) Subcellular localization of SIPSKR1 and SIPSKR2. SIPSKRs-GFP and FLS2-mCherry (marker for plasma membrane localization) plasmids were transiently co-expressed into *N. benthamiana* leaves. The GFP and mCherry signals were visualized under confocal microscopy after 48 h infiltration. Scale bar = 50 μm .

(B) Surface plasmon resonance analysis of the binding of PSK to potential tomato PSK receptors, SIPSKR1 and SIPSKR2. The curves represent the concentrations of the injected PSK and dPSK. From bottom to top, 0.39, 0.78, 3.125, 6.25, 12.5, and 25 μM were used for PSK. An additional 50 μM concentration was also used for dPSK. The recombinant extracellular portion of the SIPSKR1 and SIPSKR2 was immobilized onto the sensor chip. The obtained kinetic constants for specific binding are shown in each panel. RU, resonance units.

(C-F) Effects of *SIPSKR1*- and/or *SIPSKR2*-silencing on tomato innate immunity against *B. cinerea*. (C) Representative chlorophyll fluorescence imaging of the photochemical quantum yield of photosystem II (Φ PSII) at 2 days post *B. cinerea* inoculation (dpi). (D) Representative images of trypan blue staining for cell death in leaves at 2 dpi. Scale bar = 250 μm . (E) The quantification data of Φ PSII at 2 dpi. (F) Relative *B. cinerea actin* transcript abundance in infected leaves at 1 dpi.

The results in (E, F) are presented as the mean values \pm SD; $n = 3$. Different letters indicate significant differences between treatments ($P < 0.05$, Tukey's test). The above experiments were repeated two times with similar results.

Figure 3

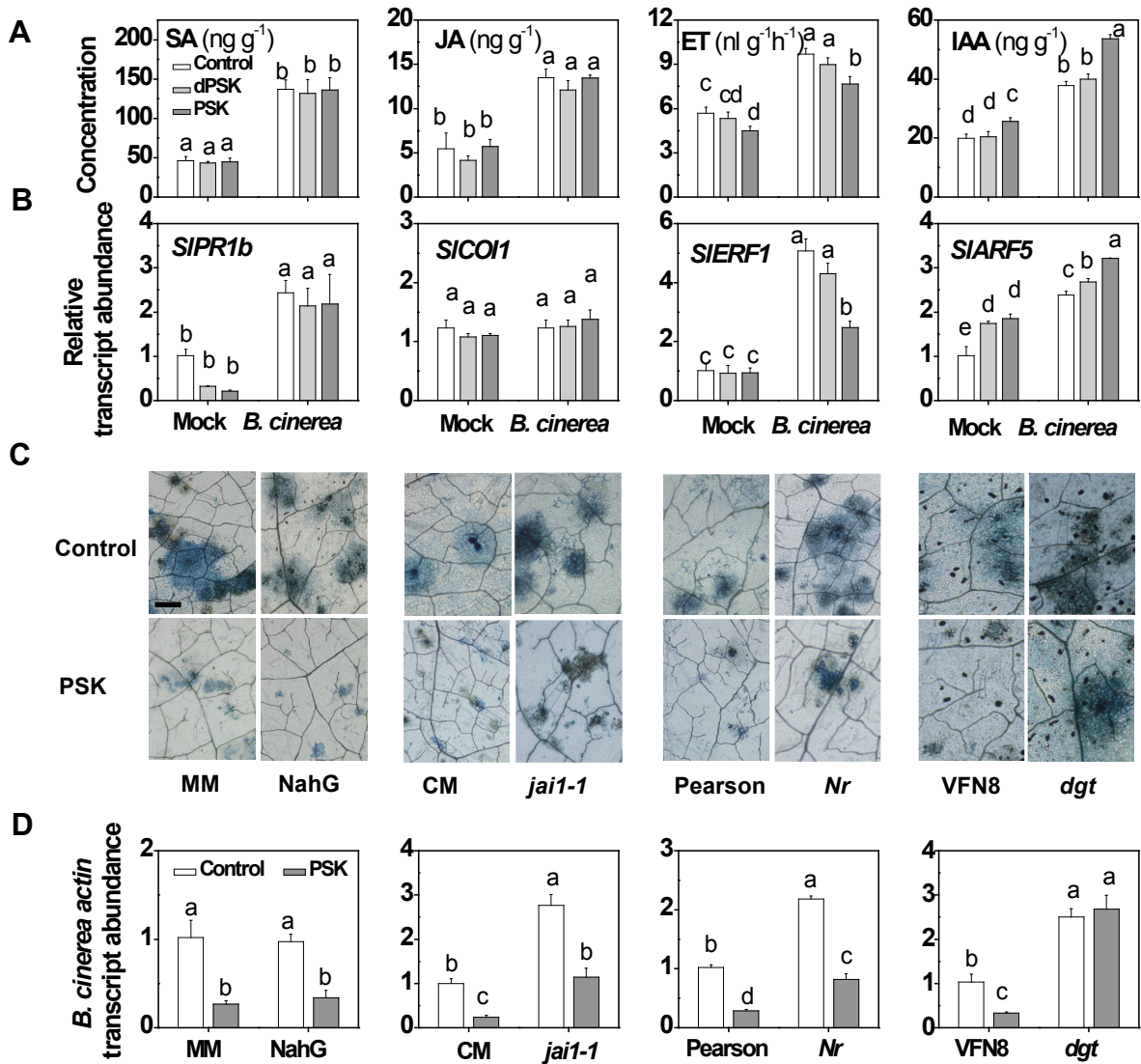


Figure 3. PSK promotes endogenous IAA accumulation and the PSK-induced tomato immunity against *B. cinerea* is blocked in auxin signaling mutant.

(A) Effects of PSK application on endogenous leaf hormone contents (SA, JA, ET, and IAA). Four-week-old tomato plants were treated with 10 μ M PSK, dPSK, or water as control 12 h before *B. cinerea* inoculation, and leaf samples were collected at 0.5 days post *B. cinerea* inoculation (dpi).

(B) Effects of PSK application on the transcript abundance of hormone signaling-related marker genes in leaves at 0.5 dpi. The elicitor application is as in (A). SA-related genes *SIPR1b*, JA-related genes *SICO1*, ET-related genes *SIERF1*, Auxin-related genes *SIARF5*.

(C) Effects of PSK application on trypan blue staining for cell death in hormone signaling-defective and control plants at 2 dpi. Tomato wild type (WT), mutants, or transgenic lines were treated with 10 μ M PSK or water control 12 h before *B. cinerea* inoculation. The following tomato lines were used: SA accumulation-defective transgenic NahG and its WT line cv. Moneymaker (MM), JA-signaling mutant *jai1-1* and its WT line cv. Castlemart (CM), ET-signaling mutant *Nr* and its WT line cv. Pearson, auxin signaling-insensitive mutant *dgt* and its WT line VFN8. Scale bar = 250 μ m.

(D) Relative *B. cinerea actin* transcript abundance in infected hormone-related mutants in the presence or absence of PSK at 1 dpi, the elicitor application is as in (C).

The results in (A, B, and D) are presented as the mean values \pm SD; $n = 3$. Different letters indicate significant differences between treatments ($P < 0.05$, Tukey's test). The above experiments were repeated three times with similar results.

Figure 4

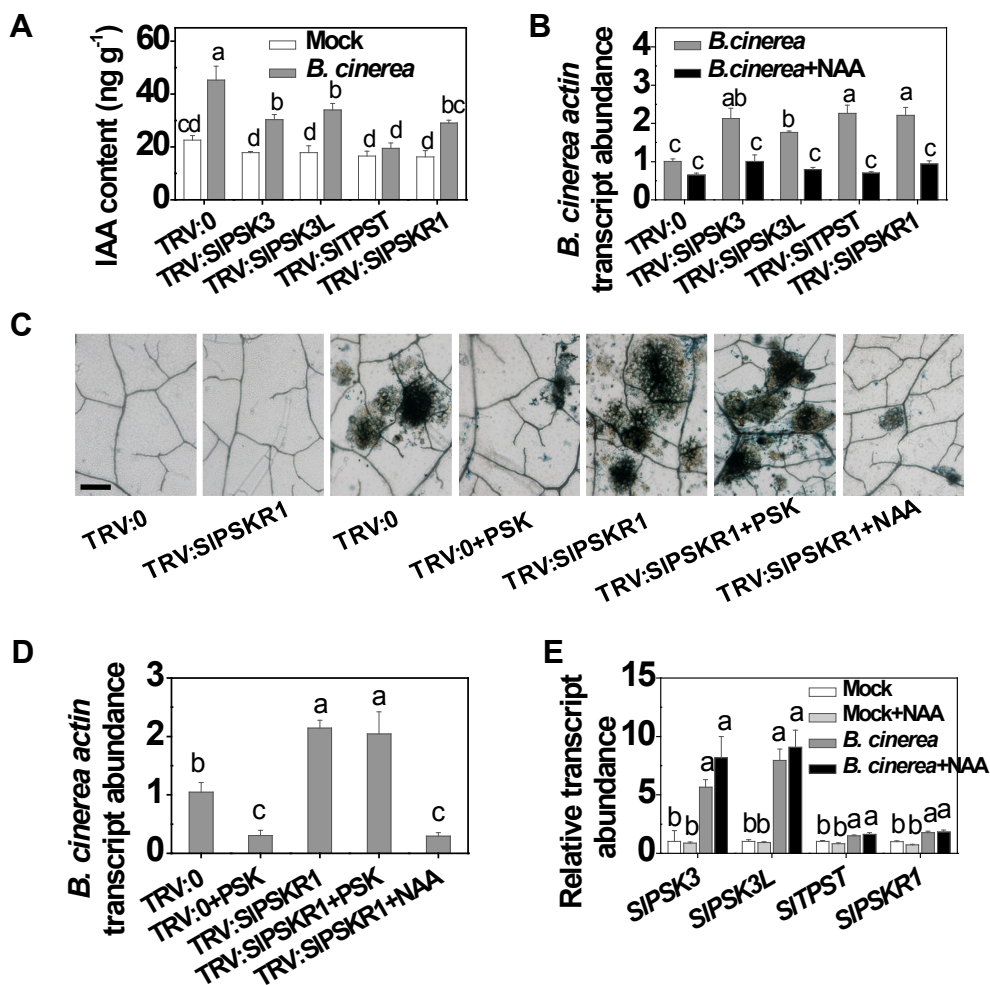


Figure 4. Auxin functions downstream of PSK-SIPSKR1 signaling in tomato immunity against *B. cinerea*.

(A) The changes of IAA content in PSK signaling component gene-silenced tomato plants. Leaf samples were collected at 0.5 days post *B. cinerea* inoculation (dpi).

(B) Effects of NAA application on leaf *B. cinerea actin* transcript abundance in target gene-silenced tomato plants at 1 dpi. PSK signaling component gene-silenced and TRV:0 control plants were treated with 10 nM NAA or water control 12 h before *B. cinerea* inoculation.

(C-D) *SIPSKR1* silencing-compromised immunity was complemented by NAA but not by PSK. (C) Trypan blue staining for cell death as affected by *SIPSKR1* silencing and application of PSK and NAA. Tomato *SIPSKR1*-silenced plants were treated with 10 μ M PSK or 10 nM NAA 12 h before *B. cinerea* inoculation, and leaf samples were collected at 2 dpi. Scale bar = 250 μ m. (D) Relative *B. cinerea actin* transcript abundance in infected leaves at 1 dpi.

(E) The effects of 10 nM NAA application on the transcript abundance of PSK signaling component genes in leaves under both mock- and *B. cinerea*-inoculated condition, samples were taken at 0.5 dpi.

The results in (A, B, D, and E) are presented as the mean values \pm SD; $n = 3$. Different letters indicate significant differences between treatments ($P < 0.05$, Tukey's test). The experiments in (A, B, and E) were repeated three times, and others were repeated two times with similar results.

Figure 5

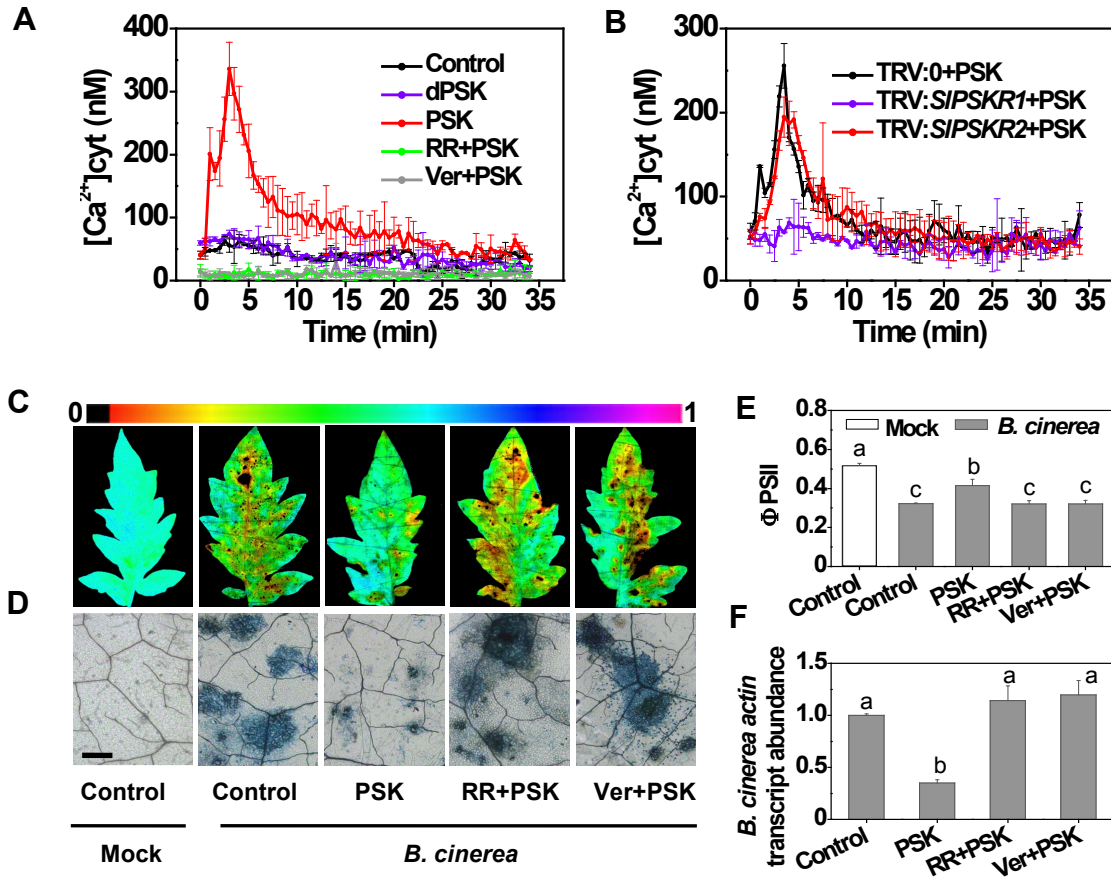


Figure 5. Cytosolic Ca²⁺ elevation is induced and required for PSK-induced tomato immunity against *B. cinerea*.

(A-B) PSK-induced cytosolic Ca²⁺ elevation in leaves of aequorin-expressing tomato plants as affected by Ca²⁺ channel inhibitors (A) or *SIPSKRs* genes silencing (B). Tomato leaf discs were preincubated for 30 min with ruthenium red (RR) or verapamil (Ver) at 20 μM, and 10 μM PSK ligand was then added at time 0. The signals shown at 0.5-min intervals are the mean values ± SD, (*n* = 10~12). In (A), totally 50 leaf discs obtained from at least 5 plants were used for experiment and each treatment had 10 leaf discs; In (B), each 12 leaf discs obtained from independent 5 plants served as one treatment.

(C-F) Effects of Ca²⁺ channel inhibitor on tomato innate immunity against *B. cinerea*. The four-week-old tomato plants were treated with 10 μM PSK, 20 μM each Ca²⁺ channel inhibitor, or water as control 12 h before *B. cinerea* inoculation. (C) Representative chlorophyll fluorescence imaging of the photochemical quantum yield of photosystem II (ΦPSII) at 2 days post *B. cinerea* inoculation (dpi). (D) Representative images of trypan blue staining for cell death in leaves at 2 dpi. Scale bar = 250 μm. (E) The quantification data of ΦPSII at 2 dpi. (F) Relative *B. cinerea actin* transcript abundance in infected leaves at 1 dpi. The results in (E and F) are presented as the mean values ± SD; *n* = 3. Different letters indicate significant differences between treatments (*P* < 0.05, Tukey's test).

The above experiments were repeated three times with similar results.

Figure 6

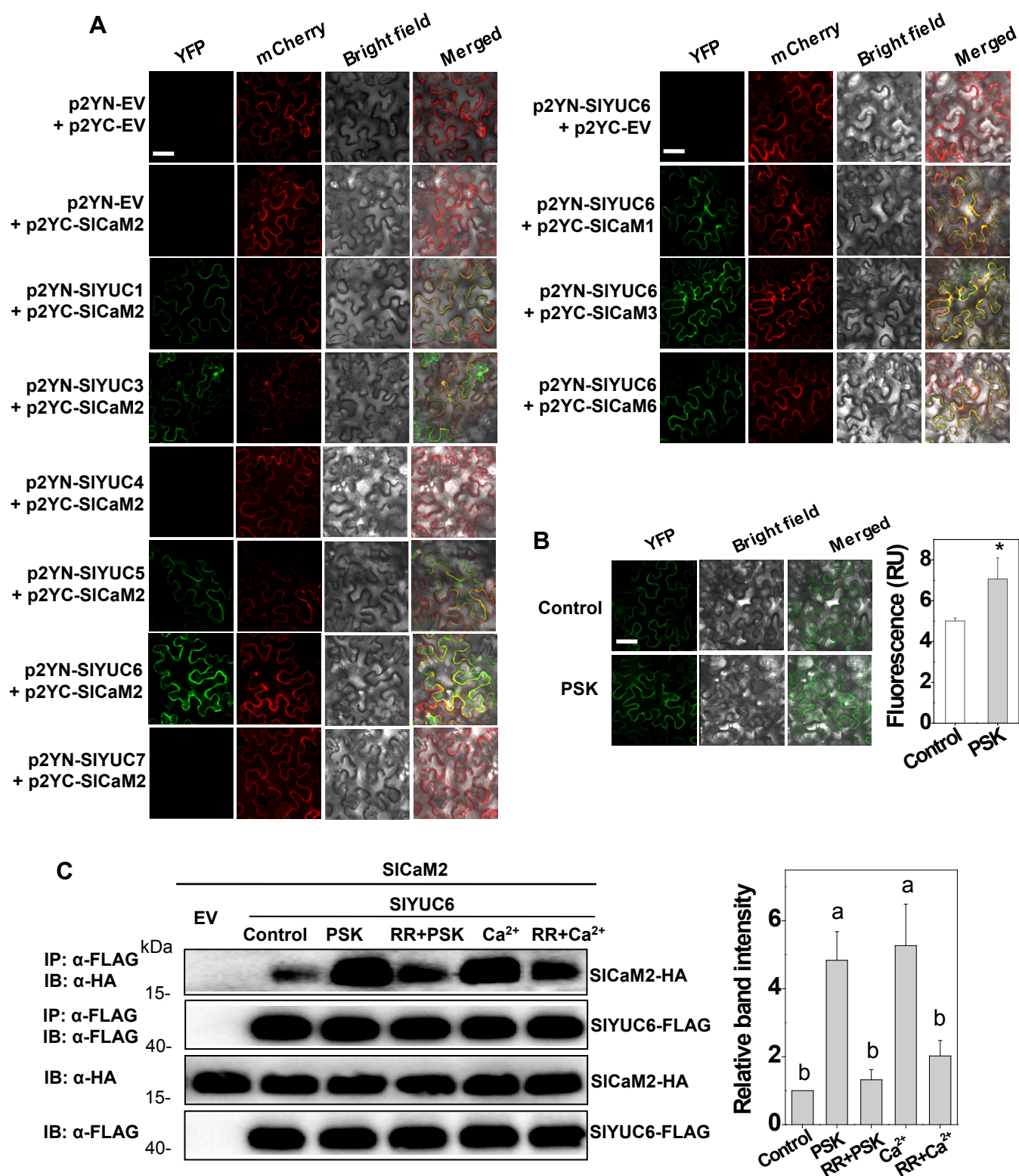


Figure 6. SiCaMs binds to auxin biosynthetic protein SIYUCs.

(A) BiFC analyses of the binding between SiCaM2 and SIYUCs (left panel), and between SiCaMs and SIYUC6 (right panel). Both spliced YFP constructs and FLS2-mCherry (marker for plasma membrane localization) plasmids were transiently co-expressed into *N. benthamiana* leaves. The YFP and mCherry signals were visualized under confocal microscopy after 48 h infiltration. Scale bar = 50 μm.

(B) The changes of BiFC fluorescence signal between p2YC-SiCaM2 and p2YN-SIYUC6 with or without 2 h

of PSK (10 μ M) application. Scale bar = 50 μ m. The fluorescence signal intensity from three independent repeats was quantified and the data are shown as mean \pm SD ($n = 3$). Asterisks indicates a significant effect of PSK application ($P < 0.05$, Tukey's test).

(C) Co-IP analysis of association between HA-tagged SiCaM2 and FLAG-tagged SiYUC6 with or without application of 10 μ M PSK, 20 μ M CaCl₂, and 20 μ M Ca²⁺ channel inhibitor ruthenium red (RR) for 2 h. Total proteins were extracted from leaves transiently expressed with the SiCaM2-HA, SiYUC6-FLAG construct alone or their combinations after 48 h infiltration. The extracted proteins were immunoprecipitated with an anti-FLAG antibody and the presence of SiCaM2-HA and SiYUC6-FLAG in the immune complex was determined by immunoblot (IB) with the indicated antibody. The Co-IP band intensity (top) from three independent repeats was quantified by Image J software. The data are shown as mean \pm SD ($n = 3$). Different letters indicate significant differences between treatments ($P < 0.05$, Tukey's test).

The experiments in **(A, B)** were repeated three times, and experiments in **(C)** were repeated two times with similar results.

Figure 7

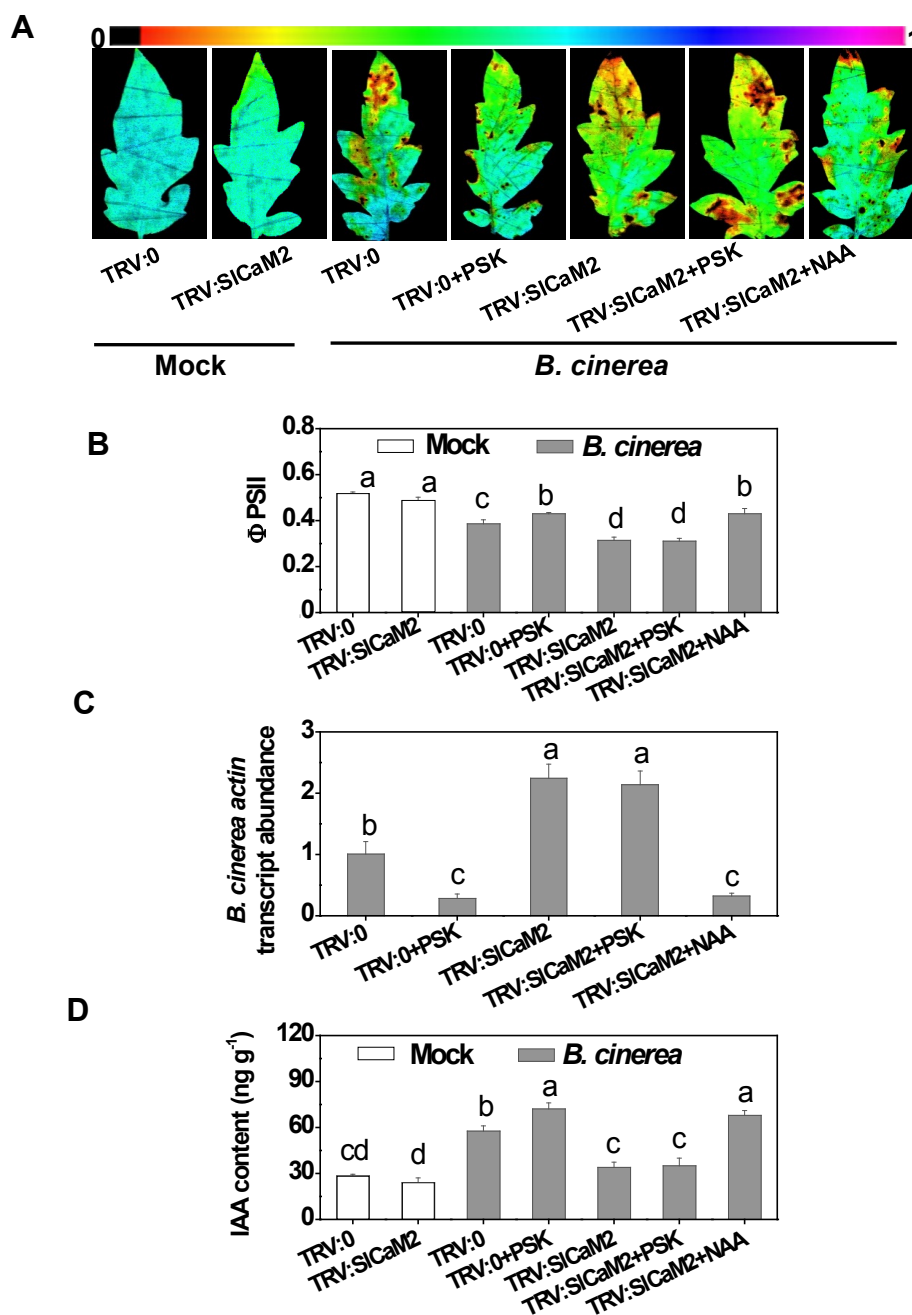


Figure 7. *SICA2* silencing compromises the PSK-induced immunity and IAA accumulation.

(A) Representative chlorophyll fluorescence imaging of the photochemical quantum yield of photosystem II (Φ PSII) as affected by *SICA2* silencing, and application of PSK and NAA. Tomato *SICA2*-silenced plants were treated with 10 μ M PSK or 10 nM NAA 12 h before *B. cinerea* inoculation, and leaf samples were collected at 2 days post *B. cinerea* inoculation (dpi).

(B) The quantification data of Φ PSII at 2 dpi.

(C) Relative *B. cinerea actin* transcript abundance in infected leaves at 1 dpi.

(D) The changes of IAA content in *SICA2*-silenced tomato plants at 0.5 dpi, as affected by exogenous PSK and NAA application.

The results in (B to D) are presented as the mean values \pm SD; $n = 3$. Different letters indicate significant differences between treatments ($P < 0.05$, Tukey's test). The above experiments were repeated three times with similar results.

Figure 8

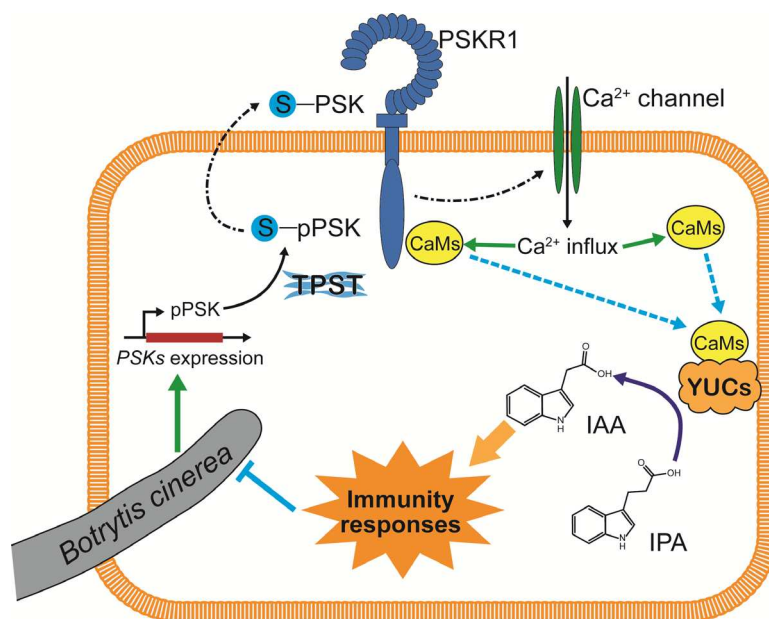


Figure 8. A working model of PSK-induced immunity against *B. cinerea* in tomato plants. PSK plays as a damage-associated molecular pattern (DAMP), its precursors and protein processing are activated upon *B. cinerea* inoculation. At the apoplast, PSK signaling peptide is mainly perceived by its receptor PSKR1, which transduce the signal into cytoplasm by initiating cytosolic Ca^{2+} influx. The transient cytosolic Ca^{2+} are further transduced to CaMs which binds to YUCs, promoting auxin biosynthesis and associated signaling to combat *B. cinerea* infection.