**Text S1**

**Supporting Materials and Methods**

**Bacterial Strains, Plant Material and Growth Conditions.** All strains and plasmids used in this study are listed in Tables S1–S2. *Pseudomonas syringae* pv *tomato* DC3000 (*Pto*DC3000) and its derivatives (see Table S1), were grown on agar and liquid King’s broth [[1](#_ENREF_1)] with 34 μg/mL rifampicin at 30**°**C. *E. coli* DH5α and *E. coli* BL21 strains were grown on agar and liquid Luria-Bertani broth [[2](#_ENREF_2)] with appropriate antibiotics at 37**°**C. *Arabidopsis thaliana* accession Col and transgenic derivatives (Lifeact-GFP, kindly provided by Dr. Michael J. Deeks at the University of Durham [[3](#_ENREF_3)]; dex:HopW1-HA, stably transformed using JJ74 (Table S2)) were grown in soil for 2 to 3 weeks for assays of bacterial growth and protoplast isolation. Arabidopsis Col and Col/Lifeact-GFP grown on MS agar plates in a growth chamber for 6- to 8-d were used for actin quantification and endocytosis inhibition assays after bacterial infection. All Arabidopsis plants were grown under 16 h light, 8 h dark at 21**°**C. *N. benthamina* used for transient expression was grown for 3-4 weeks at 24**°**C with 16h light/8h dark cycle.

**Bacterial Growth Assay *in Planta.*** One ml blunt syringes were used to inoculate *Pto*DC3000 and its derivatives (see Table S1) at OD600 of 0.0001 in 10 mM MgSO4 and/or 10 μM LatB into leaves of 3-week old soil grown Arabidopsis. After infiltration, inoculated plants were covered and returned to the growth room. Bacterial populations were quantified three days post-inoculation from 3 samples each of which had 3 whole infected leaves. For statistical analysis, data from three independently performed experiments were analyzed together. Each inoculated leaf area was measured by Image J software, and then the leaves were homogenized in 1 mL of 10 mM MgSO4. Samples were serially diluted and enumerated by colony forming units after plating on KB agar with tetracycline (12.5 μg/mL).

**Immunoprecipitation and Western Blot Analysis.** Immunoprecipitation (IP) of HopW1-HA-containing complexes with anti-HA matrix (Roche) was done as described [[4](#_ENREF_4)]. HopW1-HA was immunoprecipitated from transgenic Arabidopsis (JJ74) 22 h after treatment with 0.3 μM dexamethasone (dex). *N. benthamiana* leaves were infiltrated with Agrobacterium GV3101 harboring HopW1-HA or its fragments. Tissue for IP was collected 36-38 h after Agroinfiltration with 35S construct (JJ31), or leaves were sprayed with 20 μM dex 22h after Agroinfiltration (JJ74, HopW1-C, HopW1-N) and collected 15h later. After IP, proteins were separated by SDS/PAGE and analyzed either by Coomassie blue staining and LC-MS/MS or by immunoblotting with anti-HA (1:1200, Covance) and anti-actin (1:1000, [[5](#_ENREF_5)]) monoclonal antibodies and secondary horseradish peroxidase-conjugated anti-mouse antibody (1:20,000, Thermo Fisher Scientific). Actin antibody was kindly provided by Dr. M V. Parthasarathy, Cornell University. Membranes were subsequently stained with Coomassie blue to control for loading using standard procedures for gels.

**Purification of Recombinant HopW1-C Protein.** The His-tagged HopW1-C (HopW1407-774) protein was produced in *Escherichia coli* BL21 (DE3) (Stratagene, USA). After isopropyl β-D-1-thiogalactopyranoside induction (1 μM, for 3 h) *E. coli* proteins were extracted in protein extraction buffer (50mM phosphate buffer, 300mM NaCl2, 2M urea, 10% glycerol, pH 8.0) and purified using Ni-NTA spin columns (Qiagen, USA). Protein extraction buffer was exchanged to protein buffer (50mM phosphate buffer, 300mM NaCl2, 5mM DTT, 10% glycerol, pH 8.0) using a Zeba™desalting spin column (Thermo Scientific, USA) or dialysis.

**Arabidopsis Protoplast Isolation and Transformation.** Arabidopsis protoplasts were prepared from 2 - 3 week-old Arabidopsis leaves (Col, Col/Lifeact-GFP or Col/dex:HopW1). 0.5 − 1 mm leaf strips were digested in enzyme solution containing 1.5 % cellulase R10 (SERVA, Germany), 0.4 % macerozyme R10 (SERVA, Germany), 0.4 M mannitol, 20 mM KCl, 10 mM CaCl2, 0.1 % BSA, and 20 mM MES, pH 5.7. The digestions were continued for 3 h with gentle shaking in the dark. The resulting protoplasts were filtered through a 40 μm nylon filter and washed by centrifugation (100 × g, 5 min) and resuspended in buffer W5 (154 mM NaCl, 125 mM CaCl2, 5 mM KCl, and 2 mM MES, pH 5.7). The intact protoplasts were obtained using 20% sucrose-gradient centrifugation (500 × g, 5 min). Plasmids encoding HopW1*-*CFPor trafficking markers (SPO-GFP and AALP-GFP) (see Table S1) were introduced by polyethylene glycol mediated transformation [[6](#_ENREF_6),[7](#_ENREF_7)]. The expression of the fusion constructs was monitored by fluorescence microscopy at various time points after transformation.

**Confocal Microscopy.** For protein trafficking and endocytosis analysis, images were captured with an electron amplified CCD camera (ImagEM, Hamamatsu) and a confocal fluorescence microscope (Olympus DSU Spinning Disk Confocal System) using large pinhole, with the filter sets for GFP (ex:480/25 and em:525/40), CFP (ex:436/10 and em:470/30), and chlorophyll autofluorescence (ex:635/20 and em:685/40), respectively.

For actin cytoskeleton (marked by Lifeact-GFP) visualization in the presence of HopW1 in Arabidopsis protoplasts and *N. benthamiana* leaves or during infection of Arabidopsis cotyledons, a Zeiss LSM710 laser-scanning confocal microscope (Zeiss, Germany) was used. Fluorescence was visualized as follows: GFP excitation 488 nm/emission 505-530 nm ; mCherry/RFP: ex 561/em 570-620 nm; CFP: ex 405 nm/em 550-500 nm; and chlorophyll autofluorescence: ex 633/em 650-750 nm. Images (512x512 pixels scanning resolution in maximum speed mode) were taken using a LD C-Apochromat 40x/1.1 W Korr objective. Z-series optical sections of infected Arabidopsis and transformed *N. benthamiana* epidermal cells were captured. GFP and RFP or GFP and CFP fluorescence was acquired for the same field using a sequential acquisition mode.

Images were processed using Slidebook 5.0 (Intelligent Imaging Innovations, Inc., USA), ImageJ (http://rsb.info.nih.gov/ij) and Adobe Photoshop software.

**Muscle F-actin Disruption Assays.** For assays using chicken muscle actin, Ca-ATP actin was extracted from chicken muscle acetone powder (Pel-Freez Biologicals, Rogers, Arkansas) as described [[8](#_ENREF_8)]. Ca-ATP actin was converted to Mg-ATP actin by incubation with 0.1 volume of 0.5 mM MgCl2 and 2 mM EGTA for 2 min at room temperature prior to each experiment. F-actin was assembled from Mg-ATP-actin in buffer containing 10 mM Imidazole-HCl pH 7.0, 50 mM KCl, 1 mM EGTA, and 1 mM MgCl2 for 1 hour at room temperature. Preassembled muscle F-actin was incubated for 1h with HopW1-C or BSA and visualized as described for non-muscle actin.

**Supporting References**

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