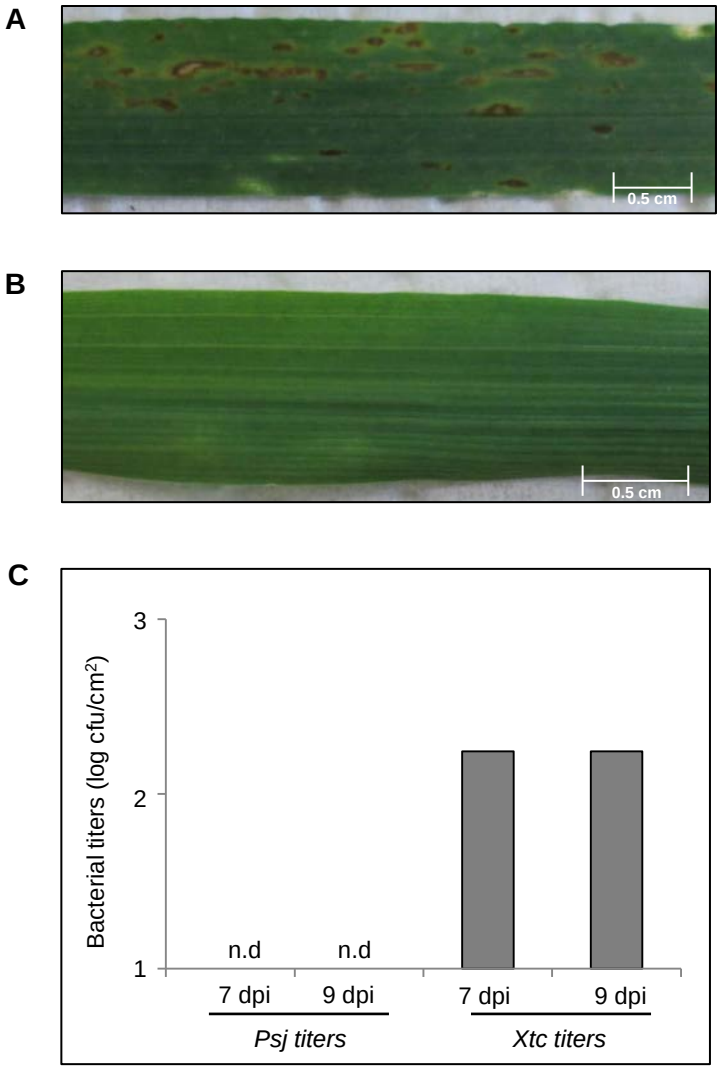

Figure S1 Primary infections. Representative pictures of Barley cv GP leaves 7 days after spray inoculation with (A) *Pseudomonas syringae* pathovar *japonica* (*Psj*) and (B) *Xanthomonas translucens* pathovar *cerealis* (*Xtc*). The experiment was repeated twice with similar results. (C) Bacterial titers at 7 and 9 days post-inoculation (dpi) in systemic untreated leaves of plants that were locally inoculated by infiltration with *Psj* or *Xtc.* n.d., not detected

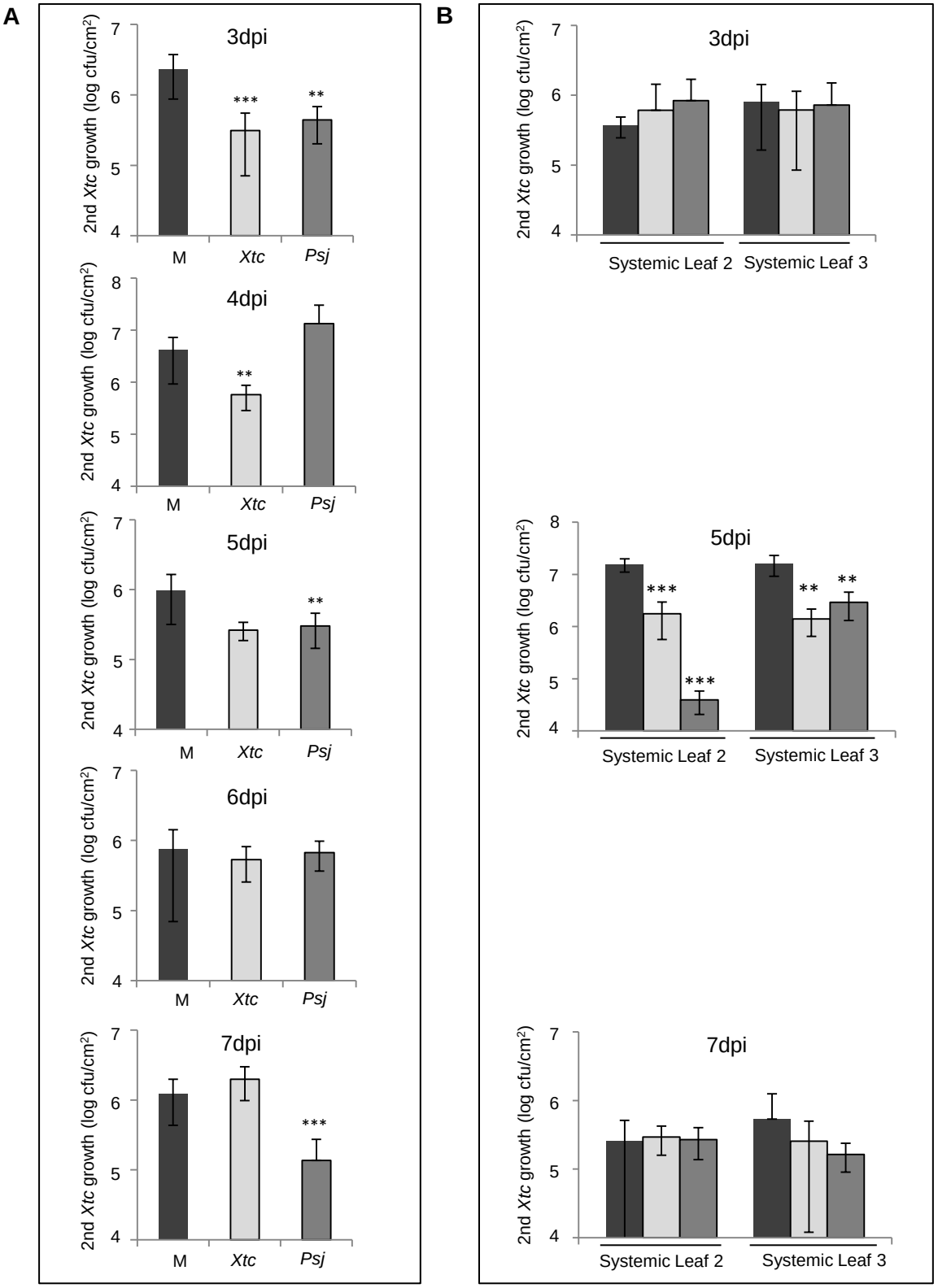

Figure S2 Secondary challenge time course experiments. A and B represent two biologically independent replicate experiments. Barley cv Barke plants were pretreated in leaf 1 with 10 mM $MgCl_2$ (Mock, black bars), *Xtc* (light grey bars), or *Psj* (dark grey bars). At different time points (3-7 days post-infection; dpi) systemic leaves 2 and 3 were infected with *Xtc*. *Xtc* titers in the systemic secondary (2nd) infected leaves are shown at 4 dpi. Bars in (A) include data from leaves 2 and 3 combined. Values indicated are a mean of 4-5 replicates per systemic leaf ± standard deviation. Asterisks indicate a statistically significant difference to Mock (***P*<0.01, ****P*<0.001, student's *t* test).

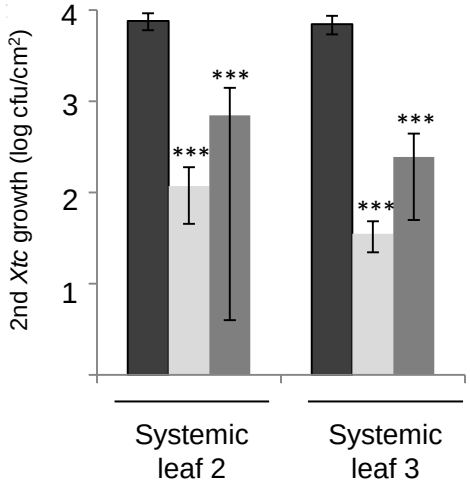

Figure S3 Systemic resistance in barley cv Ingrid. Barley cv Ingrid was pretreated in leaf 1 with 10 mM $MgCl_2$ (Mock, black bars), with *Xtc* (light grey bars), or with *Psj* (dark grey bars). Five days later, (systemic) leaves 2 and 3 were infected with *Xtc*. *Xtc* titers in both systemic secondary (2nd) infected leaves are shown at 4 dpi. Values indicated are a mean of 5 replicates ± standard deviation. Asterisks indicate a statistically significant difference to Mock (***P<0.001, student's t test).

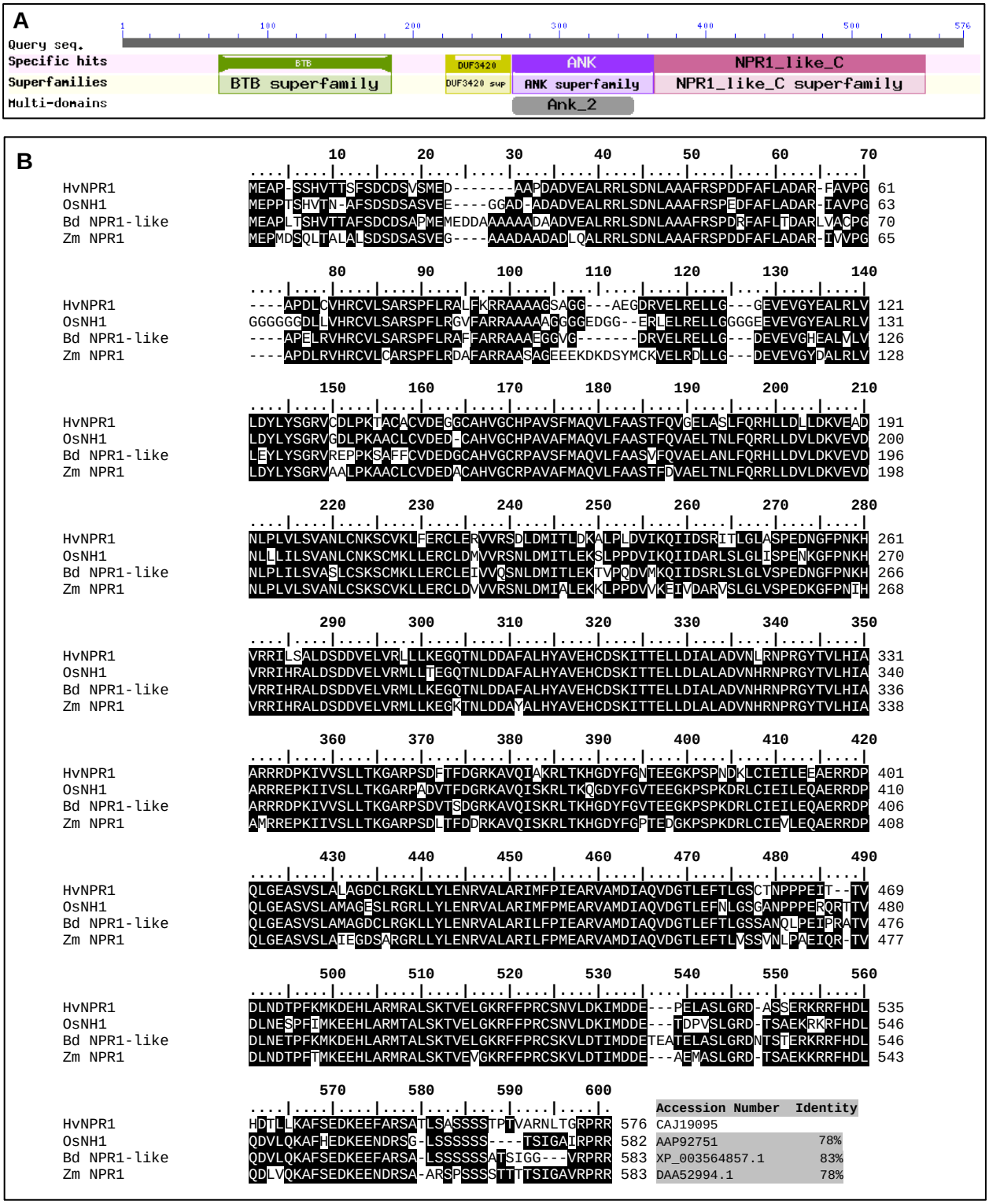

Figure S4 *Hv*NPR1 (CAJ19095). A) Overview of the spatial distribution of the consensus Broad-Complex, Tramtrack and Bric a brac (BTB) domain, Ankyrin repeats (ANK), and the NPR1_like_C domain (NPR1/NIM1 like defence protein C terminal) on the 576 amino acid sequence of CAJ19095 (depicted as query sequence) as obtained on blastp in NCBI. B) Multiple sequence alignment of *Hv*NPR1 showing identities in black, with rice *Os*NH1, *Brachypodium distachyon* NPR1-like and maize *Zm*NPR1. Respective accession numbers and percent identity to CAJ19095 are indicated to the far right of the alignment.

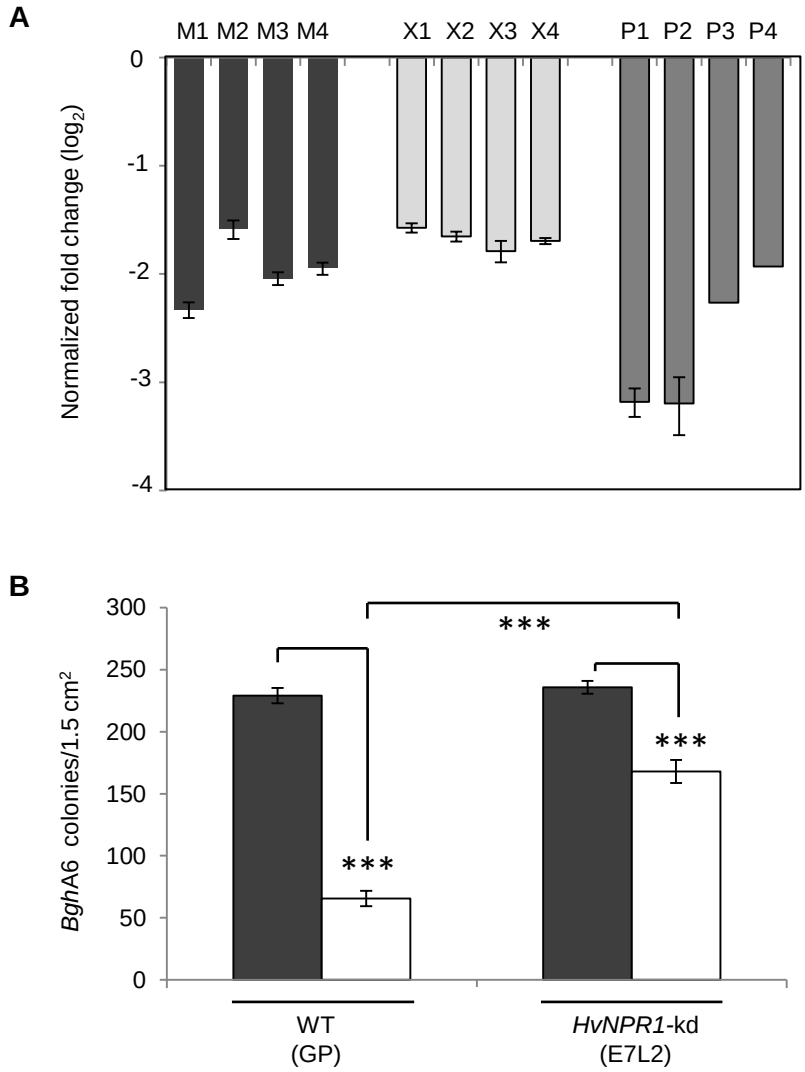

Figure S5 *HvNPR1* transcript levels and BTH-induced resistance to *Bgh*A6 in homozygous T4 plants of *HvNPR1*-kd line E7L2 A) *HvNPR1* transcript accumulation in T4 plants of *HvNPR1*-kd line E7L2. *HvNPR1* transcript accumulation was normalized to that of *HvEF1α* in samples from plants that were used to generate Fig. 2E. *HvNPR1* transcript accumulation in each plant is shown relative to the average *HvNPR1* transcript abundance in 10 GP wild type plants from the same experiment. Samples were taken from uninoculated leaves after the systemic immunity experiment that is depicted in Fig. 2E was finished. M, mock pre-treated plants (black bars), X, *Xtc* pre-treated plants (light grey bars), and P, *Psj* pre-treated plants (dark grey bars). B) Five-day-old T4 seedlings of the genotypes indicated below the panel were treated by soil drench with water-solved wettable powder (black bars) or with BTH (white bars) and first leaf segments were infected with *Bgh*A6 two days later. The resulting number of *Bgh*A6 colonies per 1.5 cm^2 was determined at 7 dpi. Values indicated are the mean (± standard error) of 40 plants per genotype and pretreatment and asterisks indicate statistically significant differences between data sets that are indicated by connecting lines (*** $P<0.001$, student's *t* test). The *HvNPR1*-kd line appeared slightly more susceptible to *Bgh*A6 compared to WT in the mock-treated control plants, but this was not significant in this experiment possibly due to higher virulence of the *Bgh*A6 inoculum used here compared to Fig. 2D (compare y-axis scales in Supplemental Fig. S5B and Fig. 2D).

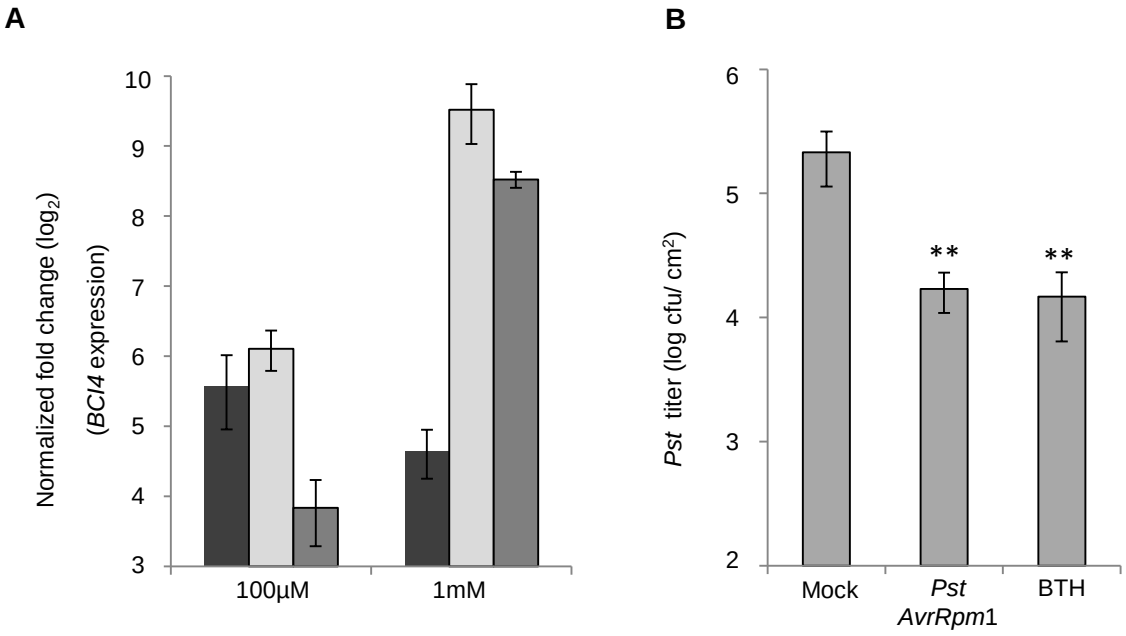

Figure S6 Barley and Arabidopsis responses to BTH application. A) BTH locally induces *BCI4* transcript accumulation in barley. qRT-PCR-assisted determination of *BCI4* transcript levels in barley cv GP leaves treated with 100 µM or 1 mM of BTH as measured at 1 day post-infiltration (dpi; black bars), 2 dpi (light grey bars), and 5 dpi (dark grey bars). Transcript accumulation was normalized to that of *HvEF1α* and is shown relative to transcript accumulation in mock-treated leaves. B) BTH induces SAR in *Arabidopsis thaliana*. Four to five-week-old Arabidopsis plants were infiltrated in the first two true leaves with 10 mM $MgCl_2$, 10^6 cfu/ml of *Pseudomonas syringae* pv *tomato* (*Pst*) delivering the effector AvrRpm1 (positive control), or with 100 µM of BTH. Three days later, the next two upper leaves were inoculated with *Pst*; the resulting *Pst* titers are shown at 4 dpi. Asterisks indicate significant differences to the mock control (** $P < 0.01$, student's *t* test).

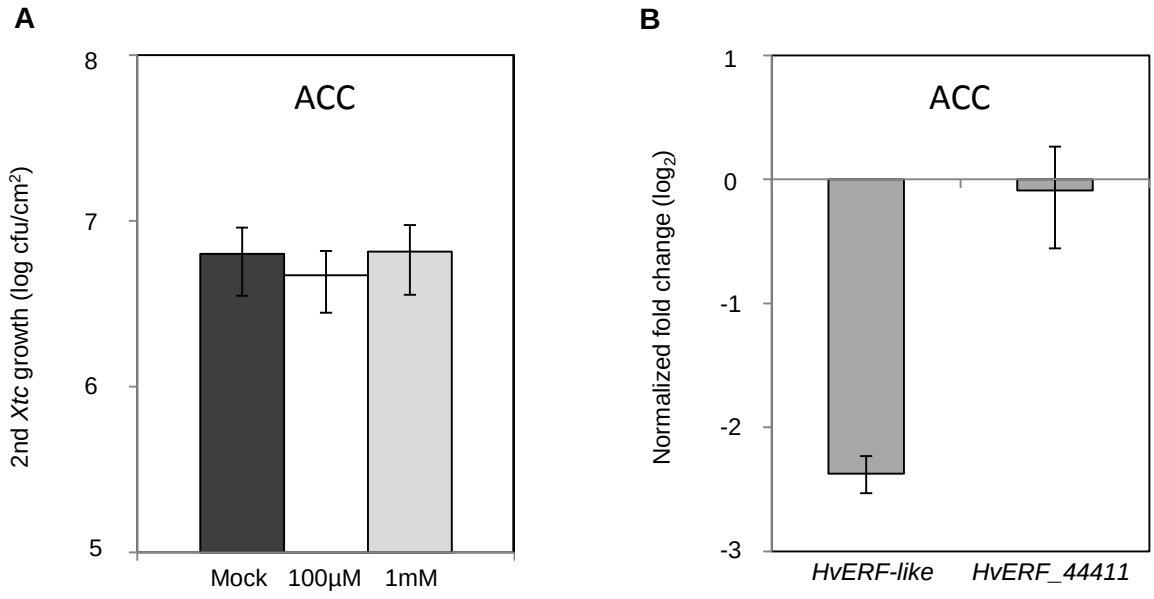

Figure S7 ACC does not induce systemic immunity in barley to *Xtc*. A) Four-week-old barley cv GP plants were infiltrated in leaf 1 with water (Mock) or with 100 µM or 1 mM of ACC as indicated below the panel. Five days later, systemic leaves were infected with *Xtc. Xtc* titers in the challenge-infected tissue are shown at 4 dpi as the average of 5 replicates ± standard deviation. This experiment was repeated three times with similar results. B) qRT-PCR-assisted determination of the transcript levels of *HvERF-like and HvERF_44411* in systemic leaf 2 of GP plants 5 days after a local treatment with 100 µM ACC in leaf 1. Transcript accumulation was normalized to that of *HvEF1α* and is shown relative to the normalized transcript levels of the respective genes in mock-treated plants. This experiment was repeated twice with similar results.

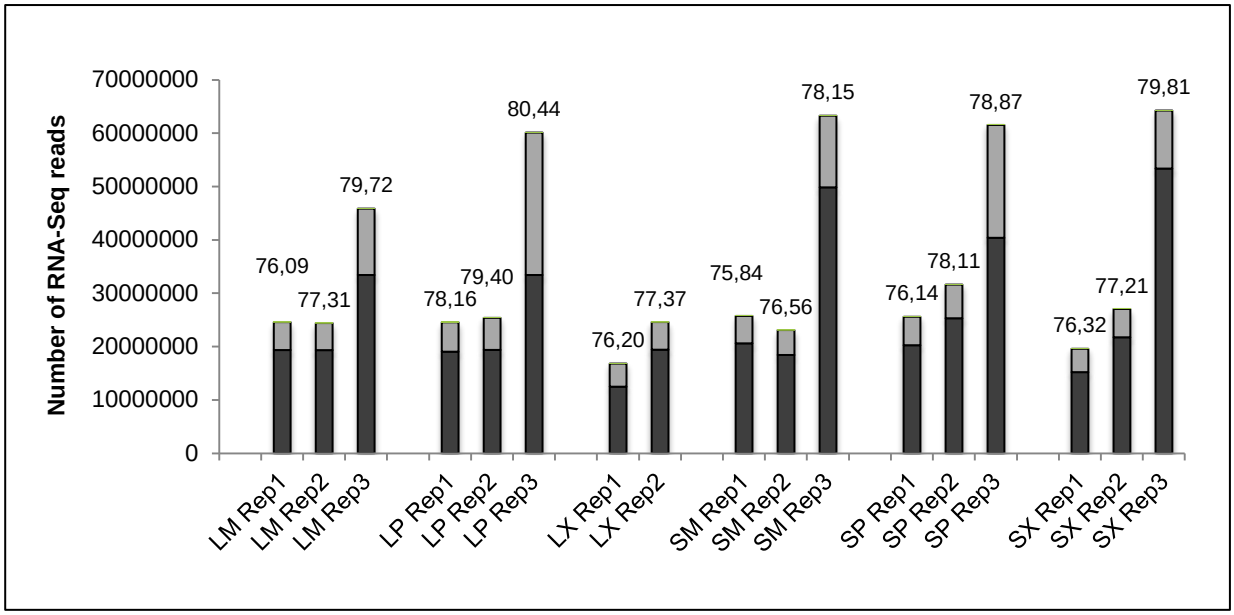

Figure S8 Mapping statistics of RNA-Seq data. Bars represent the number of reads that were sequenced from each sample, indicating the number of reads which could be mapped (in black) and reads which could not be mapped (in grey) to the *H. vulgare* cultivar Morex genome. The number above each bar indicates the percentage of genes that were expressed out of the total 24,244 annotated barley genes. Abbreviations LM, Local Mock-treated; LP, Local *Psj*-treated; LX, Local *Xtc*-treated; SM, Systemic to Mock treatment; SP, Systemic to *Psj*-treatment; SX, Systemic to *Xtc* treatment; Rep, replicate

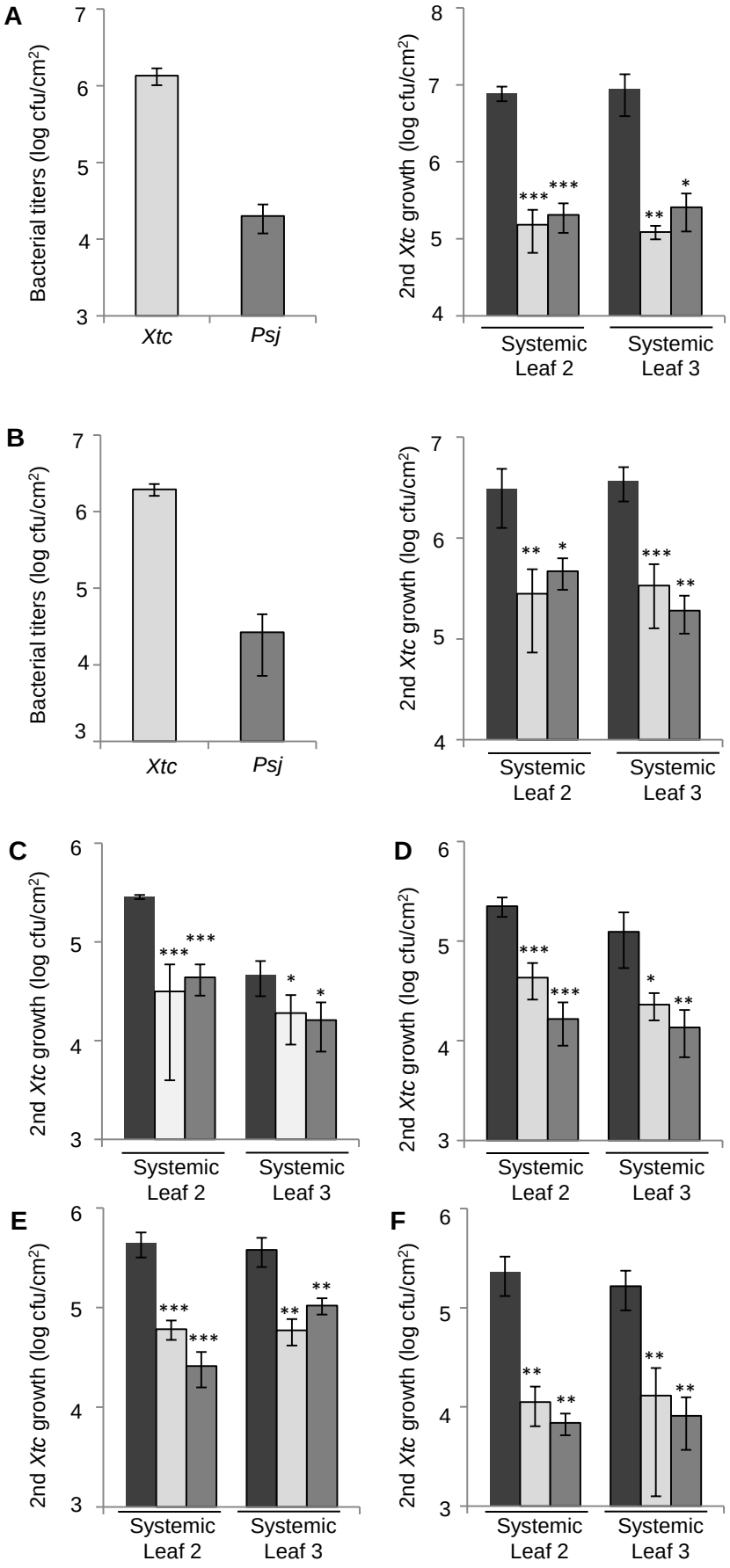

Figure S9 Systemic immunity controls A/B Panels to left: local *Xtc* (light grey bars) and *Psj* (dark grey bars) titers at 5 dpi in two of the replicate experiments that were used for RNA-seq analysis. Panels to right: *Xtc* titers in systemic secondary (2nd) infected leaves at 4 dpi confirming systemic immunity in the same experiments. C-F *Xtc* titers in systemic secondary (2nd) infected leaves at 4 dpi in the experiments that were used for qRT-PCR analysis in Figure 5. Panels to right in A/B and C-F Black bars, mock pre-treated plants; light grey bars, *Xtc*-pre-infected plants; dark grey bars, *Psj*-infected plants. Asterisks indicate statistically significant differences to the Mock controls (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, student's *t* test).

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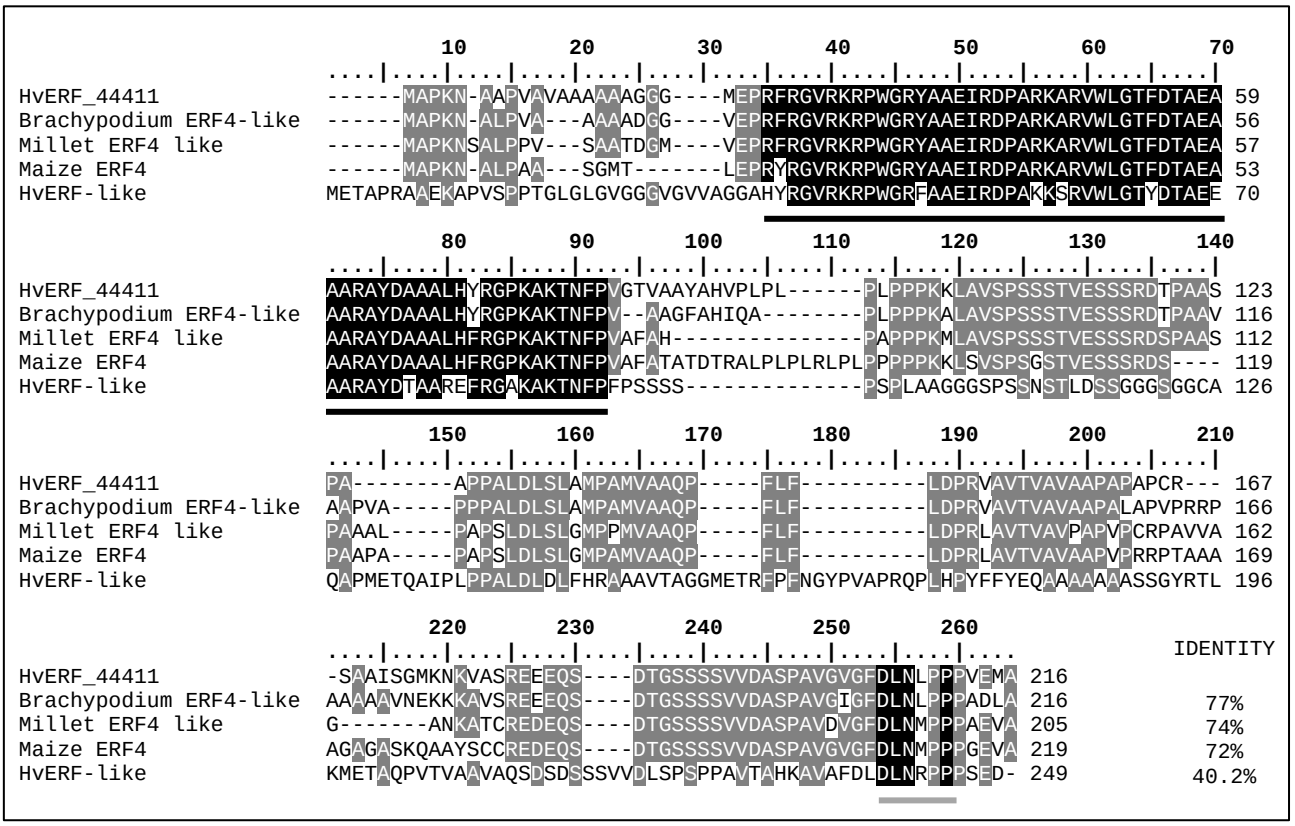

Figure S10 Multiple sequence alignment of *Hv*ERF_44411 (Accession No. AK364181 corresponding to the full length amino acid sequence of MLOC_44411) with *Brachypodium distachyon* ERF4-like (Accession No. XP_003580517.1), millet ERF4-like (Accession No. XP_004976762.1), maize ERF4 (Accession No. NP_001147685.1), and *Hv*ERF-like (MLOC_24530.1). Conserved residues are highlight in grey and the conserved AP2 domain as well as the EAR motif in black. The AP2 domain is underlined in black and the EAR motif in grey. The amino acid identities (in %) of the respective sequences to the query *Hv*ERF_44411 (AK364181) are indicated to the far right of the alignment.

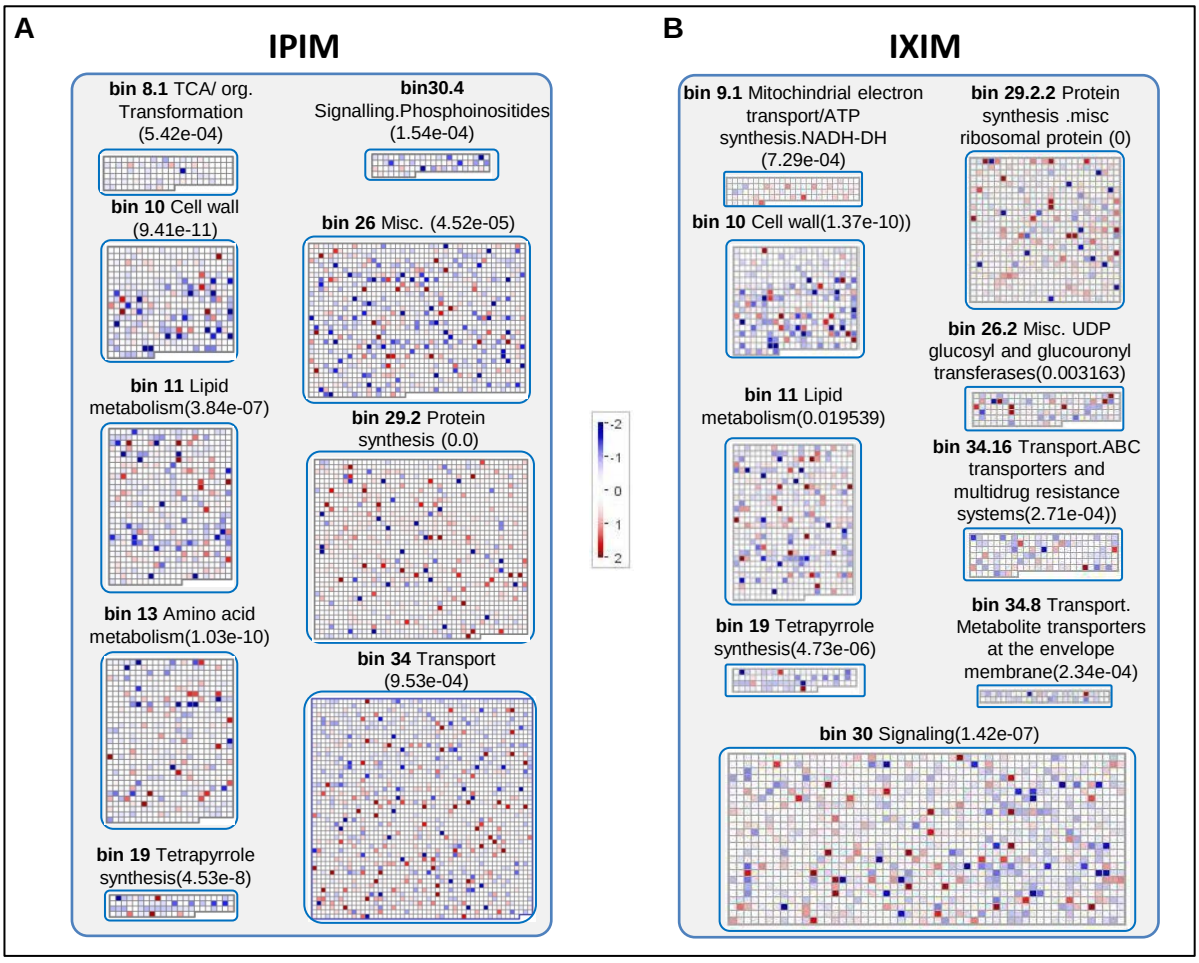

Figure S11 Summary of MapMan output of significantly regulated `bins`/biological processes on secondary *Xtc* infection of plants pre-infected with either *Psj* (A) or *Xtc* (B) compared to mock-pre-treated plants. Statistically significant `bins´ (Wilcoxon Rank test and FDR-corrected *P*<0.05) derived from the average of three biologically independent micro array replicates are shown if the same 'bins' or one or more of its 'sub-bins' also were enriched in two of the three individual replicates (Wilcoxon Rank test and FDR-corrected *P*<0.05). Red and blue indicate increase and decrease, respectively, in relative transcript abundance and are represented as $\log_2$ ratios. Each 'bin' is depicted with its corresponding MapMan number (in bold), name, and with the Wilcoxon Rank test and FDR-corrected *P* value from the analysis of the average data set (in brackets; Supplemental Table S3).

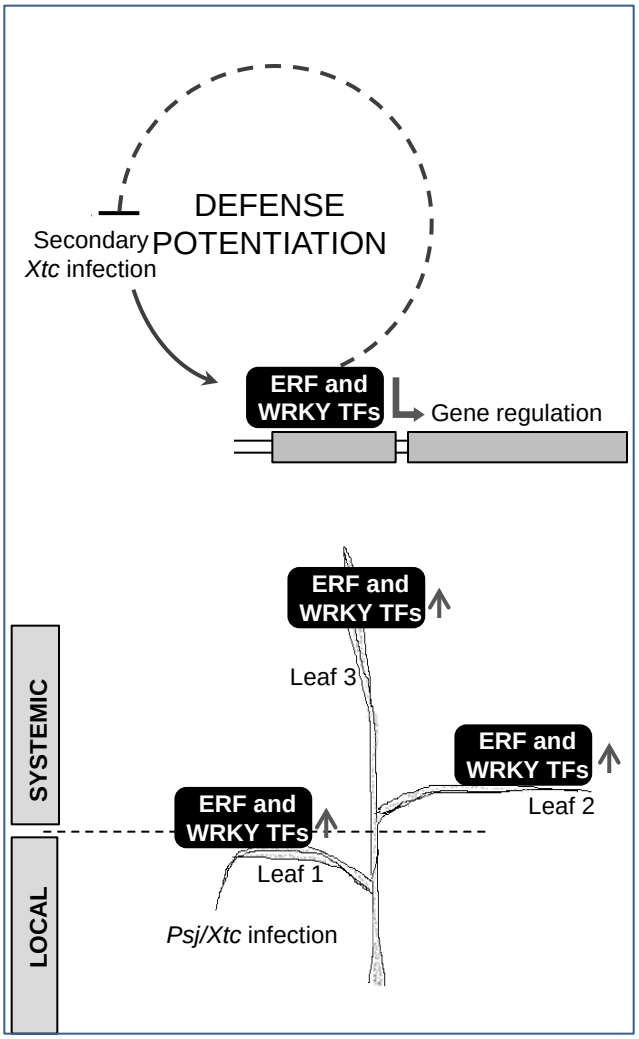

Figure S12 Working model of bacteria-induced systemic immunity in barley. Local *Psj/Xtc* infection in leaf 1 induces expression in the infected and systemic uninfected tissue (leaf 2 and 3) of ERF and WRKY TFs. Upon secondary *Xtc* challenge infection, these TFs might potentiate gene expression changes (solid grey arrow) and immunity (dashed arrow).