

Supporting Information

Wilton et al. 10.1073/pnas.0904739107

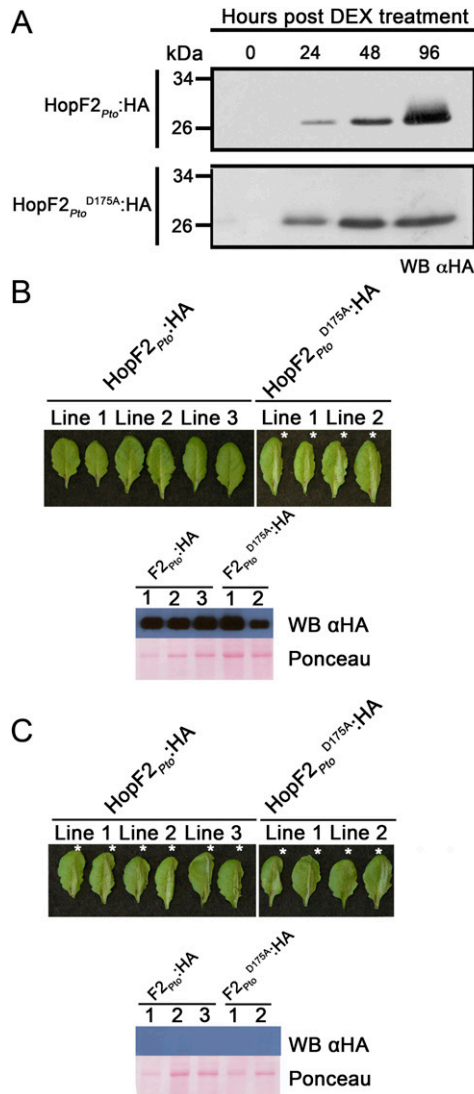


Fig. S1. AvrRpt2-induced HR in multiple independent HopF2_{Pto}:HA transgenic lines. (A) Western blot of transgenic expression of HopF2_{Pto}:HA or HopF2_{Pto}^{D175A}:HA after application of 30 μM DEX. (B and C). Half-leaves of independent *Arabidopsis* HopF transgenic lines were treated with DEX for 24 h (B) or water (C) and subsequently infiltrated with *Pto* DC3000 (5×10^7 cfu/mL) expressing AvrRpt2 and monitored for macroscopic tissue collapse associated with an HR. Experiments presented in Figs. 1–3 were conducted with transgenic line 1 of both HopF2_{Pto}:HA or HopF2_{Pto}^{D175A}:HA. Asterisks indicate leaf tissue displaying macroscopic HR. Pictures were taken ≈20 h after inoculation. Western blot analysis confirms transgenic expression of HopF2_{Pto}:HA and HopF2_{Pto}^{D175A}:HA, ≈48 h after application of 30 μM DEX. Ponceau staining is presented as a loading control.

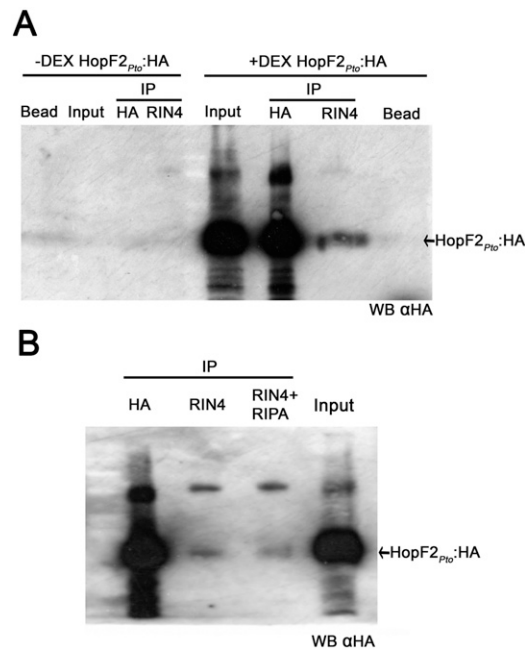


Fig. S2. Interaction of RIN4 with HopF2_{Pto} is specific. (A) Proteins from plants that are not expressing HopF2_{Pto}:HA (–DEX) or expressing HopF2_{Pto}:HA (+DEX) were extracted in IP buffer. Immunoprecipitation was performed by incubating 750 μg of protein in 50 μL of HA-conjugated magnetic beads (HA) or 1 μL of RIN4 polyclonal antibodies for 1 h and followed by 100 μL of Protein A-conjugated magnetic beads (RIN4). The same amount of protein was incubated with 100 μL of Protein A-conjugated magnetic beads alone, which served as negative control (B). Five micrograms of input protein was used as positive control for the HopF2_{Pto}:HA (Input). The incubation time and the washes for the immunoprecipitation experiments and immunoblot analysis with the HA antibodies are detailed in *Methods*. (B) Proteins from tissues expressing HopF2_{Pto}:HA were immunoprecipitated as described in A, except the washing steps following immunoprecipitation with the RIN4 antibodies were conducted in RIPA buffer (RIN4 RIPA). Immunoblot analysis with the HA antibody was performed as described in *Methods*.

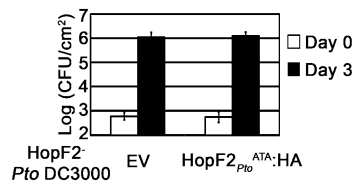


Fig. S3. HopF2⁻ mutant *Pto* DC3000 expressing HopF2_{Pto}^{ATA}:HA does not show altered virulence in *Arabidopsis* relative to *Pto* DC3000 HopF2⁻ expressing the EV (EV). Growth of HopF2_{Pto}⁻ mutant *Pto* DC3000 expressing HopF2_{Pto}^{ATA}:HA or the empty vector in *Arabidopsis* Col-0. Bacteria were syringe-infiltrated by using a suspension containing 1 × 10⁵ cfu/mL. Bacterial counts were taken 1 h postinoculation (day 0) and 3 days postinoculation. Error bars represent the SD of eight samples.

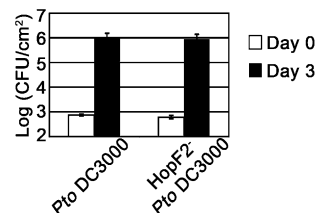


Fig. S4. HopF2⁻ mutant *Pto* DC3000 does not show altered virulence in *Arabidopsis* relative to wild-type *Pto* DC3000. Growth of *Pto* DC3000 and the HopF2⁻ mutant *Pto* DC3000 in *Arabidopsis* Col-0. Bacteria were syringe-infiltrated by using a suspension containing 1 × 10⁵ cfu/mL. Bacterial counts were taken 1 h postinoculation (day 0) and 3 days postinoculation. Error bars represent the SD of eight samples.

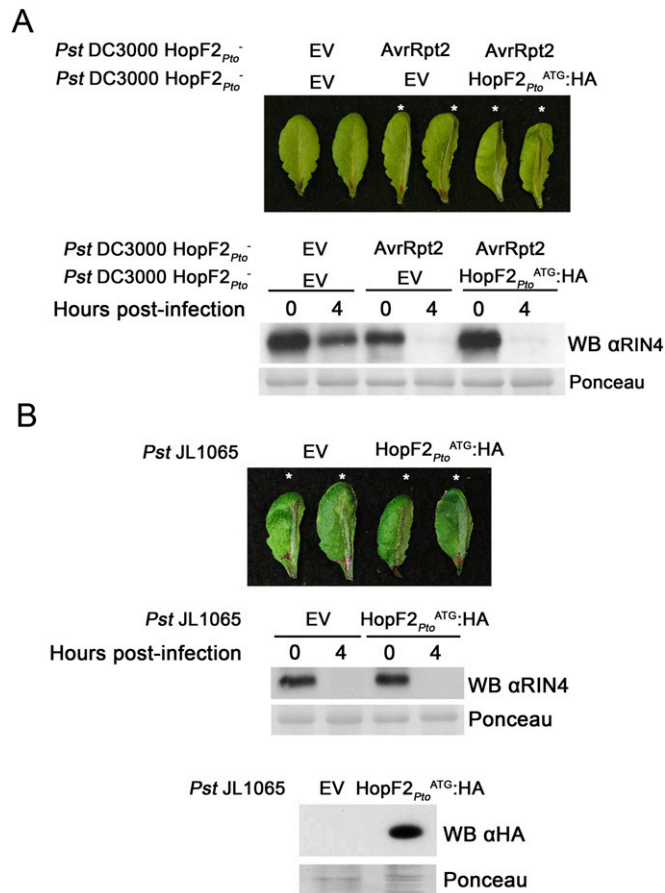


Fig. S5. HopF2_{Pto}:HA does not suppress AvrRpt2-elicited ETI when delivered by *Pseudomonas syringae*. (A) 1×10^8 cfu/mL (0.2 OD₆₀₀) *Pto* DC3000 HopF2⁻ strain expressing AvrRpt2 mixed with 1×10^8 cfu/mL of either *Pto* DC3000 HopF2⁻ expressing HopF2_{Pto}^{ATG}:HA (HopF2_{Pto}) or empty vector (EV) (final titer of each strain is 5×10^7), and syringe-infiltrated into pairs of *Arabidopsis* Col-0 leaves [Ritter C, Dangl JL (1996) Interference between two specific pathogen recognition events mediated by distinct plant disease resistance genes. *Plant Cell* 8:251-257]. Leaves were monitored for macroscopic HR. No observable difference of AvrRpt2-induced HR was observed in the presence of bacteria expressing HopF2_{Pto}^{ATG}:HA. Below is an immunoblot analysis of *Arabidopsis* extracts with anti-RIN4 antibody indicating no alteration in AvrRpt2-mediated RIN4 degradation in the presence of *Pseudomonas* expressing HopF2_{Pto}^{ATG}:HA at 4 h postinfection. (B Top) 2×10^8 cfu/mL (0.4 OD₆₀₀) *Pto* JL1065 expressing HopF2_{Pto}^{ATG}:HA (HopF2_{Pto}) or empty vector (EV) syringe-infiltrated into pairs of *Arabidopsis* Col-0. *Pto* JL1065 natively expresses AvrRpt2 and induces an AvrRpt2-dependent HR [Lim MT, Kunkel BN (2005) *The Pseudomonas syringae avrRpt2* gene contributes to virulence on tomato. *Mol Plant-Microbe Interact* 18:626-633]. No significant difference in AvrRpt2-induced HR was observed upon inoculation of *Pto* JL1065 expressing HopF2_{Pto}^{ATG}:HA or EV. (B Middle) Anti-RIN4 immunoblot indicating that AvrRpt2-mediated RIN4 degradation is not altered by HopF2_{Pto}^{ATG}:HA expression in *Pto* JL1065. (B Bottom) Anti-HA immunoblot to detect the expression of HopF2_{Pto}^{ATG}:HA in *Pto* JL1065. Ponceau staining is presented as a loading control. Asterisks indicate leaves displaying macroscopic HR. These experiments were repeated four times with similar results. Photographs were taken at ≈ 20 h postinfection.

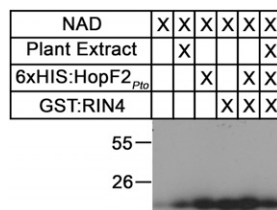


Fig. S6. Lack of HopF2_{Pto} ADP ribosyltransferase activity. Purified recombinant 6xHIS:HopF2_{Pto} displayed no ADP ribosyltransferase activity in an assay with purified recombinant GST:RIN4 supplemented with radiolabeled NAD. Addition of DEX-induced transgenic HopF2_{Pto}:HA *Arabidopsis* plant extract (Plant Extract) with purified 6xHIS:HopF2_{Pto} and/or GST:RIN4 still exhibited no ADP ribosyltransferase activity using previously described in vitro assays (1, 2).

- Singer AU, et al. (2004) Crystal structures of the type III effector protein AvrPphF and its chaperone reveal residues required for plant pathogenesis. *Structure* 12:1669-1681.
- Coburn J, Wyatt RT, Iglewski BH, Gill DM (1989) Several GTP-binding proteins, including p21c-H-ras, are preferred substrates of *Pseudomonas aeruginosa* exoenzyme S. *J Biol Chem* 264: 9004-9008.

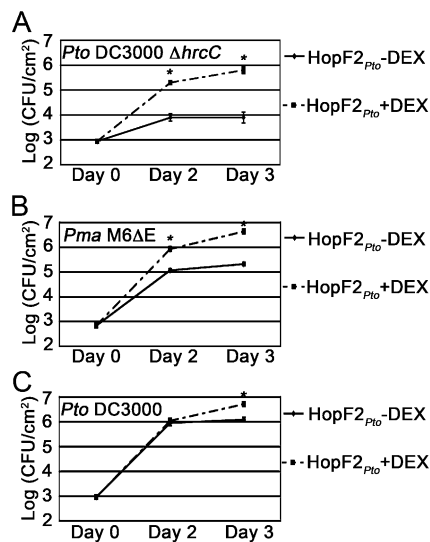


Fig. S7. Growth of *P. syringae* strains in HopF2_{Pto}:HA transgenic *Arabidopsis* Col-0. *Pto* DC3000 Δ *hrcC* (A), *Pma* M6 Δ E (B), and *Pto* DC3000 (C) were syringe-infiltrated by using a suspension containing 1×10^5 cfu/mL into transgenic HopF2_{Pto}:HA *Arabidopsis* Col-0 leaves. Plants were sprayed with 30 μ M DEX (+DEX) or water (-DEX) immediately after infiltration. Bacterial counts were taken 1 h postinoculation (day 0), 2 days postinoculation, and 3 days postinoculation. Error bars represent the SD of eight samples. Asterisks denote statistically significant differences in bacterial growth between the -DEX and +DEX values at a given time point [Fisher's protected least significant difference post hoc (FLSD) test, $P < 0.05$].

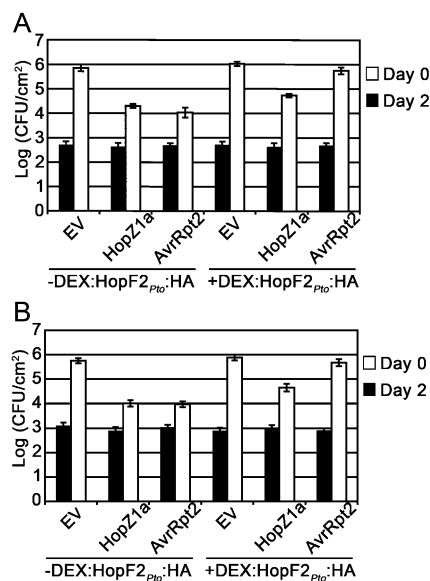


Fig. S8. Transgenic expression of HopF2_{Pto}:HA suppresses AvrRpt2-elicited ETI in an independent HopF2_{Pto}:HA transgenic line (A, transgenic line 2 in Fig. S1) or application of 3 μ M DEX (B, transgenic line 1 in Fig. S1). Plants were sprayed with 30 μ M (A) or 3 μ M DEX (B) (+DEX) or water (-DEX) immediately after infiltration of *Pto* DC3000 at 1×10^5 cfu/mL. Bacterial counts were taken 1 h postinoculation (day 0) and 2 days postinoculation. Error bars represent the SD of eight samples.

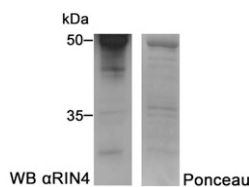


Fig. S9. Degradation products observed by Coomassie staining of purified GST:RIN4 recombinant protein are detected by anti-RIN4 antibody. Expression, purification, and immunoblot analysis of RIN4:GST was conducted as described in *Methods*.