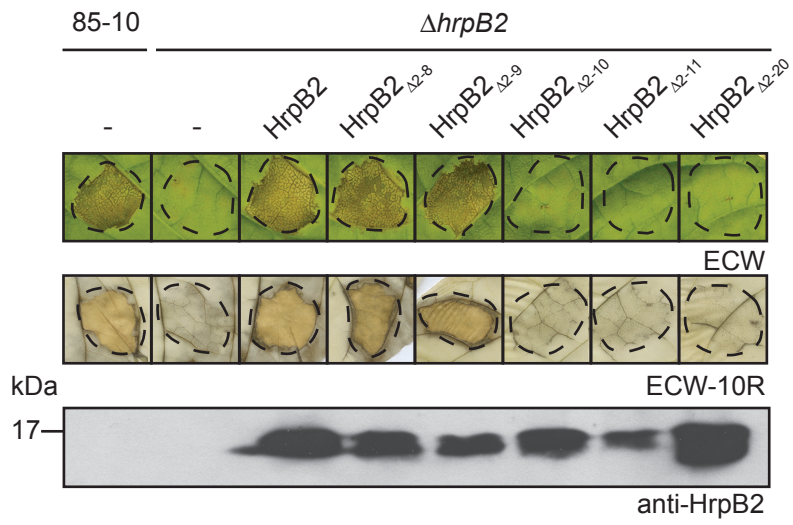


Supplemental material

Type III-dependent translocation of HrpB2 by a non-pathogenic *hpaABC* mutant of the plant-pathogenic bacterium *Xanthomonas*

Felix Scheibner, Steve Schulz¹, Jens Hausner, Sylvestre Marillonnet² and Daniela Büttner[#]

A



B

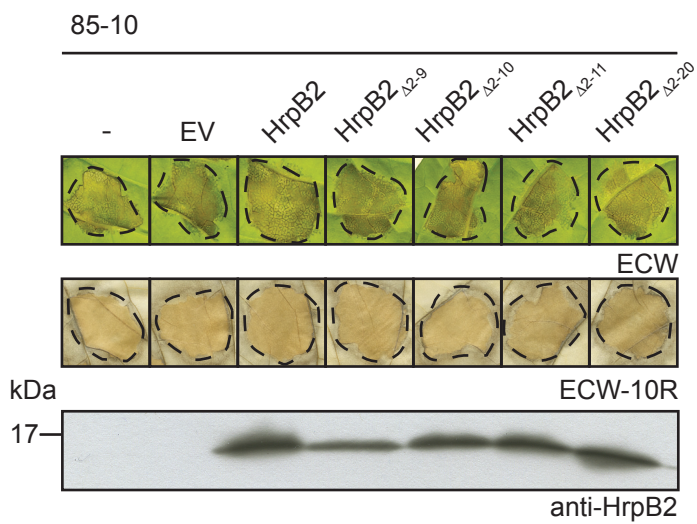


Figure S1
Scheibner *et al.*

Fig. S1 The N-terminal region of HrpB2 contributes to protein function.

(A) The N-terminal 10 amino acids of HrpB2 are essential for protein function. Strains 85-10 and 85-10 Δ *hrpB2* (Δ *hrpB2*) without plasmid (-) or with expression constructs encoding HrpB2 or N-terminal deletion derivatives thereof as indicated were inoculated into leaves of susceptible ECW and resistant ECW-10R pepper plants. Disease symptoms were photographed 12 dpi. For the better visualization of the HR, infected leaves of ECW-10R plants were destained in ethanol 2 dpi. Dashed lines mark the infiltrated areas.

(B) HrpB2 derivatives do not exert a dominant-negative effect on pathogenicity of strain 85-10. Strain 85-10 with or without plasmid pBBRMCS-5 (EV, empty vector) or containing HrpB2 or HrpB2 derivatives as indicated was inoculated into leaves of susceptible ECW and resistant ECW-10R pepper plants. Disease symptoms were photographed 10 dpi. Leaves of ECW-10R pepper plants were destained in ethanol 2 dpi.

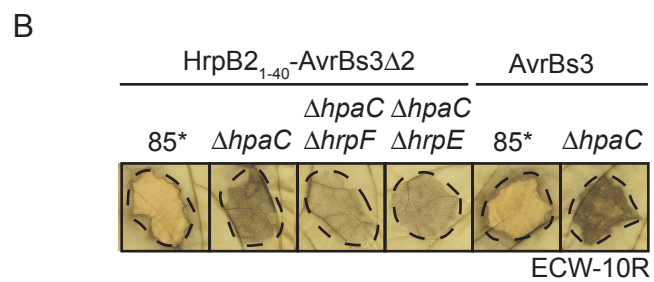
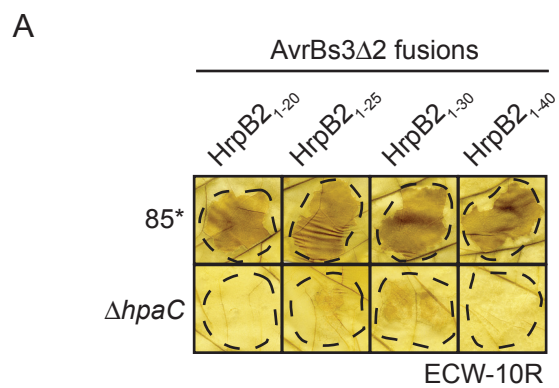


Figure S2
Scheibner *et al.*

Fig. S2 HR induction in AvrBs1-responsive pepper plants by *X. campestris* pv. *vesicatoria* strains containing HrpB2-AvrBs3 Δ 2 fusion proteins.

(A) HrpB2-AvrBs3 Δ 2 fusion proteins do not interfere with the interaction of *X. campestris* pv. *vesicatoria* with AvrBs1-responsive plants. Strains 85* and 85* Δ hpaC (Δ hpaC) containing HrpB2-AvrBs3 Δ 2 fusion proteins as indicated were inoculated into leaves of AvrBs1-responsive ECW-10R pepper plants. Leaves were destained in ethanol 2 dpi.

(B) Infection assays with *X. campestris* pv. *vesicatoria* strains containing HrpB2₁₋₄₀-AvrBs3 Δ 2 or AvrBs3. Strains 85*, 85* Δ hpaC (Δ hpaC), 85* Δ hpaC Δ hrpF (Δ hpaC Δ hrpF) and 85* Δ hpaC Δ hrpE (Δ hpaC Δ hrpE) containing HrpB2₁₋₄₀-AvrBs3 Δ 2, and 85* and 85* Δ hpaC delivering AvrBs3 were inoculated into leaves of ECW-10R pepper plants. Phenotypes were documented as described for panel A.

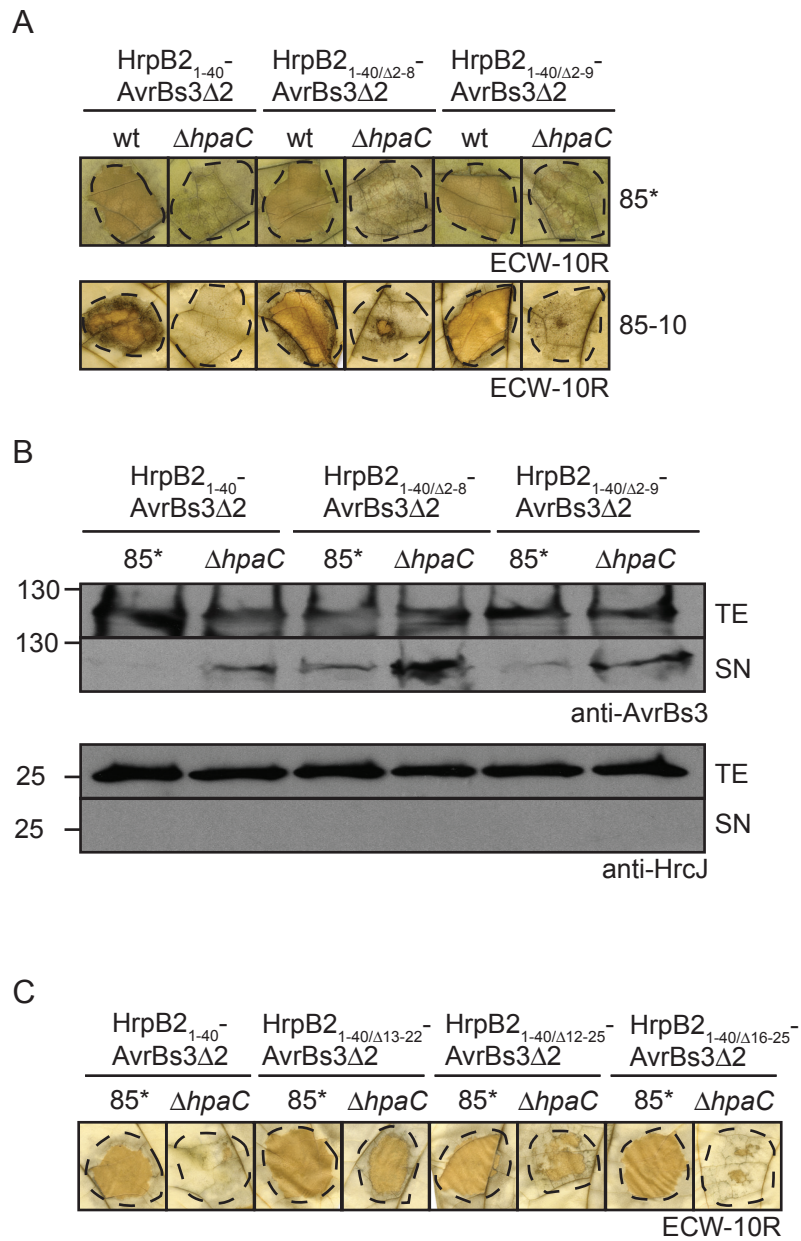


Figure S3
Scheibner *et al.*

Fig. S3 Analysis of HrpB2-AvrBs3 Δ 2 fusion proteins.

(A) Infection assays with *X. campestris* pv. *vesicatoria* wild-type and *hpaC* mutant strains containing HrpB2-AvrBs3 Δ 2 fusion proteins. Strains 85* (wt), 85-10 (wt), 85* Δ *hpaC* (Δ *hpaC*) and 85-10 Δ *hpaC* (Δ *hpaC*) containing HrpB2-AvrBs3 Δ 2 fusion proteins as indicated were inoculated into leaves of AvrBs1-responsive ECW-10R pepper plants. Leaves were destained in ethanol 2 dpi. Dashed lines mark the infiltrated areas.

(B) The N-terminal nine amino acids are dispensable for secretion of HrpB2₁₋₄₀-AvrBs3 Δ 2. Strains 85* and 85* Δ *hpaC* (Δ *hpaC*) containing HrpB2-AvrBs3 Δ 2 fusion proteins as indicated were incubated in secretion medium. Total cell extracts (TE) and culture supernatants (SN) were analysed by immunoblotting using antibodies specific for AvrBs3 and HrcJ, respectively.

(C) Infection assays with *X. campestris* pv. *vesicatoria* strains containing HrpB2-AvrBs3 Δ 2 fusion proteins. Strains 85* and 85* Δ *hpaC* (Δ *hpaC*) containing HrpB2-AvrBs3 Δ 2 fusion proteins as indicated were inoculated into leaves of AvrBs1-responsive ECW-10R pepper plants. Leaves were destained in ethanol 2 dpi. Dashed lines mark the infiltrated areas.

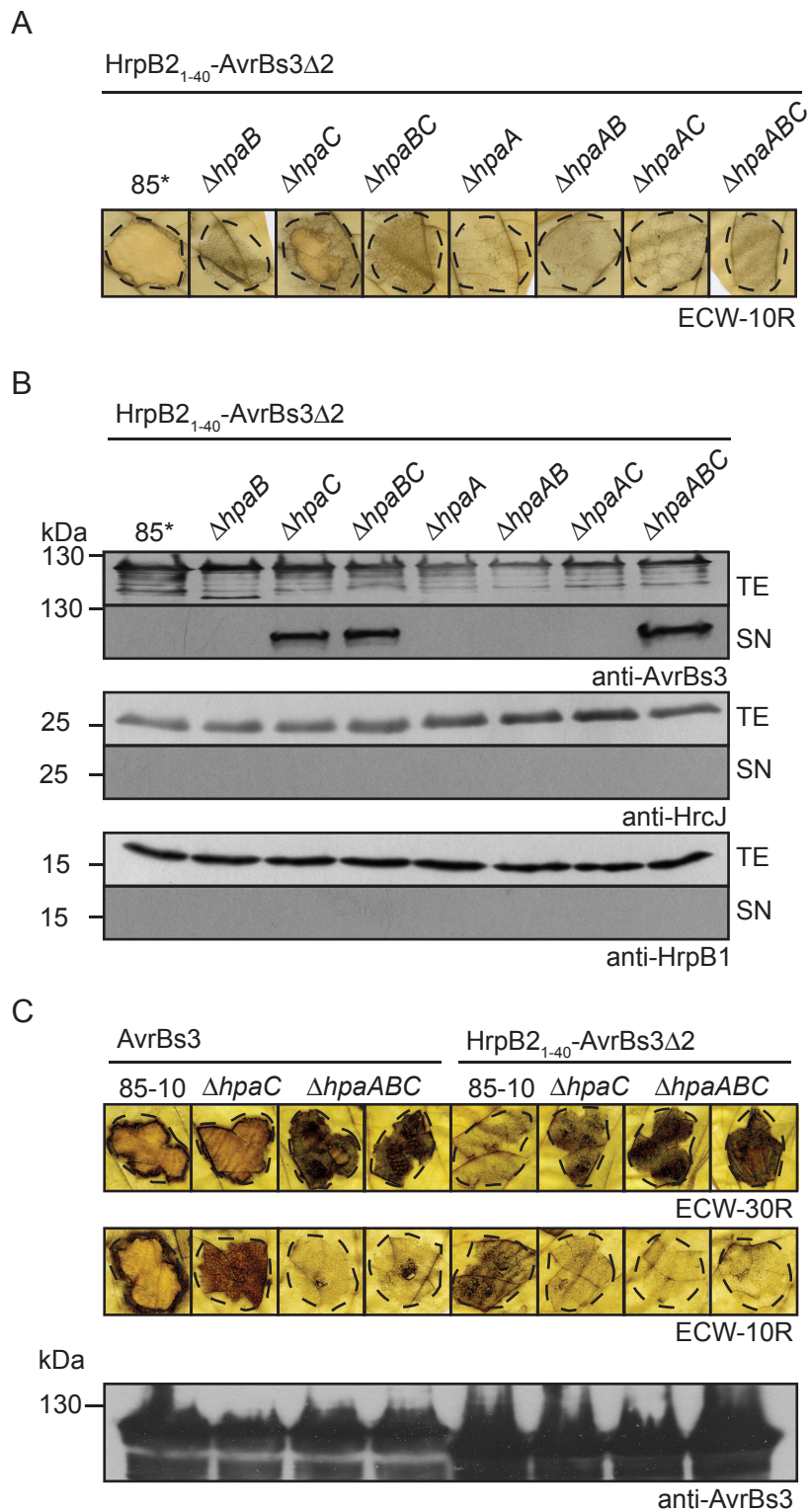


Figure S4
Scheibner *et al.*

Fig. S4 Analysis of HrpB2-AvrBs3 Δ 2 fusion proteins in double and triple *hpa* deletion mutants.

(A) Phenotypes of double and triple *hpa* deletion mutants containing HrpB2₁₋₄₀-AvrBs3 Δ 2 in AvrBs1-responsive pepper plants. Strains 85*, 85* Δ *hpaB* (Δ *hpaB*), 85* Δ *hpaC* (Δ *hpaC*), 85* Δ *hpaBC* (Δ *hpaBC*), 85* Δ *hpaA* (Δ *hpaA*), 85* Δ *hpaAB* (Δ *hpaAB*), 85* Δ *hpaAC* (Δ *hpaAC*) and 85* Δ *hpaABC* (Δ *hpaABC*) containing HrpB2₁₋₄₀-AvrBs3 Δ 2 were inoculated into leaves of AvrBs1-responsive ECW-10R pepper plants. Leaves were destained in ethanol 2 dpi. Dashed lines mark the inoculated areas.

(B) HrpB2₁₋₄₀-AvrBs3 Δ 2 is secreted by *hpaC*, *hpaBC* and *hpaABC* deletion mutants. *X. campestris* pv. *vesicatoria* strains as described in (A) were incubated in secretion medium. Total cell extracts (TE) and culture supernatants (SN) were analysed by immunoblotting using antibodies specific for AvrBs3, HrcJ and HrpB1, respectively.

(C) HR induction by AvrBs3 and HrpB2₁₋₄₀-AvrBs3 Δ 2 is comparable when both proteins are delivered by strain 85-10 Δ *hpaABC*. Strains 85-10, 85-10 Δ *hpaC* (Δ *hpaC*) and 85-10 Δ *hpaABC* (Δ *hpaABC*) containing AvrBs3 or HrpB2₁₋₄₀-AvrBs3 Δ 2 as indicated were inoculated at a bacterial density of 8×10^8 CFU ml⁻¹ into leaves of AvrBs3-responsive ECW-30R and AvrBs1-responsive ECW-10R pepper plants. Leaves were destained 2 dpi (ECW-10R plants) and 4 dpi (ECW-30R plants).

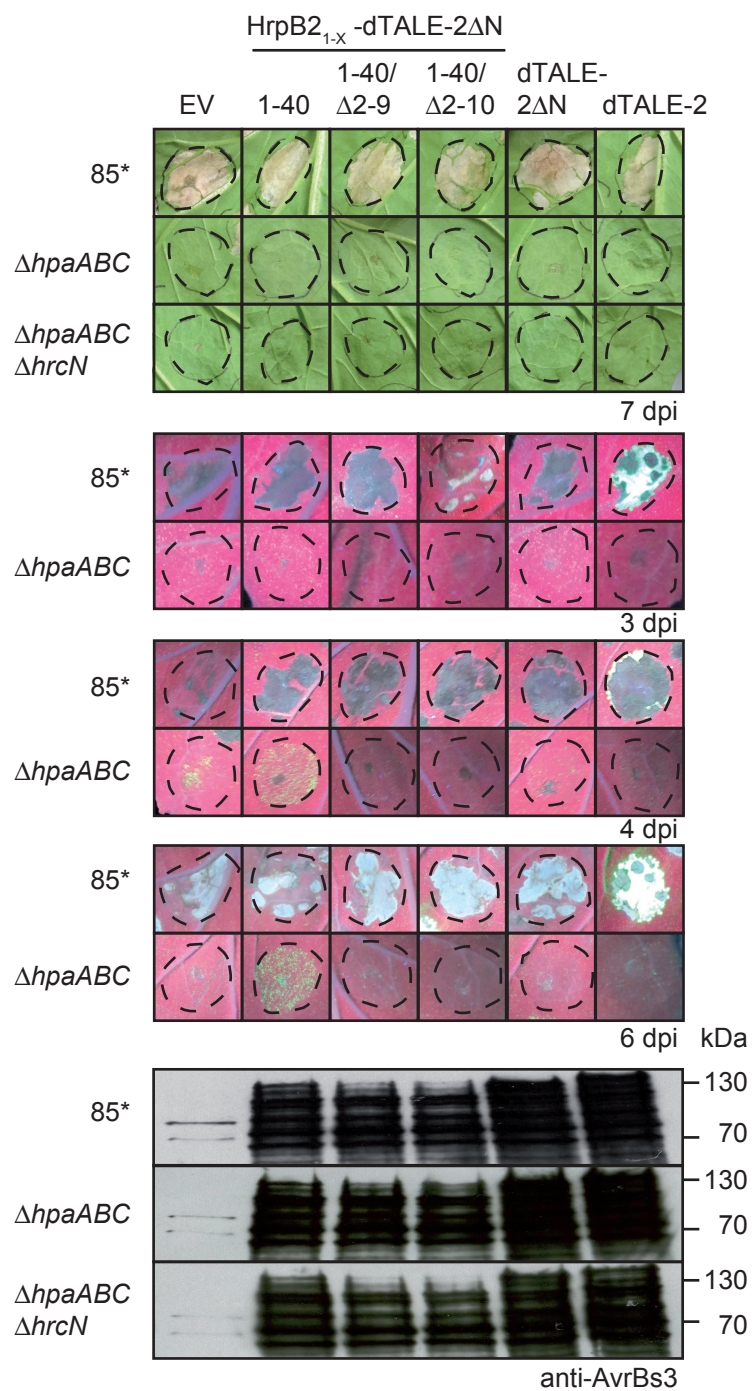


Figure S5
Scheibner *et al.*

Fig. S5 Analysis of dTALE-2 derivatives in *gfp*-transgenic *N. benthamiana* plants.

Strains 85*, 85* $\Delta hpaABC$ ($\Delta hpaABC$) and 85* $\Delta hpaABC\Delta hrcN$ ($\Delta hpaABC\Delta hrcN$) containing the empty vector (EV), HrpB2₁₋₄₀-dTALE-2 Δ N, HrpB2_{1-40/Δ2-9}-dTALE-2 Δ N, HrpB2_{1-40/Δ2-10}-dTALE-2 Δ N, dTALE-2 Δ N or dTALE-2 as indicated were inoculated at a density of 8×10^8 CFU ml⁻¹ into leaves of *gfp*-transgenic *N. benthamiana* plants. Photographs of plant reactions were taken 7 dpi, photographs of GFP fluorescence 3, 4 and 6 dpi as indicated. Equal amounts of cell extracts (adjusted according to the cell density) were analysed by immunoblotting using AvrBs3-specific antibodies. The upper bands correspond to dTALE-2 and derivatives thereof, lower bands are degradation products.

Sequences of dTALE2 constructs

DNA sequences of constructs encoding dTALE-2, dTALE-2 Δ N, HrpB2₁₋₄₀-dTALE-2 Δ N, HrpB2_{1-40/ Δ 2-9}-dTALE-2 Δ N and HrpB2_{1-40/ Δ 2-10}-dTALE-2 Δ N. The *lac* promoter is shown in red, *hrpB2* sequences in green and dTALE-2 sequences in blue. DNA sequences were assembled from individual modules containing the *lac* promoter, *hrpB2* sequences and sequences encoding N-terminal, repeat and C-terminal regions of dTALE-2, respectively, using Golden Gate cloning. For the assembly of the construct encoding dTALE-2 Δ N, an oligonucleotide linker (shown in bold) encoding two lysine residues was inserted upstream of the dTALE-2 Δ N-encoding sequence.

dTALE-2

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dTALE-2ΔN

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HrpB2₁₋₄₀-dTALE-2ΔN

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HrpB2_{1-40/Δ2-9}-dTALE-2ΔN

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