Supplementary Figure 1: Characterization of the XopH protein phosphatase activity.

a, Dephosphorylation of pNPP by XopH in different buffering systems. b, Dephosphorylation of pNPP by XopH (WT) and catalytic variants in 50 mM HEPES pH 7.0. c, Dephosphorylation of 0.5 mM commercial phosphor-peptides by XopH. pThr, PRApTVA; pSer, PRApSV; pTyr1,
pTyr2, DADEpYLIPQQP; pTyr3, RRLIEDAEpYAARG; pTyr4, TSTEPQpYQPGNEL. d, pTyr dephosphorylation by XopH (WT) and mutant derivatives. GFP served as negative control. e, Two-sample logo of pTyr dephosphorylation by XopH displaying enriched and depleted amino acid residues surrounding the dephosphorylated tyrosine. The logo was created from 72 top substrates out of >6000 peptides tested. f, pTyr2 dephosphorylation by XopH under high and low salt conditions. g, Kinetics of pTyr2 and pTyr-chip (KVDVDEpYDENKFVW) dephosphorylation by XopH. The maximal velocity $V_{\text{max}}$ and Michaelis constant $K_M$ were $0.63 \pm 0.16 \ \mu M \ \text{min}^{-1}$ and $25.7 \pm 12.2 \ \mu M$ for pTyr2 (circles), $0.21 \pm 0.12 \ \mu M \ \text{min}^{-1}$ and $29.7 \pm 3.0 \ \mu M$ for pTyr-chip (triangles). The experiments in a-d, f and g were performed twice with similar results, using two independent protein preparations each. Values are means of two technical replicates. Error bars indicate s.d.
Supplementary Figure 2: Structures and symmetry of InsP₆ and dephosphorylated derivatives.
Supplementary Figure 3: InsP$_5$ is the main product of XopH-dependent InsP$_6$ dephosphorylation.

a. InsP$_6$ was incubated with XopH for various time points as indicated. Reaction products were separated by PAGE and visualized by toluidine blue. Undigested InsP$_6$ served as control. Orange
G, loading dye. The experiment was repeated once with similar results. b, MALDI-ToF-MS of gel-purified hydrolysis product of InsP₆ after treatment with recombinant XopH enzyme. The obtained ion mass (578.9) corresponds to an InsP₅. c, LC-ToF-MS analysis of myoinositolpolyphosphates in recombinant XopH enzyme assays. Expected retention times: InsP₆: 19.33 min, InsP₅: 19.26 min (all isomers coelute in one peak), InsP₄: 19.08 min (all isomers coelute in one peak), InsP₃: 17.5-18.2 min (dependent on isomer). The experiment was repeated twice with similar results. d, LC-QToF-MS/MS analysis of InsP₅ [1-OH]. Left panel: chromatographic retention of InsP₅ monoisotopic precursor ion mass ([M-H] = 578.888 Da ± 0.02 Da), right panel: MS/MS spectra of InsP₅ from recombinant XopH enzyme assays and InsP₅ [1-OH] standard. e, High-performance ion chromatography (HPIC) analysis of XopH-dependent hydrolysis products of InsP₆, InsP₅ [1-OH] and InsP₅ [3-OH]. An InsP₅ mix served as standard: a, InsP₆; b, Ins(1,3,4,5,6)P₅; c, Ins(1,2,4,5,6)P₅; d, Ins(1,2,3,4,5)P₅; e, Ins(1,2,3,4,6)P₅; f, Ins(1,2,5,6)P₄; g, Ins(1,3,4,5)P₄; h, Ins(1,2,4,5)P₄; i, Ins(1,2,3,4)P₄/Ins(1,3,4,6)P₄; j, Ins(1,2,3,5)P₄/Ins(1,2,4,6)P₄; k, Ins(4,5,6)P₃; l, Ins(1,5,6)P₃.
Supplementary Figure 4: InsP₅ [1-OH] is the likely XopH-dependent InsP₆ dephosphorylation product.

a, Reaction products (RP) obtained by phytate digestion with XopH (0.13µg/µL; 30 min) were mixed with InsP₅ isomers as indicated, separated by PAGE and visualized by toluidine blue staining. InsP₆ and InsP₅ isomers alone served as controls. Double bands report different isomer identities of RP and the respective InsP₅ species tested. 
b, All six different InsP₅ isomers (10 nmol) were digested with XopH, separated by PAGE and visualized by toluidine blue staining. 
c, An InsP₅ [1-OH]/XopH reaction mixture was incubated for 30 min at 28°C, then supplemented with InsP₆ (10 nmol) for additional 30 min, separated by PAGE and visualized by toluidine blue staining. InsP₆, InsP₅ [1-OH] and the InsP₅ [1-OH]/XopH reaction mixture (incubated for 1 h at
28°C prior to loading onto the gel) served as controls. The experiments were done twice with similar results.
Supplementary Figure 5: InsP₆ synthesis and quality control.

a, Chemical synthesis of highly pure InsP₆: (i) Bis-fluorenylmethyl-diisopropylamino-P-amidite (CAS: 197709-11-8), 4,5-Dicyanoimidazole, then mCPBA, 32 % yield; (ii) 5% Piperidine in DMF, then precipitation (and ion exchange with NaI), 92% yield. b-d, Quality control of synthetic InsP₆. b, InsP₆, ³¹P-NMR in D₂O. c, InsP₆, ¹H-NMR in D₂O. d, InsP₆, ¹³C NMR in D₂O.
Supplementary Figure 6: L-Arg-N enables the discrimination of InsP$_5$ [1/3-OH] by $^{31}$P-NMR.
Overlay of separately recorded mixtures of either InsP$_5$ [1-OH] (A) or InsP$_5$ [3-OH] (B), 100 µg each, in ammonium acetate buffer in the presence of excess of L-Arg-N (ca. 100 fold). The relative position of the peaks is identical to those found in spiking experiments.
Supplementary Figure 7: XopH leads to InsP₅ accumulation in planta.
a, HPLC profiles of neutralized extracts from 16-day old N. benthamiana transgenic seedlings labelled with [³H]-myo-inositol. Based on published chromatographic mobilities¹, InsP₅a represents InsP₅ [2-OH] and InsP₅c represents InsP₅ [1-OH] or its enantiomer InsP₅ [3-OH]. The
isomeric nature of InsP$_{3a-c}$, InsP$_{4a-b}$, InsP$_7$ and InsP$_8$ is unknown. **b,** Zoom-in into the HPLC profile. **c,** Relative amounts of InsP$_{5c}$ and InsP$_6$ in control line expressing *gfp* and two independent *xoph*-expressing lines. Error bars indicate s.e.m. **d,** LC-ToF-MS analysis of myo-inositolpolyphosphates in transgenic *N. benthamiana* seedlings. Plant extracts were generated as in **a,** but with unlabeled myo-inositol. Expected retention times: InsP$_6$: 19.33 min, InsP$_5$: 19.26 min (all isomers coelute in one peak), InsP$_4$: 19.08 min (all isomers coelute in one peak), InsP$_3$: 17.5-18.2 min (isomer-dependent). The experiments were performed three times with similar results.
Supplementary Figure 8: The enantiomer identity of XopH-dependent InsP₆ hydrolysis in planta is InsP₅ [1-OH].

Digestion and HPLC analyses of plant-purified InsP₅c. InsP₅c was purified from [³H]-myo-inositol-labeled xopH- and GFP-expressing *N. benthamiana* seedlings (see methods). XopH-treated or non-treated InsP₅c was then separated by SAX-HPLC. XopH was inactivated by incubating the reaction mixture at 95°C for 15 min. The figure shows the full chromatograms of the respective selections (min 45-71) depicted in Figure 7.
Supplementary Figure 9: XopH reduces the InsP₆ content of pepper leaves during Xcv infection.

HClO₄ extracts of *C. annuum* ECW leaves infected with Xcv strains as indicated were subjected to TiO₂ bead enrichment. Inositol polyphosphates were eluted from TiO₂ beads, resolved by PAGE and stained with toluidine blue. Protein extracts were visualized by Coomassie blue as a loading control. The experiment was repeated twice with similar results.
Supplementary Figure 10: XopH affects plant growth.
Photographs of two-months-old transgenic *N. benthamiana* plants grown on 0.5 MS + 1% sucrose. Scale bars, 1 cm.
Supplementary Figure 11: HopAO1 from *Pseudomonas syringae* pv. *tomato* hydrolyzes InsP₆ into lower phosphorylated myo-inositol derivatives.

Recombinant XopH (0.13 µg/µL) or indicated amounts of recombinant HopAO1 were incubated with 10 nmol InsP₆ in reaction buffer (10 mM NaCl, 5% glycerol, 5 mM β-mercaptoethanol and 50 mM HEPES, pH 7.0) at 28°C for 30 min. Reaction products were separated by PAGE and visualized by toluidine blue staining. Orange G, loading dye. The experiment was repeated once with similar results.
Supplementary Figure 12: Unprocessed Western blots.

a, Reproduction of Fig. 1e (top) and the full blot of the membrane including a longer exposure (bottom). Please note that we removed three lanes that were not relevant for the results presented (stippled lines). b, c, Full Western blots used in Fig. 3c (b) and Fig. 8b (c).
Supplementary Figure 13: Uncropped gels.
Supplementary Figure 13: Uncropped gels (continued).
## Supplementary Table 1

### Oligonucleotides used in this study.

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**xopH-early-stop-B-fw**

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**pUC57-BamHI-early-stop-rv**

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CACCAGATCTGCCACTTGGATTAATGGTG

si NbCOI1 rev

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si CaEIN2 fwd

CAACGAGGTGTACTTCTCTTC

si CaEIN2 rev

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si CaEBF1 fwd

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si NbMYC2 fwd

GATGGGATGCTATGATTCGTATAC

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si NbPR1 fwd

CATTACGGCCAAACCTAGC

si NbPR4 fwd

GCCAAGATTCTCGTGTAGAT

si NbLR23 fwd

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si NbLR23 rev

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si NbCOI1 fwd

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si NbCOI1 rev

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si NbEBF1 rev

CAAGGGAGAAGATGCTGC

XopH ATG fw

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XopH rev-3

GTCACGTGATTTGCCACG

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5' phosphorylation; b 1-bp exchange in *N. benthamiana*; c adapted for *N. benthamiana* from references 2 and 3.
Supplementary References

